Die Hyperbare Sauerstofftherapie

bei akuten Apoplex
und
chronischen Apoplex-Folgen

in den Druckkammerzentren

des VDD e.V.

Verband Deutscher
Druckkammerzentren

Zusammenstellung von Informationen

für Ärzte

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(Dr. Steinberg, gesehen: Heiden Schlotterbeck) **(Stand 19.1.98)**

Die Hyperbare Sauerstofftherapie (HBO) als Behandlungsmethode im Rahmen des Therapiekonzeptes bei

Apoplex (akute und subakute cerebrale Ischämie)

Der Einsatz der hyperbaren Sauerstofftherapie ist bei der o.g. Indikation in folgenden Fällen sinnvoll:

- Begleitend zur Antikoagulation und rheologischen Therapie beim akuten ischämischen Insult
- Als alleinige Therapiemaßname bei Vorliegen von Kontraindikationen gegen die Antikoagulation mit Heparin (hypertensive Enzephalopathie mit Hirnödem, hämorrhagischer Infarkt)
- Als begleitende Maßnahme neben der rheologischen Therapie bei der subakuten cerebralen Ischämie

Inhaltsübersicht

1. Behandlungsindikationen

1.1 Epidemiologie

In Deutschland steht der cerebrale Gefäßinsult nach den Herz-Kreislauf-Erkrankungen und den bösartigen Tumoren an dritter Stelle der Todesursachen. In der Bundesrepublik Deutschland stirbt damit jeder zehnte Bürger über 50 Jahren an einem cerebralen Gefäßinsult. Ab dem 7. Lebensjahrzehnt ist der Schlaganfall bereits die zweithäufigste Todesursache. Die Zahl der jährlichen Neuerkrankungen wird auf das Dreifache der Sterblichkeitsziffer geschätzt, d.h., aufgrund der bekannten Mortalitätsziffer (1989: 83.605) kann man mit dem Auftreten ca. 270.000 neuer Schlaganfälle pro Jahr rechnen (28). Männer und Frauen sind etwa gleich häufig betroffen. Als Risikofaktoren gelten arterielle Hypertonie (erhöht die Inzidenz um den Faktor 4-8 im Vergleich zu normotensiven Personen gleichen Alters), Herzerkrankungen (insbesondere bei Vorliegen einer absoluten Arrhythmie bei Vorhofflimmern mit Vorhofthromben) Herzklappenfehler, Hyperlipoproteinämien, Diabetes mellitus, Übergewicht, Nikotinabusus und die Einnahme medikamentöser Ovulationshemmer (insbesondere bei übergewichtigen Frauen und Raucherinnen) (28). Eine erhebliche sozialmedizinische Bedeutung erlangt der Schlaganfall durch die Tatsache, daß etwa die Hälfte aller Patienten, die einen cerebralen Insult überleben, auf Dauer arbeitsunfähig bleiben. Zerebrale Gefäßinsulte beruhen in 85% der Fälle auf einer Mangeldurchblutung infolge thrombotischen oder embolischen Verschlusses von Hirngefäßen (28) mit daraus resultierender unzureichender O2-und Substratversorgung des Gewebes sowie einer lokalen Anhäufung zellschädigender Stoffwechsel-endprodukte (17).

1.2 Allgemeine Pathophysiologie des ischämischen Insults

1.2.1 Arteriosklerose

Die Gefäßerkrankung, die dem ischämischen Insult zugrunde liegt, ist in der überwiegenden Mehrzahl der Fälle eine fortgeschrittene Arteriosklerose. Sie tritt in erster Linie an den großen, extrakraniellen hirnversorgenden Arterien (A. carotis interna, A. vertebralis) und den größeren intrakraniellen Gefäßen (proximale A. cerebri media, A. basilaris und A. cerebri posterior) (6) auf. Dabei kommt es durch ein Endothelläsion und einer wachsenden Thrombose in diesem Bereich zu einem Territorialinfarkt = Insitu Thrombose.

1.2.2 Embolie

In einem kleineren Prozentsatz löst die Einschwemmung embolischen Materials in die cerebrale Endstrombahn einen Insult aus. Das eingeschwemmte Material stammt dabei entweder aus aufgebrochenen, arteriosklerotischen Plaques in den großen, extrakraniellen Arterien (v. a. der A. carotis interna = arterio-arterielle Embolie) oder aus dem Herzen = kardiale Embolie. Seltenere Quellen sind Ablagerungen auf den Klappensegeln bei Linksherzvitien oder kardiale Septumdefekte, die für eine paradoxe Embolie verantwortlich sein können (12).

1.2.3 haemodynamische Stenose
Bei einer hämodynamischen Stenose der extra- oder intrakraniellen Carotiden kann es bei einem Blutdruckabfall zu einem sogenannten Grenzzoneninfarkt kommen. Dieser pathogenetische Subtyp wird als hämodynamische Stenose bezeichnet.

1.2.4 Verschluß kleiner Gefäße
Bei einer sogenannten „small vessel disease“ kann es zu kleinen Gefäßverschlüssen mit der Entstehung von sogenannten Lacunen kommen. Dieser Typ ist der lacunäre Infarkt.

1.2.5 entzündliche Erkrankungen
Als deutlich seltenere Ursache einer akuten cerebralen Ischämie kommen entzündliche Gefäßkrankheiten (Panarteriitits nodosa, Beteiligung hirnversorgender Gefäße bei visceralen Lupus erythematodes oder die fibromuskuläre Dysplasie cerebraler Arterienwände) in Betracht (27, 48).

1.2.6
Vereinzelte Fälle treten zusätzlich im Rahmen von Bluterkrankungen (akute Leukosen, Polyzythämie mit Hyperviskositätssyndrom) und Gerinnungsanomalien auf (29).

1.3 Spezielle Physiologie und Pathophysiologie der cerebralen Zirkulation

Der globale Sauerstoffverbrauch des menschlichen Gehirns beträgt ca. 160 µmol 100 g-1 min-1 und der Glucoseverbrauch etwa 30 µmol 100 g-1 min-1. Bei normalem cerebralem Blutfluß (55 ml 100 g-1 min-1) extrahiert das Gehirn etwa 50% des Sauerstoffs und ca. 10% der Glucose des durchströmenden arteriellen Blutes. In 24 Stunden werden somit rund 75 Liter O2 und 115 Gramm Glucose verbraucht (36, 37). 90% der Glucose wird nach Umwandlung in Glucose-6-Phosphat der oxidativen Energiegewinnung zugeführt (37, 38). 75% des gesamten cerebralen Sauerstoffverbrauches entfällt auf die Aktivität von Neuronen (4, 36, 37). Eine Unterbrechung der Substratzufuhr im Rahmen eines ischämischen Insults führt zunächst zum Verbrauch des noch im Gewebe vorhandenen Restsauerstoffs (24). Während des Aufbrauchens dieser sehr begrenzten O2-Reserven innerhalb von 10-30 Sekunden kommt es zunächst zum Ausfall der spontanen hirnelektrischen Aktivität mit entsprechenden klinischen Ausfallserscheinungen bis hin zum Bewußtseinsverlußt. Das Abschalten der hirnelektrischen Spontanaktivität, die etwa 40% des gesamten cerebralen Sauerstoffkonsums ausmacht, geht dem Ausfall der evozierten Hirnaktivität voraus und ist möglicherweise ein zelleigener Schuzmechanismus (3, 4). Der Restsauerstoff wird dann durch die Na+-K+-ATPasen aufgebraucht (4). Ist kein Sauerstoff mehr für die oxidative Energiegewinnung vorhanden, setzt die anaerobe Glykolyse unter Verbrauch der restlichen Glucosevorräte ein, um basale Zellfunktionen und die Integrität der Membranpotentiale aufrechtzuerhalten (24, 36, 37, 44). Bei reversiblen Durchblutunsgstörungen, entsprechend den klinischen Stadien I (asymtomatische Gefäßstenose), II (transitorisch ischämische Attacke, TIA) und III (prolongiertes ischämisches Defizit, PRIND), kommt es in Abhängigkeit von der Ischämiedauer zur kompletten Erholung der zellulären und damit auch der neurologischen Funktionen (43). Bleibt jedoch die Gewebsperfusion dauerhaft unterbrochen (klinisches Stadium 4: kompletter Infarkt), wird die freie Glucose innerhalb von ca. 3-5 Minuten verbraucht und es kommt zu progredienten funktionellen Änderungen der neuronalen Membranen (3, 4, 9, 43, 44), deren funktionelle Integrität die Voraussetzung für die Generation und Weiterleitung der elektrischen Aktivität der Neurone bildet. Es kommt zur Anhäufung von Glutamat, Calcium strömt intracellulär vermehrt ein, und Phospholipasen und freie Radikale werden freigesetzt. Im Rahmen der ischämiebedingten progredienten Störung der energieabhängigen Membranfunktionen läßt die neuronale Erregbarkeit entsprechend nach. Dies kann über die Ableitung der evozierten hirnelektrischen Aktivität (evozierte Potentiale) anhand der reduzierten Erregbarkeit der Neurone bzw. der abnehmenden Erregungsleitung über deren Axone im zeitlichen Verlauf nachvollzogen werden (20, 44). Anhand eines retinalen Ischämiemodells konnte gezeigt werden, daß sich nach ca. 30 min kompletter Ischämie die Proteinbiosynthese in neuronalem Gewebe nicht wieder erholt und irreversible Zellschäden auftreten (1). Die Erholungsfähigkeit integrierter neurologischer Funktionen ist im Gegensatz zu der einzelner Zellen noch enger begrenzt. Obwohl das Zeitintervall zwischen vollständigem Ausfall des Funktionsstoffwechsels und Verlust der Zellintegrität im zweistelligen Minutenbereich liegt (1), ist eine Wiederherstellung der funktionellen Systemintegrität bereits vor dem Auftreten morphologisch faßbarer Zellschäden nicht mehr möglich (43, 44). Der Übergang zwischen reversiblem und irreversiblem Zellschaden läßt sich auch anhand der Änderung physikalischer Gewebseigenschaften, die möglicherweise auf Alterationen an Zytoskelett bzw. Zellmembranstrukturen beruhen, nachweisen (1). Das aus der Zellhypoxie resultierende zytotoxische Ödem kann über eine entsprechende Volumenzunahme umliegendes Hirngewebe durch Beeinträchtigung der Mikrozirkulation mit Verlängerung der Diffusionsstrecken für O2 und Substraten für den Energiestoffwechsel zusätzlich schädigen (5).

Bezogen auf die räumliche Ausdehnung des von der Ischämie betroffenen Hirnareals lassen sich ein Infarktkern und eine ischämische Penumbra voneinander abgrenzen (5, 9, 43). Im Infarktkern kommt es aufgrund der maximalen Ausprägung der vorangehend beschriebenen pathophysiologischen Abläufe zum raschen Zelluntergang. Dieses Gewebe ist einer Therapie in der Regel nicht mehr zugänglich (5). Daher konzentrieren sich sämtliche therapeutischen Interventionen auf die Erhaltung des Gewebes im Bereich der Penumbra. Die Penumbra zeichnet sich durch eine grenzwertig reduzierte Gewebsperfusion mit Ausbildung eines vasogen-zytotoxischen Ödems aus (5). Das Ödem ist auf den Einstrom von Natrium und freiem Wasser in die Zelle zurückzuführen, der aufgrund des vorliegenden Energiemangels nicht durch die entgegengesetzte Aktivität der Ionenpumpen ausgeglichen werden kann (3, 4, 5, 24).

CCT- Frühzeichen (1. – 8. Std.) sind:

- verstrichene Rindenzeichnung
- verstrichene Grenze grau/weiße Substanz in der Inselregion
  (loss of insular ribbon)
- Hypodensität Nucleus lentiformis (ab 3.-4. Std.)
- trübe diffuse Hypodensität
- hyperdenses Mediazeichen (Hyperdensität posterior) (bis 3. Tag)

In dieser Ödemzone, die sich in CT und MRT als perifokale Hypodensität / Hypointensität darstellen läßt, reicht der residuale Blutfluß zur (möglicherweise zeitlich begrenzten) Aufrechterhaltung der strukturellen Integrität aus (5, 43, 44). In Abhängigkeit von Schwere und Dauer der Ischämie kann sich der primäre Infarktkern durch sukzessive Ausdehnung in die Penumbra hinein vergrößern (24, 28).

Ziel aller derzeit standardmäßig eingesetzten therapeutischen Modalitäten ist es, die Versorgung des Gewebes im Bereich der Penumbra mit Sauerstoff und Glucose über eine Verbesserung der Durchblutungsverhältnisse zu steigern und damit eine Ausdehnung des primären Infarktkerns zu verhindern.

Voraussetzung einer Therapieeinleitung ist:
das Durchführen eines CCT zum Ausschluß einer Blutung und Suche nach Frühzeichen
Die Standardbehandlung beinhaltet zunächst die sogenannten Basismaßnahmen:
Blutdruck auf hohem Niveau stabilisieren > 150 mmHg syst.
Blutglucose normalisieren
gute Oxygenierung, Nomo
Temperatur normalisieren
Die Akuttherapie durch Thrombolyse durch rt-PA ist bei ausgewählten Fällen in ca. 5 - 10 % möglich, wenn der Patient innerhalb von drei Stunden behandelt wird
Die rheologische Therapie mit Häes ist verlassen
Die Standardtherapie mit Heparin perfusorgesteuert wird nach Ausschluß einer Blutung durchgeführt. Ziel ist die Unterbrechung des thrombotischen Prozesses oder Verhinderung von Reembolien. Bei ausgedehnten Territorialinfarkten wird die Therapie in der Regel nach 48 Stunden beendet.

Als Standardtherapie bei nachgewiesenem Infarkt (Stadium 4) gilt die perfusorgesteuerte, intravenöse Antikoagulation mit Heparin zur Verlängerung der partiellen Thromboplastinzeit (PTT) auf das 2-3-fache der Norm (32, 33). Ist noch kein Infarkt aufgetreten (TIA, PRIND), wird über den Einsatz rheologischer Maßnahmen (HAES, Senkung des Hämatokrit) versucht, die Fließeigenschaften des Blutes prophylaktisch zu verbessern. Der Wert der Hämodilutionstherapie mit HAES oder niedermolekularen Dextranen (z.B. Dextran 40) sowie der Antikoagulation mit Heparin konnte bislang nicht mit ausreichender Sicherheit nachgewiesen werden (32, 39, 42).

2. Therapeutischer Nutzen und Vorzüge der HBO

2.1 Grundlagen des Einsatzes der HBO-Therapie bei der akuten cerebralen Ischämie

Unter Berücksichtigung der pathophysiologischen Abläufe im Rahmen eines cerebralen ischämischen Insults erscheint der Einsatz der hyperbaren Sauerstofftherapie (HBO) als adjuvante therapeutische Maßnahme neben Antikoagulation und Hämodilution bzw. als alleinige Maßnahme bei Vorliegen von Kontraindikationen gegen eine

effektive Heparinisierung (Hypertensive Enzephalopathie mit Hirnödem, Gewebseinblutung im Bereich des Infarkts) gerechtfertigt (8, 10, 13, 18, 25, 30, 40, 41). Im Rahmen einer HBO kann der arterielle pO2 in Abhängigkeit vom Kammerdruck bei Beatmung mit 100 % Sauerstoff bis auf etwa 1.500 mmHg gesteigert werden (15). Der entsprechende Gewebe-pO2 (geschätzt aus dem im Liquor cerebrospinalis gemessenen pO2) beträgt dabei bis über 500 mmHg (15). Über diesen O2-Gradienten vom Gefäß über das Interstitium zur Zelle wird die O2-Versorgung des Gewebes trotz erheblicher Verlängerung der Diffusionsstrecke im Bereich ischämischer Hirnareale, insbesondere innerhalb der Penumbra beträchtlich gesteigert. Über die Reduktion sowohl der zytotoxischen, als auch der vasogenen Ödemkomponenten werden darüber hinaus die lokalen Zirkulationsverhältnisse günstig beeinflußt (21, 22, 40). Anhand dieser Mechanismen ist eine direkte, kausale Beeinflussung der Zellhypoxie durch rechtzeitigen Einsatz der HBO in der akuten Phase des cerebralen ischämischen Insults möglich (14). Unter Berücksichtigung der tierexperimentellen Daten muß im Einklang mit den vorangehend ausgeführten pathomechanistischen Überlegungen davon ausgegangen werden, daß ein maximaler therapeutischer Effekt nur dann zu erreichen ist, wenn die HBO innerhalb weniger Stunden nach Eintritt einer akuten cerebralen Ischämie eingesetzt wird (23). Im Gegensatz dazu kann eine HBO-Therapie bei chronischen cerebralen Zirkulationsstörungen auch noch Jahre nach Einsetzen der neurologischen Symptomatik zu erheblichen klinischen Befundbesserugen führen. In diesen Fällen geht es nicht um die Beeinflussung einer kompletten fokalen Ischämie mit progredienter Gewebsinfarzierung, sondern um die Kompensation einer chronischen bzw. rezidivierenden regionalen Mangeldurchblutung durch eine massive Steigerung des O2-Angebots (s. u.).

2.2 Grundlagen des Einsatzes der HBO bei der subakuten und chronisch rezidivierenden cerebralen Ischämie

Diese o. g. zeitliche Beschränkung bei der Einleitung einer HBO-Therapie gilt nicht bei Vorliegen einer arteriosklerotischen cerebrovaskulären Insuffizienz mit rezidivierendem Auftreten hämodynamisch bedingter neurologischer Symptome (TIA, PRIND). Hier führt die HBO durch die physikalisch bedingte Erhöhung des Sauerstofftransports in das Gewebe auch bei seit längerer Zeit bestehenden Beschwerden zu einer deutlichen Besserung der Symptomatik. Dieser Effekt ließ sich im Rahmen einer Therapiestudie an einem entsprechenden Patientengut nachweisen (25). Die hämodynamisch bedingte Reduktion der cerebrovaskulären Sauerstofftransportkapazität kann unter diesen Bedingungen durch den höheren Sauerstoffgehalt des Plasmas im Rahmen einer HBO kompensiert werden.

2.3 Übersicht / Zusammenfassung

Die hyperbare Sauerstofftherapie (HBO) soll als adjuvante Behandlung neben der Antikoagulation mit Heparin und der rheologischen Therapie im akuten und subakuten Stadium der cerebralen Ischämie eingesetzt werden. Bei reversiblen cerebralen Durchblutungsstörungen (TIA, PRIND) kann sie eingesetzt werden, um den Erholungsvorgang zu unterstützen. Chronische oder rezidivierende Symptome infolge einer arteriosklerotisch bedingten cerebralen hämodynamischen Insuffizienz können durch Steigerung des Sauerstoffgehaltes im Blut durch wiederholte hyperbare Oxygenierung günstig beeinflußt werden. Im Rahmen der Behandlung des manifesten Hirninfarktes kann die HBO als adjuvante Therapieform neben der Antikoagulation durch Reduktion des vasogen-cytotoxischen Ödems und über ein gesteigertes Sauerstoffangebot zur Minderung der Infarktgröße beitragen. Über eine Vervielfachung des arteriellen O2-Partialdruckes können mit Hilfe der HBO auch minderperfundierte Gewebsbezirke innerhalb der Penumbra mit Sauerstoff versorgt werden.

2.4 Spezifische Effekte der HBO

1. Unter Druck im Plasma gelöster Sauerstoff kann den arteriellen O2-Partialdruck derart erhöhen, daß die Versorgung des Gewebes nicht mehr an das Vorhandensein von Hämoglobin als Transportmolekül gebunden ist, sondern ausschließlich über den im Plasma gelösten O2 erfolgen kann (7). Bedeutsam wird dieser Effekt, wenn aufgrund einer durch Endothelschäden verursachten Viskositätszunahme des Blutes im Bereich der Mikrozirkulation der Strom der Erythrozyten stark verlangsamt bzw. unterbrochen wird: Der noch vorhandene Plasmastrom in den Kapillaren kann den Sauerstofftransport zum Gewebe dann aufrechterhalten (7).

2. Die Versorgung ischämischer Gewebeareale per Diffusion wird über die Anhebung des Gradienten zwischen dem hohem O2-Partialdruck in offenen, blutdurchströmten Kapillaren und dem niedrigen pO2 innerhalb verschlossener Kapillaren ermöglicht. Da das Gehirn ein kapillarreiches Gewebe darstellt, kann dieser Mechanismus zur Versorgung ischämischer Hirnareale nach Verschluß der sie versorgenden Gefäße beitragen (16).

3. Die HBO entfaltet einen antiödematösen Effekt über eine Vasokonstriktion. Der Hypoxie - induzierten Vasodilatation mit Extravasation von Plasmaproteinen und freiem Wasser im Bereich ischämischer Hirnareale wird entgegengewirkt. Auf diese Weise wird die Blut – Hirn - Schranke stabilisiert (22).

4. Die HBO reduziert das zytotoxische Ödem. Durch die Erhöhung des intrazellulären pO2 wird der zelluläre oxidative Energiestoffwechsel aufrechterhalten. Dadurch stehen weiterhin ausreichende Mengen von ATP für den Betrieb der membranständigen Ionenpumpen zur Verfügung (11, 19).

2.5 Studien und Expertenaussagen

Kategorie 1 (Randomisierte und kontrollierte Studien)

1. Nighoghossian N, Trouillas P, Adeleine P, Salord F (1995). Hyperbaric oxygen in the treatment of acute ischemic stroke. A double-blind pilot study. Stroke: 26 (8): 1369-72
Nighoghossian et al. untersuchten den Effekt der HBO hinsichtlich der Verhinderung von Langzeitbehinderungen bei 34 Patienten mit akutem Verschluß der A. cerebri media in einer randomisierten Studie. Innerhalb von 3 Jahren wurden insgesamt 34 Patienten in die Studie aufgenommen und nach Randomisierung auf Test- und Kontrollgruppe einer hyperbaren Behandlung mit 10 Tagessitzungen von je 40 Minuten bei 1.5 ATA mit entweder 100% Sauerstoff (HBO - Gruppe) oder Raumluft (Kontrollgruppe) unterzogen. Es erfolgte eine Verlaufskontrolle anhand eingehender klinisch - neurologischer Untersuchungen 6 Monate bzw. 1 Jahr nach Abschluß der Behandlung. Der im Rahmen dieser Nachuntersuchungen erhobene Befund wurde jeweils mit dem korrespondierenden neurologischen Befund bei Aufnahme (vor der Behandlung) verglichen. Dieser Vergleich erfolgte anhand parametrischer Testverfahren. Die HBO - Gruppe zeigte im Vergleich zur Kontrollgruppe einen tendenziell günstigeren klinischen Verlauf. Im Hinblick auf die Beurteilung der Effektivität der HBO bei der Behandlung der akuten cerebralen Ischämie anhand der vorliegenden Studie hat sich gezeigt, daß die Erfolgsaussichten der HBO (wie auch der anderen Therapieverfahren) dann am größten sind, wenn sie rasch, d. h. innerhalb weniger Stunden nach Ischämiebeginn eingesetzt wird. Das erforderliche Zeitfenster wurde im Rahmen der aufgeführten Studie nicht eingehalten (Einschlußkriterium: Aufnahme innerhalb von 24 Stunden nach Auftreten der klinischen Symptomatik).

2. Anderson DC, Bottini AG, Jagiella WM, Westphal B, Ford S, Rockswold GL, Loewenson RB (1991). A pilot study of hyperbaric oxygen in the treatment of stroke. Stroke 22: 1137-42
Anhand dieser Studie von Anderson et al. werden die o. g. Probleme bei der Durchführung eines geeigneten Protokolls zur Untersuchung der Effektivität der HBO im Rahmen der Behandlung des ischämischen Insults deutlich. Die Autoren prüften den Effekt der HBO - Behandlung bei 21 Patienten mit ischämischen Hirninfarkten. Die Einschlußkriterien umfaßten das Vorliegen einer einseitigen cerebralen Ischämie. Zugelassen wurden alle pathognentischen Mechanismen der Infarktentstehung (Arteriosklerose, rezidivierende Mikroembolien, kardiale Embolien und unbekannte Mechanismen). Der Beginn der Behandlung erfolgte bis zu 2 Wochen nach Auftreten der klinischen Symptomatik. Die Patienten wurden nach Aufnahme zunächst der üblichen Diagnostik unterzogen und anschließend auf der neurologischen Intensivstation einer nicht näher beschriebenen Behandlungsroutine zugeführt. Die mittlere Zeitspanne zwischen dem Auftreten der klinischen Symptomatik und dem Therapiebeginn in der HBO - Gruppe betrug 52 Stunden. Analog zur Studie von Nighoghossian et al. ist von einer hyperbaren Oxygenierung zu diesem späten Zeitpunkt kein durchgreifender therapeutischer Effekt mehr zu erwarten, da die von ihr beeinflußten pathogenetischen Abläufe bereits weit fortgeschritten und nicht mehr reversibel sind. Entsprechend dieser Anmerkungen ist das unbefriedigende Studienergebnis zu bewerten. Die Untersuchungsreihe wurde letztlich aufgrund des ausbleibenden Behandlungserfolges abgebrochen.

Kategorie 4 (nicht kontrollierte klinische Studien)

1. Saltzman HA, Anderson B, Whalen RE et al (1966). Hyprbaric oxygen therapy of acute cerebral vascular insufficiency. In BrowN IW, Cox BG (eds): HypeRbaric Medicine. National Research Council,
Washington DC
Saltzman et al. zeigten in einer retrospektiven Studie mit 25 Patienten mit akuter cerebrovaskulärer Insuffizienz eine deutliche, bleibende Verbesserung des klinischen Zustandes in 8 Fällen nach einmaliger HBO-Therapie (1 Stunde, 2-3 ATA). 5 weitere Patienten zeigten unter der Behandlung zunächst eine deutliche Besserung, verschlechterten sich jedoch im weiteren Verlauf wieder. In den übrigen 12 Fällen kam es zu keiner klinischen Befundänderung.

2. Holbach KH, Wassmann H (1979) Neurochirurgische Therapie der zerebralen Mangeldurchblutung. Neurol Psychiatr 5: 347
Holbach und Wassmann untersuchten 131 Patienten mit thrombotischem Verschluß der Aa. carotis interna und cerebri media. Die Patienten wurden einer mehrmaligen 1-stündigen HBO zugeführt. Als Parameter dienten

neben dem klinischen -neurologischen Befund der Verlauf des Elektroencephalogramms. Sie berichteten über eine Befundbesserung in 80% der akuten und 43% der chronischen Fälle.

3. Neubauer RA, End E (1980). Hyperbaric oxygenation as an adjunct Therapy in strokes due to thrombosis. Stroke 11:297
Neubauer und End nahmen 122 Patienten mit akuter und chronischer cerebraler Ischämie in ihre Studie auf. Bei Vorliegen einer akuten cerebralen Ischämie erfolgte eine 10-malige, jeweils 1-stündige Behandlung mit 1.5 ATA. Bei chronischen Fällen erfolgten 20 Behandlungszyklen. Die Liegedauer der behandelten Patienten (177 Tage) konnte gegenüber der Vergleichspopulation (287 Tage) erheblich gesenkt werden. Bei 65% der Patienten mit chronischen Hirngefäßerkrankungen konnte eine Zustandsverbesserung verzeichnet werden.

Wiederum muß betont werden, daß in den aufgeführten Studien die Patienten nicht unmittelbar nach Auftreten der neurologischen Symptomatik einer HBO unterzogen wurden. Da die HBO jedoch ihren maximalen Effekt nur in den ersten Stunden nach Beginn der Ischämie entfalten kann, geben auch die Ergebnisse dieser Untersuchungen den potentiellen Wert der Behandlung mit hyperbarem Sauerstoff nicht adäquat wieder.

Kategorie 5 (Studien zum Nachweis der Beeinflussung pathophysiologisch relevanter Größen)

1. Shiokawa O, Fujishima M, Yanai T, Ibayashi S, Ueda K, Yagi H (1986). Hyperbaric oxygen therapy in experimentally induced acute cerebral ischemia. Undersea Biomed Res 3: 337-344
Shiokawa et al. untersuchten den Effekt der HBO anhand der Überlebenszeiten sowie der cerebralen Laktat- und ATP- Spiegel nach beidseitiger Carotisligatur bei Ratten. Die Tiere der Testgruppe wurden 1 bzw. 3 Stunden nach Induktion einer cerebralen Ischämie durch Carotisligaturen beidseits einer 30-minütigen HBO (2 ATA, 100% O2) zugeführt. Die Kontrollgruppe wurde nicht behandelt. In der Kontrollgruppe wurden signifikant höhere Laktatwerte ($P<0.05$) und niedrigere ATP- Spiegel als in der HBO - Gruppe gemessen. Die Tiere der HBO - Gruppe wiesen dementsprechend längere Überlebszeiten auf ($P<0.05$).

2. Takahashi M, Iwatsuki N, Katsuhiko O, Tajima T, Akama M, Koga Y (1992). Hyperbaric oxygen therapy accelerates neurologic recovery after 15-minute complete global cerebral ischemia in dogs. Crit Care Med 20: 1588-94
Einen signifikanten Effekt der HBO konnten Takahashi et al. im Rahmen der Erholungsphase nach 15-minütiger globaler cerebraler Ischämie in einer Studie an Hunden nachweisen (n=20). Die Induktion einer globalen cerebralen Ischämie erfolgte über eine temporäre Unterbindung von Aorta ascendens und V. cava superior für 15 Minuten. Im Anschluß wurden je 10 Tiere einer HBO - Gruppe mit 3 Behandlungszyklen (3, 24 und 29 Stunden nach Rezirkulation für je 1 Stunde bei 3 ATA, 100% Sauerstoff) bzw. der Kontrollgruppe (gleiches Behandlungsschema bei Raumluft, entsprechend 21% Sauerstoff) zugeordnet. Der therapeutische Effekt der HBO im Vergleich zur Kontrollgruppe wurde anhand des elektroenzephalographischen und klinisch-neurologischen Verlaufs überprüft. Es zeigte sich eine Überlebensrate von 78% in der HBO - Gruppe gegenüber 30% in der Kontrollgruppe. Im Rahmen der 14-tägigen postischämischen Verlaufsbeobachtung zeigten die HBO - behandelten Hunde eine deutlich bessere Erholungstendenz sowohl des EEG als auch der klinisch-neurologischen Parameter als die Tiere der Kontrollgruppe ($p<0.02$). Diese Studie belegt den therapeutischen Wert der HBO bei frühem Behandlungsbeginn nach abgelaufenem ischämischen Insult.

3. Risiken

Die Risiken der HBO-Therapie für die hier genannten Behandlungsindikationen sind mit denen bei anderen Behandlungsindikationen vergleichbar. Sie werden daher in dieser Zusammenfassung nicht gesondert aufgeführt, sondern in einer eigenen Darstellung erörtert (s. Kapitel „Risiken der HBO-Therapie“).

4. Wirtschaftlichkeit

Die Wirtschaftlichkeit der HBO in der Therapie der akuten und subakuten cerebralen Ischämie muß anhand der potentiellen Reduktion der Anzahl dauerhaft cerebral geschädigter Patienten gemessen werden, die nicht mehr in die Berufstätigkeit zurückkehren. Dieser Anteil beträgt derzeit mindestens 50%. Der daraus resultierende volkswirtschaftliche Schaden ist gerade angesichts des Alters der Betroffenen beträchtlich, da eine große Zahl der Patienten noch berufstätig ist. Erhebliche finanzielle Mittel müssen für die Akutversorgung und Rehabilitation cerebral geschädigter Patienten aufgebracht werden. Dabei entfällt der größte Anteil auf die Liegezeiten in den Einrichtungen zur Akutversorgung und auf die Langzeittherapie dauerhaft geschädigter Patienten in speziellen Pflegeeinrichtungen. Maßnahmen, die eine Reduktion der Anzahl intensiv- bzw. dauerhaft pflegebedürftiger Patienten zur Folge haben, bedeuten daher eine enorme finanzielle Entlastung der jeweiligen Kostenträger (Kassen, Rentenversicherungen, Sozialämter etc.). Angesichts der Kosten und Erfolgsquoten der gegenwärtig üblichen Therapiemaßnahmen kann erwartet werden, daß die HBO ein kosteneffektives Heilverfahren darstellt.

6. Anlagen (komplette Literaturstellen zur Belegung der Aussagen in der Geschäftsstelle des - Verbandes Deutscher Druckkammerzentren (VDD e.V.) Cuno Niggl Str. 3, 83278 Traunstein - zu beziehen)
(1, 4, 5, 9, 10, 15, 18, 20, 21, 22, 23, 25, 30, 37, 39, 44, 46, 47)

7. Literaturverzeichnis

1) Ames A, Nesbett FB (1982). Pathophysiology of ischemic cell death (I-III). Stroke 14: 219-240
2) Anderson DC, Bottini AG, Jagiella WM, Westphal B, Ford S, Rockswold GL, Loewenson RB (1991). A PILOT STUDY OF HYPERBARIC OXYGEN IN THE TREATMENT OF STROKE. STROKE 22: 1137-42
3) Astrup J, Branston L, Symon L (1977). Extracellular potassium activity, evoked potential and tissue blood flow: Relationship during progressive ischaemia in baboon cerebral cortex. J Neuro Sci 32: 305-321
4) Astrup J, Siesjö BK, Symon L (1981). Thresholds in cerebral ischemia: The ischemic penumbra. Stroke 12: 723-725
5) Astrup J, Sørensen PM, Sørensen HR (1981). Oxygen and glucose consumption related to Na^+ - K^+ transport in canine brain. Stroke 12: 726-730
6) Barnett HJM, Mohr JP, Stein BM, Yatsu FM (eds; 1986): Stroke - Pathophysiology, Diagnosis and Management. Chapter III: Clinical Manifestations of Stroke. Churchill Livingstone, New York
7) Boerema I, Meyne NG, Brummelkamp WK, Bouma S, Mensch MH, Kammermans F, Sternhanf M, Van Aaldern W (1960). Life without blood: a study of influence of high atmospheric pressure and hypothermia on dilution of blood. Cardiovasc Surg 1: 133-146
8) Branston NM, Symon L, Crockard HA, Pasztor E (1974). Relationship between the cortical evoked potential and local cortical blood flow following acute middle cerebral artery occlusion in the baboon. Exp Neurol 45: 195-208
9) Burt JT, Kapp JP, Smith RR (1987). Hyperbaric oxygen and cerebral infarction in the gerbil. Surg Neurol 28: 265-268
10) Contreras RL, Kadekaro M, Eisenberg HM (1988). The effect of hyperbaric oxygen on glucose utilization in a freeze-traumatized rat brain. J Neurosurg 68: 137-141
11) Corkill G, Van Housen K, Hein L, Reitan J (1985). Videodensitometric estimation of the protective effect of hyperbaric oxygen in the ischemic gerbil brain. Surg Neurol 24: 206-210
12) Furlan AJ, Conomy JP (1986). Stroke related to cardiac dysrhythmia and valvular heart disease. In Barnett HJM, Mohr JP, Stein BM, Yatsu FM (eds): Stroke - Pathophysiology, Diagnosis and Management. Churchill Livingstone, New York
13) Holbach KH, Caroli A, Wassmann H (1977). Cerebral energy metabolism in patients with brain lesions at normo-and hyperbaric oxygen pressures. J Neurol 217: 17-30
14) Holbach KH, Wassmann H (1979) NEUROCHIRURGISCHE THERAPIE DER ZEREBRALEN MANGELDURCHBLUTUNG. NEUROL PSYCHIATR 5: 347
15) Hollin SA, Espinosa OE, Sukoff MH, Jacobson JH (1968). The effect of hyperbaric oxygenation on cerebrospinal fluid oxygen. J Neurosurg 29: 229-235
16) Homer LD, Weathersby PK, Kieslow LA (1981). Oxygen gradients between red blood cells in the microcirculation. Microvasc Res 22: 308-323
17) Hurn PD, Koehler RC, Norris SE, Blizzard KK, Traystman RJ (1991). Dependence of cerebral energy phosphate and evoked potential recovery on end-ischemic pH. Am J Physiol 260: H532-H541
18) Kapp JP, Phillips M, Markow A, Smith RR (1982). Hyperbaric oxygen after circulatory arrest: modification of postischemic encephalopathy. Neurosurgery 11: 496-499
19) Lanse SB, Lee JC, Jacobs EA, Brody H (1978). Changes in the permeability of the blood-brain-barrier under hyperbaric conditions. Aviat Space Environ Med 49: 890-894
20) McPherson RW, Zeger S, Traystman RJ (1986). Relationship of somatosensory evoked potentials and cerebral oxygen consumption during hypoxic hypoxia in dogs. Stroke 17: 30-36

21) Miller JD, Ledingham IM, Jennet WB (1970). Effects of hyperbaric oxygen on intracranial pressure and cerebral blood flow in experimental cerebral oedema. J Neurol Neurosurg Psychiatry 33: 745-755
22) Mink RB, Dutka AJ (1995). Hyperbaric oxygen after global cerebral ischemia in rabbits reduces brain vascular permeability and blood flow. Stroke 26: 2306-12
23) Nakahara I, Kikuchi H, Taki W, Nishi S, Kito M, Yonekawa Y, Goto Y, Ogata N (1992). Changes in major phospholipids of mitochondria during postischemic reperfusion in rat brain. J Neurosurg 76: 244-250
24) Nemoto EM (1978). Pathogenesis of cerebral ischemia-anoxia. Crit Care Med 6: 203-214
25) Neubauer RA, End E (1980). Hyperbaric oxygenation as an adjunct therapy in strokes due to thrombosis: a review of 122 patients. Stroke 11: 297-300
26) Nighoghossian N, Trouillas P, Adeleine P, Salord F (1995). HYPERBARIC OXYGEN IN THE TREATMENT OF ACUTE ISCHEMIC STROKE. A DOUBLE-BLIND PILOT STUDY. STROKE: 26 (8): 1369-72
27) Petty GW, Mohr JP (1986). Giant cell arteriitis and isolated granulomatous angiitis of the central nervous system. In Barnett HJM, Mohr JP, Stein BM, Yatsu FM (eds): Stroke - Pathophysiology, Diagnosis and Management. Churchill Livingstone, New York
28) Raish RJ. Hoak JC (1986). Coagulopathy and Stroke. In Barnett HJM, Mohr JP, Stein BM, Yatsu FM (eds): Stroke - Pathophysiology, Diagnosis and Management. Churchill Livingstone, New York
29) Rockswold GL, Ford SE, Anderson DC, Bergman TA, Sherman RE (1992). Results of a prospective randomized clinical trial for treatment of severely brain-injured patients with hyperbaric oxygen. J Neurosurg 76: 929-934
30) Saltzman HA, Anderson B, Whalen RE et al (1966). HYPRBARIC OXYGEN THERAPY OF ACUTE CEREBRAL VASCULAR INSUFFICIENCY. IN BROWN IW, COX BG (EDS): HYPERBARIC MEDICINE. NATIONAL RESEARCH COUNCIL, WASHINGTON DC
31) Salzman EW, Deykin D, Shapilo RM, Rosenberg R (1975). Management of heparin therapy controlled prospective trial. N Engl J Med 299: 1046
32) Sandercock PA, van den Belt AG, Lindley RI, Slattery J (1993). Antithrombotic therapy in acute ischaemic stroke: an overview of the completed randomised trials. J Neurol Neurosurg Psychiatry 56: 17-25
33) Shiokawa O, Fujishima M, Yanai T, Ibayashi S, Ueda K, Yagi H (1986). HYPERBARIC OXYGEN THERAPY IN EXPERIMENTALLY INDUCED ACUTE CEREBRAL ISCHEMIA. UNDERSEA BIOMED RES 3: 337-344
34) Siesjö BK (1978). Brain energy metabolism. John Wiley & Sons, New York
35) Smith G, Lawson DD, Renfrew S, Ledingham IM, Sharp GR (1961). Preservation of cerebral cortical activity by breathing oxygen at two atmospheres of pressure during cerebral ischemia. Surg Gynecol Obstet 113: 13-16
36) Sokoloff L (1989). Circulation and energy metabolism of the brain. In Siegel GJ, Albers RW, Katzman R, Agranoff BW (eds): Intermediary metabolism in basic neurochemistry: molecular, cellular and medical aspects. 4th edition, Raven Press, pp541-564
37) Sokoloff L, Reivich M, Kennedy C, Des Rosiers MH, Patlak CS, Pettigrew KD, Sakurada O et al (1977). The [^{14}C]-deoxiglucose method for the measurement of local cerebral glucose utilization: Theory, procedure, and normal values in the conscious and anaesthetized albino rat. J Neurochem 28: 897-916
38) Spudis EV, de la Torre E, Pikula L (1973). Management of completed strokes with dextran 40. A community hospital failure. Stroke 4: 895
39) Sterman AB, Furlan AJ, Pessin M, Kase C, Caplan L, Williams G (1987). Acute stroke therapy trials: An introduction to recurring design issues. Stroke 18: 524-527
40) Sukoff MH, Hollin SA, Espinosa OE, Jacobson JH (1968). The protective effect of hyperbaric oxygenation in experimental cerebral edema. J Neurosurg 29: 236-241
41) Sukoff MH, Ragatz RE (1982). Hyperbaric oxygenation for the treatment of acute cerebral edema. Neurosurgery 10: 29-38
42) Symon L (1980). The relationship between CBF, evoked potentials and the clinical features in cerebral ischemia. Acta Neurol Scand 78: 175-190
43) Symon L, Branston NM, Strong AJ, Hope TD (1977). The concepts of ischemia in relation to brain structure anf function. J Clin Pathol (Suppl) 11: 149-154

44) Takahashi M, Iwatsuki N, Katsuhiko O, Tajima T, Akama M, Koga Y (1992). HYPERBARIC OXYGEN THERAPY ACCELERATES NEUROLOGIC RECOVERY AFTER 15-MINUTE COMPLETE GLOBAL CEREBRAL ISCHEMIA IN DOGS. CRIT CARE MED 20: 1588-94
45) Weinstein PR, Anderson GG, Telles DA (1987). Results of hyperbaric oxygen therapy during temporary middle cerebral artery occlusion in unanesthetized cats. Neurosurgery 20: 518-524
46) Whalen RE, Heyman A, Saltzman H (1966). Hyperbaric oxygenation in cerebral anoxia. Arch Neurol 14: 15-20
47) Yonekawa Y, Handa H, Okuno T (1986). Moyamoya disease: Diagnosis, treatment and recent achievement. In Barnett HJM, Mohr JP, Stein BM, Yatsu FM (eds): Stroke - Pathophysiology, Diagnosis and Management. Churchill Livingstone, New York
48) Zerebraler Gefäßinsult (1992). In Poeck, K (ed). Neurologie, Kapitel III: Akute Zirkulationsstörungen im ZNS, pp 151-175

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Hyperbaric Oxygen Induces Late Neuroplasticity in Post Stroke Patients - Randomized, Prospective Trial

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Abstract

Background: Recovery after stroke correlates with non-active (stunned) brain regions, which may persist for years. The current study aimed to evaluate whether increasing the level of dissolved oxygen by Hyperbaric Oxygen Therapy (HBOT) could activate neuroplasticity in patients with chronic neurologic deficiencies due to stroke.

Methods and Findings: A prospective, randomized, controlled trial including 74 patients (15 were excluded). All participants suffered a stroke 6–36 months prior to inclusion and had at least one motor dysfunction. After inclusion, patients were randomly assigned to "treated" or "cross" groups. Brain activity was assessed by SPECT imaging; neurologic functions were evaluated by NIHSS, ADL, and life quality. Patients in the treated group were evaluated twice: at baseline and after 40 HBOT sessions. Patients in the cross group were evaluated three times: at baseline, after a 2-month control period of no treatment, and after subsequent 2-months of 40 HBOT sessions. HBOT protocol: Two months of 40 sessions (5 days/ week), 90 minutes each, 100% oxygen at 2 ATA. We found that the neurological functions and life quality of all patients in both groups were significantly improved following the HBOT sessions while no improvement was found during the control period of the patients in the cross group. Results of SPECT imaging were well correlated with clinical improvement. Elevated brain activity was detected mostly in regions of live cells (as confirmed by CT) with low activity (based on SPECT) – regions of noticeable discrepancy between anatomy and physiology.

Conclusions: The results indicate that HBOT can lead to significant neurological improvements in post stroke patients even at chronic late stages. The observed clinical improvements imply that neuroplasticity can still be activated long after damage onset in regions where there is a brain SPECT/CT (anatomy/physiology) mismatch.

Trial Registration: ClinicalTrials.gov NCT00715897

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Introduction

Intensive functional therapy and rehabilitation programs for post stroke patients are considered essential for maximizing the patients' quality of life [1,2]. Unfortunately, these programs are often just partially successful, and additional therapeutic approaches towards metabolic recovery of affected cerebral tissues are called for. While a considerable amount of preclinical research supports the use of hyperbaric oxygen therapy (HBOT) for post-stroke damaged brain tissue, so far, only 5 articles reported controlled clinical trials of HBOT for stroke patients. These studies, in which the treatment started during the early-acute phase immediately after stroke, yielded non conclusive and somewhat contradicting results [3,4,5,6,7]. In contrast, a recent phase-I study evaluating the effect of HBOT on chronic neurological deficiencies (due to traumatic brain injury) revealed promising results [8]. However, to date the effects of HBOT on neurological deficiencies due to stroke during the late-chronic phase (the focus of the current report) have not yet been investigated in a prospective randomized trial.

Years of clinical experience revealed that the dramatic spontaneous recovery from stroke occurs mainly within the first 30 days, though moderate and severe stroke survivors continue to improve for at least 90 days [9]. Most of the recovery involves brain regions rendered dysfunctional, but not dead [10]. Accumulated data from visualizations of these non-active (stunned) regions indicates that they may persist alive but dysfunctional for months, even years, after the acute injury [11,12,13]. It was

proposed that the oxygen supply to these under-active neurons was low due to stroke damage to blood vessels in these regions, leading to oxygen deficiency, anaerobic metabolism and ATP depletion [14,15]. The decreased oxygen level not only causes reduction in the neuronal activity but also prevents angiogenesis to replace the stroke-damaged blood vessels and the generation of new synaptic connections. Since 1 cm^3 of normal brain tissue contains about 1 km of blood vessels, high oxygen supply is essential for repair of the stunned regions. Indeed, as has been demonstrated by previous studies, an increase in dissolved oxygen has several beneficial effects in damaged brain tissues [13,16,17,18,19,20]. Transport of oxygen to glial mitochondria, the main sites of oxygen utilization, follows oxygen release from erythrocytes into the plasma and then diffusion of the blood-dissolved oxygen across the Blood-Brain Barrier (BBB). Breathing oxygen under hyperbaric conditions has been shown to be a potent means of increasing arterial oxygen tension and consequently the brain oxygen tension [20,21,22]. For example, at 2ATA (atmospheres absolute), the plasma O_2 partial pressure rises above 1,110 mmHg. Hence, it is reasonable to expect that HBOT can be an efficient (and clinically feasible) method for increasing tissue/cellular oxygenation and thus effectively evoking neuroplasticity in the chronically non-active areas during the late post-stroke phase.

Many physiological pathways, each with a different characteristic time, are spontaneously activated following the onset of stroke. Therefore, a challenging question to be addressed considers the optimal time lapse after stroke to start the HBOT procedure. It should also be kept in mind that signals and chemical cues associated with cell death during the acute stage of stroke might, in fact, promote repair during recovery [23] and can be negatively affected by premature application of HBOT. Unlike the case of preclinical animal studies, in clinical practice it is not feasible to apply the HBOT immediately at the stroke onset. Thus, HBOT procedure can practically begin either at the degenerative or at the regenerative stage. One can assume that any added energy during the degenerative stage could further increase the unwanted, post-injury damage. On the other hand, elevated oxygen supply during the regenerative stage would supply the energy needs for the innate brain repair processes. The differences in the time lapse between stroke onset and HBOT application in previous studies are likely to be the reason for the contradictive results obtained for HBOT application during the acute phase after stroke [3,4,5,6,7]. The aim of the current study was to evaluate the effects of HBOT started at the late-chronic phase after the acute stroke.

Methods

The study was performed as a prospective, randomized, controlled, two-group trial. The population included patients of ages 18 years or older, who had either ischemic or hemorrhagic stroke 636 months prior to their inclusion. All patients had to have at least one motor dysfunction. Exclusions were based on chest pathology incompatible with HBOT, inner ear disease, claustrophobia and inability to sign informed consent. Additional exclusions were based on dynamic neurologic improvements during the last month (based either on objective measurements by external evaluator or on subjective statement by the patients). Smoking was not allowed during the study. All patients signed written informed consent; the protocol was approved by the local Helsinki committee. The study was conducted in the hyperbaric and research units of Assaf-Harofeh Medical Center, Israel.

Protocol and End Points

After signing an informed consent form, the patients were invited for baseline evaluations. Included patients were randomized into two groups (1:1 randomization): a treated group and a cross group. The neurologic functions as evaluated by National Institutes of Health Stroke Scale (NIHSS) [24,25], ability to perform activities of daily living (ADL) [26], and brain metabolism as visualized SPECT were the primary endpoints of the study. The secondary end point of the study included Quality of life evaluation. The patients in the treated group were evaluated twice – at baseline and after 2 months of HBOT treatment. Patients in the cross group were evaluated three times: baseline, after 2 months control period of no treatment, and after consequent 2 months of HBOT sessions (Figure 1). The post-HBOT neurological evaluations as well as the SPECT scans were performed more than 1 week (1–3 weeks) after the end of the HBOT protocol. The following HBOT protocol was practiced: 40 daily sessions, 5 days/week, 90 minutes each, 100% oxygen at 2ATA. The detailed clinical study protocol (Protocol S1), randomization and placebo consideration (Text S1), copy of the informed consent (Form S1), as well as CONSORT 2010 checklist of information (Checklist S1) are attached as supporting information.

Neurologic Evaluation

The clinical severity of the stroke was blindly assessed by a trained physician according to the NIHSS [24,25]. The ADL were evaluated by a questionnaire covering the following functions: bathing, dressing, grooming, oral care, toileting, walking, climbing stairs, eating, shopping, cooking, managing medications, using phone, housework, doing laundry, driving and managing finances [26]. For each criterion, the patient defined whether he/she is independent, needs help, dependent or does not do at all (range: 0(best)-51(worst)).

Brain Functional Imaging and Analysis

Brain single photon emission computed tomography (SPECT) was conducted with 925–1,110 MBq (25–30 mCi) of technetium-99methyl-cysteinate-dimmer(Tc-99m-ECD)at 40–60 min post injection using a dual detector gamma camera (ECAM or Symbia T, Siemens Medical Systems) equipped with high resolution collimators. Data was acquired in 3-degree steps and reconstructed iteratively with Chang method (μ = 0.12/cm) attenuation correction [27]. Visual analysis was conducted by fusing pre- and post-treatment studies that were normalized to pre-treatment whole brain activity. SPECT images were reoriented into Talairach space using NeuroGam (Segami Corporation) for identification (based on visual inspection) of abnormal perfusion regions and in order to compute volume rendered brain images.

More specifically, the assessment was done independently by two nuclear medicine physicians who compared the scans and graded them as either: 1 = no change, 2 = mild change and 3 = significant change. This was done “blindly”(without preconditioned information about the patients). “No change”was assigned to no visual difference in the number or size of perfusion deficits; “mild change”to a reduction in number or size of perfusion defects; “significant change”to a global perfusion increment in addition to diminution of defect numbers or size. Differences in evaluation were resolved after mutual reviewing. A comparison of the SPECT results with anatomical imaging CT was conducted in order to evaluate the extent of perfusion deficit in relation to the anatomical lesion. All SPECT

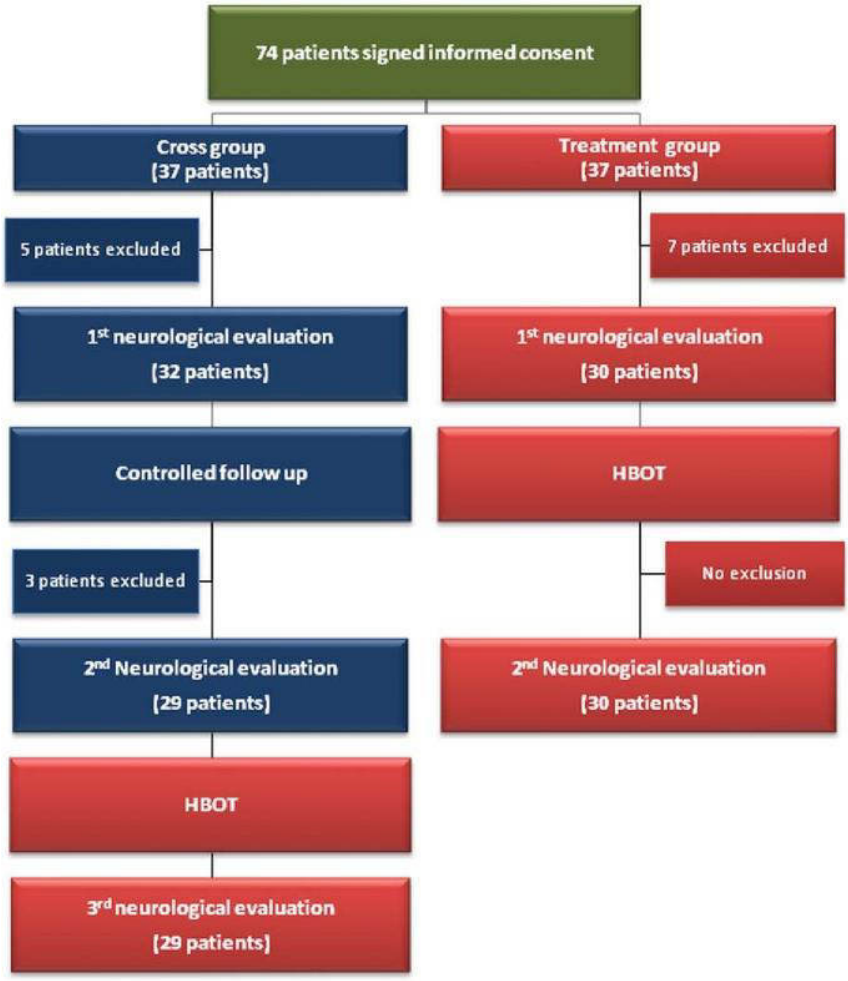

Figure 1. Flowchart of the patients in the study.
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analysis were done while blinded to the laboratory and clinical data.

Quality of Life Evaluation

Quality of life was evaluated by the EQ-5D questionnaire [28]. EQ-5D essentially consists of 2 pages: the EQ-5D descriptive system and the EQ visual analogue scale (EQ-VAS). The EQ-5D descriptive system covers mobility, self-care, usual activities, pain/discomfort and anxiety/depression. The EQ-VAS records the respondent's self-rated health on a vertical, visual analogue scale (range: 0(worst)-100(best)).

Statistical Analysis

The statistical analysis considerations are detailed in Appendix A. SAS software (version 9.1; SAS Inc.) was used. Continuous data is expressed as means ± STD (standard deviation) and compared by unpaired t-test for inter-group comparison and by paired t-test for intra-group comparison. Categorical data is expressed in numbers and percentages and compared by chi-square test. P values<0.05 were considered statistically significant. All randomly allocated patients were included in the safety analysis and those who had post-baseline assessment were included in efficacy analyses.

Scatter Plot Analysis of the Clinical Scores

The analysis aims to better quantify and compare changes in the clinical scores, while taking into consideration the high patient-to-patient variability. The idea was to inspect, for each patient at each time stage, the scaled relative differences in each of the clinical scores. More specifically, we calculated for a specific patient (j) the scaled relative difference SRD_j, defined as:

$$SRD_j \sim \left(\frac{SF_j - SF_j}{STD\ SF_j} - \frac{SI_j - SI_j}{STD\ SI_j} \right) \Big/ \sqrt{(SF_j - I_j)^2}\ STD\ SF_j - SI_j$$

Where SF_j is the value of a clinical score at the end of the time stage (either treatment or control), and SI_j is the score at the beginning of the time stage. We note that the symbol $\langle\rangle$ indicates average over the values of the patients in the group. For example, $\langle SF_j \rangle$ means the average of SF_j over all patients (j) that belong to the group. The abbreviation STD means the standard deviation between the values of the patients in the group. This analysis enables quantitative inspection of the changes in the clinical scores as is further explained and illustrated in Text S2. We note that the results can be further signified when the averaged difference ($\langle SF_j - SI_j \rangle$) is not divided by $STD(SF_j - SI_j)$.

Results

The study included 74 patients (August 2008-October 2010). 7 patients from the treated group and 8 patients from the cross group were excluded: 8 refused the SPECT, 3 had no measurable paresis, 1 had a medical problem, 1 had a stroke during the control period, and 2 refused to quit smoking (Figure 1).

Twenty four patients (80%) from the treated group had a history of ischemic stroke; of those, 17(71%), 3(13%), 2(8%) and 2(8%) patients were classified as TOAST 1, 2, 3 and 4, respectively. Twenty five patients (86%) from the cross group had ischemic stroke; of those, 18(72%), 3(12%), 2(8%) and 2(8%) patients were classified as TOAST 1, 2, 3 and 4, respectively; p = 0.8 for comparison of the TOAST classification between the groups. Of the 6 patients (20%) in the treated group that had hemorrhagic stroke, 5(83%) had anterior circulation stroke; and of the 4 patients with hemorrhagic stroke in the cross group, 3(75%) had anterior stroke. Baseline patients' characteristics are summarized in Table 1.

Neurologic Evaluation

The results of the neurological evaluations, including the NIHSS and the ADL and the quality of life estimates EQ-5D and EQ-VAS, are summarized in Table 2. Details of the parameter estimates, significance levels and confidence intervals for NIHSS and ADL are presented in Figure 2.

NIHSS. Clinical evaluations revealed statistically significant improvements in the NIHSS measures following treatment both in the HBOT-treated group (Figure 2_a; p = 0.004 compared to control) and in the HBOT-treated cross group (Figure 2_c; p<0.0001 compared to pre-HBOT). The significance of these improvements is further noticeable when compared to the control (non-treatment) period of the cross group during which the scores did not change at all (Figure 2_b): p = 0.43 compared to baseline.

Table 1. Baseline patients' characteristics.

	Treated Group (n =30)	Cross Group (n = 29)	P Value
Age (years)	61±12	63±6.3	0.28
Males/Females	22/8	17/12	0.23
Years of education	14.2±3.7	15.1±3.3	0.39
Time from Stroke (years)	1.49±0.83	1.48±0.79	0.94
Ischemic stroke	24 (80%)	25 (86%)	0.8
Hemorrhagic stroke	6 (20%)	4 (14%)	0.75
Diabetes	10 (33.3%)	12 (41.4%)	0.52
Hypertension	24 (80%)	22 (75.9%)	0.7
Ischemic heart disease	6 (20%)	6 (20.7%)	0.94
Hyperlipidemia	24 (80%)	24 (82.8%)	0.8
History of convulsions	5 (16.7%)	2 (6.9%)	0.09
History of smoking	10 (33.3%)	9 (31%)	0.7
Medications			
Aspirin	11 (36.7%)	14 (48.3%)	0.24
Clopidrogel	10 (33.3%)	7 (24.1%)	0.43
Warfarin	3 (10%)	5 (17.2%)	0.91
Statins	23 (76.7%)	23 (79.3%)	0.8
Anti-convulsive	6 (20%)	3 (10.3%)	0.14
Anti-Hypertensive	21 (70%)	22 (75.8%)	0.43
Glucose lowering drugs	9 (30%)	10 (34.5%)	0.7
Anti-Depressants	7 (23.3%)	9 (31%)	0.13

*Data presented as Mean ± standard deviation.
doi:10.1371/journal.pone.0053716.t001

ADL. Clinical evaluations revealed statistically significant improvements in the ADL score following treatment both in the HBOT-treated group (Figure 2_d; p<0.001 compared to control) and the cross group after the cross to the HBOT-treated (Figure 2_f; p<0.0001 compared to pre-HBOT). The significance of these improvements is further noticeable when compared to the control (non-treatment) period of the cross group during which there was no change in the ADL scores (Figure 2_e; p = 0.42 compared to baseline).

Scatter Plot Analysis of the Neurological Evaluations

The statistical significance of the improvements following the treatment periods is noticeable in the scatter plot analysis represented in Figure 3 and further detailed in Appendix B. In particular, the results show that the combined score of all patients improved following HBOT, while remaining unchanged during the control period.

Quality of Life

The effect on the quality of life is summarized in Table 2. The EQ-5D score significantly improved following treatment, both for the HBOT-treated group (p<0.0001 compared to baseline) and the HBOT-treated cross group (p<0.0001 compared to pre-HBOT), while there was no improvement following the control period (p = 0.122 compared to baseline, p = 0.009 for comparison between the groups). Similar results were obtained for the EQ-VAS evaluations as is summarized in Table 2. More specifically, the EQ-VAS score significantly improved following treatment, both for the HBOT-treated group (p<0.0001 compared to baseline) and the HBOT-treated cross group (p<0.0001 compared to pre-HBOT), while there was no significant improvement

following the control period ($p = 0.053$ compared to baseline, $p = 0.016$ for comparison between the groups).

Brain Functional Imaging- rCBF SPECT Imaging

All brain SPECT evaluations were completed for 29 patients in the treated group and for 28 in the cross group. Comparison of brain activity improvement following the HBOT revealed that 55% of the treated group had significant improvement after HBOT and 35% had mild improvement. In the cross group, during the first (control) period 36% had mild improvement and only 6.2% had significant improvement ($p<0.001$). After HBOT, the cross group demonstrated 43% significant improvement and 29% mild improvement ($p<0.001$) (data not shown in tables).

The improvements in the SPECT were mostly in regions showing noticeable discrepancy between the CT and SPECT–the earlier mentioned stunned regions of low activity living cells. The following examples of three typical patients illustrate the associations between the improvements in the patients' clinical conditions and evaluations and the changes in their brain activity (indication of the activation of neuroplasticity) as reflected by changes in their corresponding SPECT images:

Example-1. Baseline brain SPECT images demonstrating hypoperfusion in the right fronto-parietal region, right postero-medial frontal and posterior-parietal perfusion lesions with no significant changes after the control period (Figure 4). In comparison, the SPECT after HBOT demonstrated disappearance of the perfusion lesions. Global cortical and subcortical perfusion improvement was seen (Figure 5).

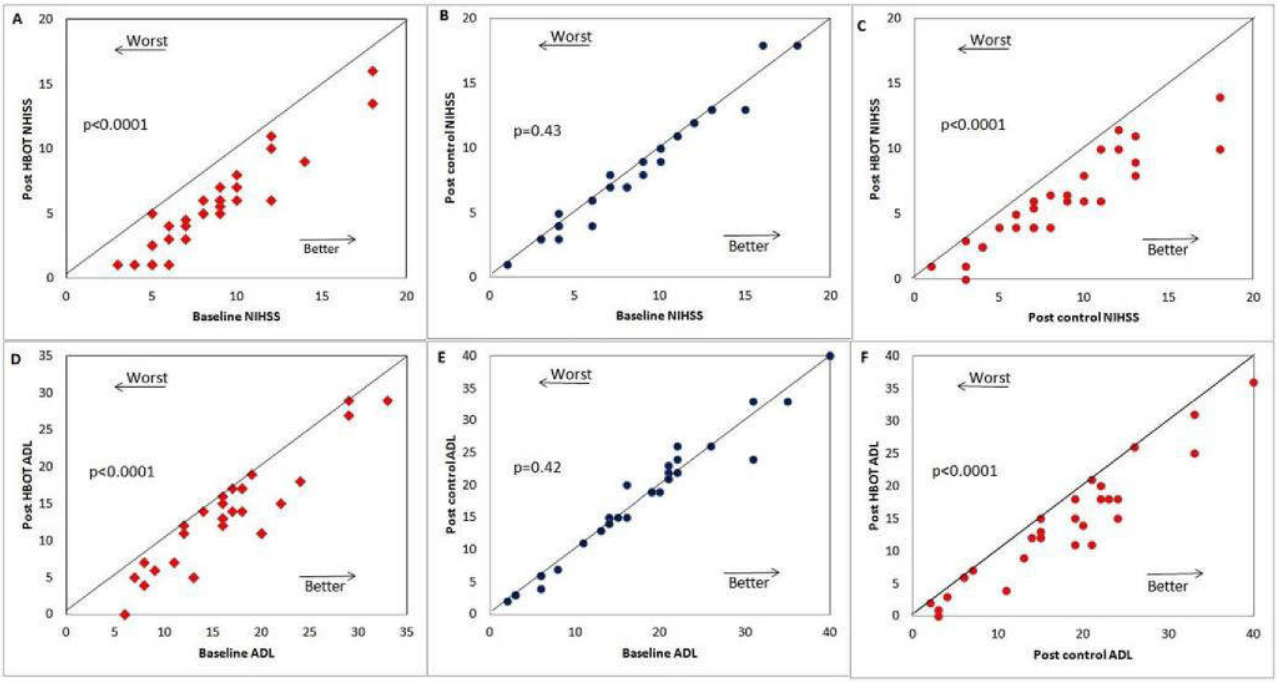

Figure 2. The results of the neurological evaluation. Each point represents a patient. (A–C) NIHSS score: (A) Scores of the treated group patients before and after the HBOT period (B) Scores of the cross group before and after the control (no treatment) period. (C) Scores of the cross group after the HBOT period. (D–F) The same as (A)-(C) for the activities of daily living (ADL) scores. We note that the lines indicate the diagonal. *Abbreviations*: NIHSS= National Institutes of Health Stroke Scale; ADL= Activities of Daily Living.
doi:10.1371/journal.pone.0053716.g002

These SPECT images are of a 61y old woman from the cross group, suffering from left hemiparesis due to ischemic stroke that occurred 1 year prior to inclusion. Baseline NIHSS showed minor facial paresis, no ability to hold her left hand against gravity, some ability to hold her left leg against gravity for less than 5 seconds and mild-to-moderate sensory loss. In ADL, she needed help in bathing, dressing and climbing stairs. She was unable to do any housework. After HBOT, she was able to hold her hand and leg against gravity without significant sensory loss. She could move her fingers, was independent in bathing, dressing, shopping and cooking.

Example-2. SPECT images at the end of HBOT demonstrating significant improvement of perfusion deficit in the left hemisphere (Figure 5) involving the medial and posterolateral frontal area (motor cortex) and lateral inferior frontal area (Broca's area). These images are from a 62y old woman from the treated group suffering from right hemiparesis due to ischemic stroke that occurred 14 months prior to inclusion. Baseline NIHSS showed no movement in her right arm, some effort against gravity in her right leg, mild-moderate aphasia, alexia and mild-moderate dysarthria. In ADL, she was completely dependent in bathing and dressing and needed help in transferring, walking, climbing stairs and eating. After HBOT, she could move her right hand against gravity, move fingers, and hold her leg against gravity. She regained speech (almost fluent) and reading capabilities. In ADL,

Table 2. Summary of the results of the National Institutes of Health Stroke Scale (NIHSS), activities of daily living (ADL) and quality of life questionnaire (EQ-5D and EQ-VAS).

	Treatment group				Cross group				
	Baseline	Post HBOT	P_1	P_2	Baseline	Control period	Post HBOT	P_1	P_3
NIHSS	8.53±3.62	5.52±3.59	<0.0001	0.004	8.71±4.11	8.34±4.25	5.85±3.44	0.43	<0.0001
ADL	16.1±6.52	12.77±7.26	<0.0001	0.02	17.38±9.49	17.45±9.53	13.82±8.75	0.42	<0.0001
EQ- 5D	9.3±1.36	7.67±1.33	<0.0001	0.009	8.78±1.55	8.64±1.69	7.57±1.51	0.122	<0.0001
EQ- VAS	4.93±1.62	6.45±1.50	<0.0001	0.016	5.14±2.25	5.34±2.27	6.79±1.85	0.053	<0.0001

*Data presented as Mean ± standard deviation.
Abbreviations: NIHSS= National Institutes of Health Stroke Scale; ADL = activities of daily living; EQ= Evaluation of Quality of life evaluation by the EQ-5D descriptive system and the EQ visual analogue scale (EQ-VAS). HBOT= Hyperbaric Oxygen Therapy.
P_1 = p value compared to baseline in the same group. P_2 = p value compared to the cross group after the control period. P_3 = p valus compared to the 2nd evaluation at the end of the control period.
doi:10.1371/journal.pone.0053716.t002

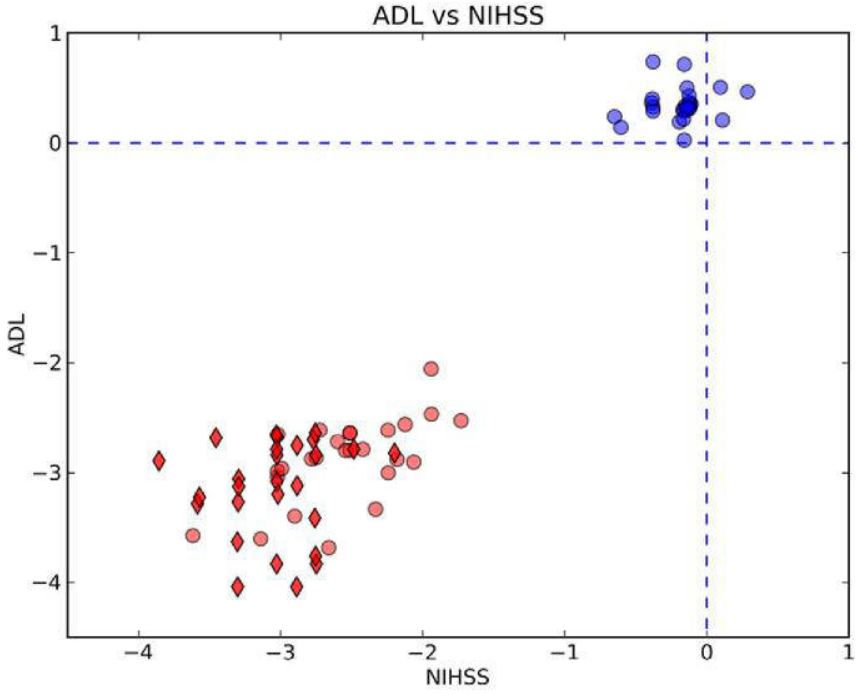

Figure 3. Scatter plot analysis of the changes in the combined neurological evaluations. The scatter plot shows changes in the NIHSS and ADL scores in terms of the scaled relative differences as is defined in the methods section (averaged difference ($\langle SF_j - SI_j \rangle$) is not divided by $STD(SF_j \cup SI_j)$). The color code is – changes during the treatment periods for the HBOT treated group (red diamonds), changes during for the HBOT-treated cross group (red circles) and changes during the control (non-treatment) period of the HBOT-treated cross group (blue circles).
doi:10.1371/journal.pone.0053716.g003

she was able to walk, climb stairs and eat by herself. She was not dependent in bathing and dressing.

Example-3. SPECT images demonstrating improvement in the peri-infarct region following HBOT. The images are of a 64y old woman from the treated group suffering from right hemiparesis due to ischemic stroke that occurred 26 months prior to inclusion. After treatment, the leg hemiparesis was resolved, her hand function improved significantly but she did not regain all fine motor skills. Figure 6 shows improvement in the peri-infarct region.

Safety

Six patients had mild-moderate barotrauma of the middle ear. After several days of rest they returned and completed the protocol. Two patients with a history of epileptic seizures prior to their inclusion in the study had mild episodes of convulsion (consciousness was fully maintained) during the study. Both patients were already treated with anti-epileptic drugs prior to their inclusion.

Discussion

In the current study, the effect of HBOT on chronic neurological deficiency due to stroke was evaluated in a prospective, randomized controlled study. Statistically significant improvements were obtained following treatment for almost all treated patients from both the HBOT-treated group and the HBOT-treated cross group (with no false negative), as was evaluated by NIHSS, ADL, brain SPECT and life quality. The significance of the improvements in this chronically debilitated population of patients is further noticeable when compared to the lack of improvement during the control (no-treatment) period of the cross group (with no false positive).

This is the first prospective, randomized clinical study evaluating the effect of HBOT in the late post-stroke period (6 months to 3 years after the acute event). There are two major reasons for selecting this study population. First, by carefully selecting patients with chronic stable neurological deficiency we were able to avoid unexpected changes in their condition. In this regard, the selection proved very useful since the control group demonstrated neurological stability with no outliers. The second reason was, as discussed in the introduction, to test our hypothesis that the optimal time for the HBOT procedure should be during the regenerative and not during the degenerative stage. While it is not possible to mark a clear line between the regenerative and the degenerative phases [23], it is quite clear that 6 months after the acute event in a stable patient the degenerative process has ended. As mentioned in the introduction, the differences in initiation times and protocols of HBOT may explain contradictive results in previous studies, where HBOT was used in the early phase after stroke [3,4,5,6,7]. The recent publication by Harch et al., evaluating the effect of HBOT on chronic neurological deficiencies due to traumatic brain injury, also supports the use of HBOT in the late stage after the acute insult [8].

The issue of "how to handle the control group"was discussed by a multidisciplinary team including physicians specializing in hyperbaric medicine, physicists specializing in neuronal-glia interactions and the ethics committee. The patients can tell if pressure is increased or not, so the pressure must be increased also in the control group. The only way to administer "placebo"of HBOT is to bring the patients to the hyperbaric chamber and to increase the environmental pressure to an extent that the patients will feel it in their ears. The minimal pressure needed to gain such

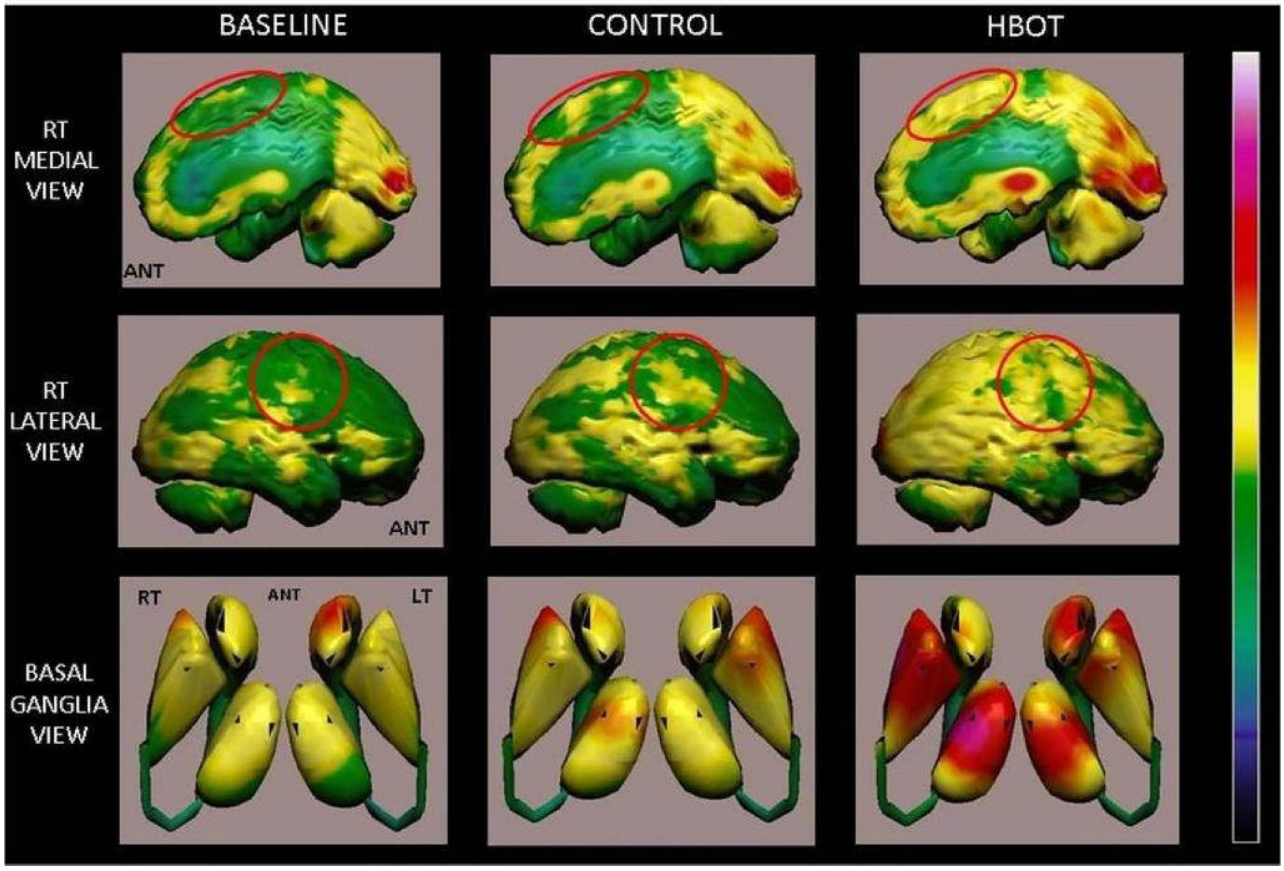

Figure 4. Volume rendered Brain SPECT perfusion maps of Example 1. The results are of a patient in the cross group, suffering from left hemiparesis due to ischemic stroke that occurred 1 year prior to inclusion in the study. Baseline and control volume rendered brain perfusion views show diffuse hypoperfusion in the right hemisphere involving the fronto-parietal region and right postero-medial frontal (right motor cortex), right medial parietal and posterior-parietal (sensory cortex and associative motor cortex) (red circles). The HBOT SPECT scan done at the end of HBOT treatments shows disappearance of the perfusion deficits that were still demonstrated at the end of the control period. In addition, a significant global cortical and subcortical (basal ganglia and thalamic nuclei) perfusion improvement is seen.
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a feeling should be 1.3 ATM. Henry's law states: "the amount of a given gas dissolved in a given type and volume of liquid is directly proportional to the pressure of that gas in equilibrium with that liquid". Thus, hyperbaric environment significantly increases the dissolved oxygen pressure even if a person holding his breath [29]. Compressed air at 1.3 ATA increases the plasma oxygen tension by at least 50% and that is certainly notable. There are many case reports illustrating significant effects following small increase in air pressure [30,31,32]. Moreover, even a slight increase in partial pressure, such as, for example, to 1.05 ATM at altitude 402 m below sea level (the Dead Sea), can lead to noticeable physiological effects [33,34,35,36,37]. However, it should be kept in mind that oxygen is not a drug, and because it is metabolized mainly in the mitochondria, there is no simple dose-response curve.

Since increasing the pressure even without adding oxygen can also increases the dissolved oxygen partial pressure, the only way to maintain normal (placebo) levels of dissolved oxygen is to supply air with lower than normal level of oxygen, which we deemed unethical. To partially compensate for this inherent limitation, the patients in the cross group started with a two-month control period of no treatment, at the end of which they were crossed to two months of HBOT sessions. To gain better validity of the results, we used the scatter plot analysis of the changes in the combined neurological evaluations. The scatter plots (figure 3) show changes in the NIHSS and ADL scores in terms of the scaled relative differences, as is defined in the methods section. In that analysis, summarized in figures 2 & 3, the correlation between changes NIHSS & ADL after HBOT is clearly demonstrated. Moreover, the analysis evidently demonstrates that the effect of HBOT on these "not completely blinded evolution"was the same in the treated group and the control group after blind randomization. The correlation between the improvement in NIHSS and ADL and the improvement in the brain SPECT results, which was done in a completely blinded fashion, further substantiates the clinical findings. Moreover, the consistency between the anatomical locations of the changes in the brain metabolism, as demonstrated by the SPECT, with the finding in the neurological evaluation provides important validation of the neurological evaluation.

During most of the 20th century, there was an ongoing debate about the time window available for induction of neuroplasticity. The improvements in the chronic late stage reported here support the view that neuroplasticity can be activated months to years after the acute event when a proper brain stimulation (such as HBOT) is applied. More specifically, the current study included patients that underwent stroke more than 6 months prior to treatment and after their condition reached a steady state (no improvements were monitored for at least a month). These important and unexpected findings arein agreement with other recent findings revealing that

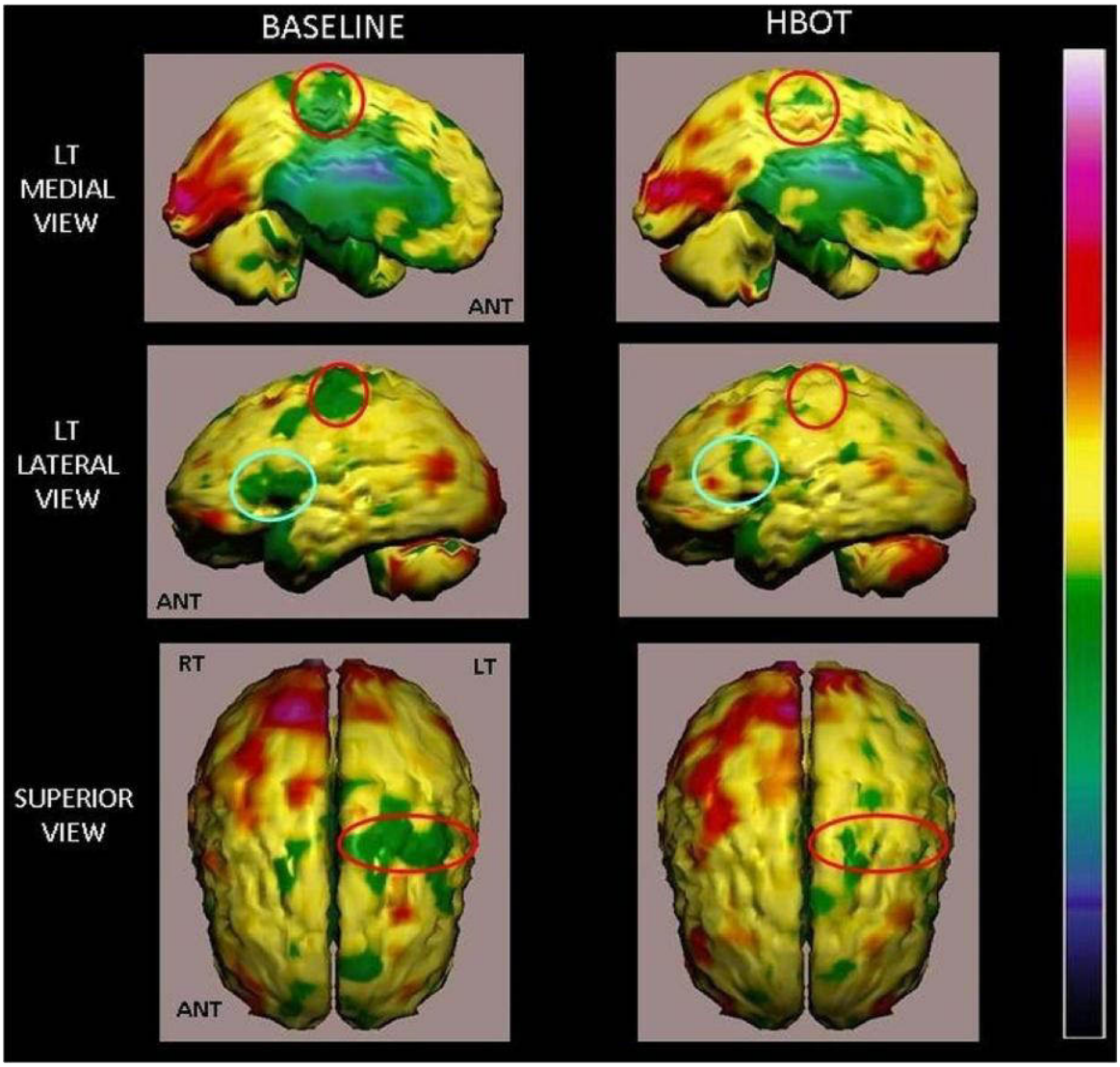

Figure 5. Volume rendered Brain SPECT perfusion maps of Example 2. The results are of a patient in the treated group, suffering from right hemiparesis due to ischemic stroke that occurred 14 months prior to her inclusion in the study. Comparison of pre- and post-hyperbaric treatment SPECT scans. These SPECT images demonstrate significant improvement of perfusion deficits in the left hemisphere involving the medial and posterolateral frontal area (motor cortex, red circles) and lateral inferior frontal region (Broca's area, blue circles) in comparison to the baseline SPECT. HBOT SPECT findings correlate positively with the patient's improved motor and verbal functions.
doi:10.1371/journal.pone.0053716.g005

many aspects of the brain remain plastic even at adulthood [38]. They are also consistent with several other studies in post stroke patients [39,40,41]. In the current study, patients were treated only with HBOT without any additional guided training and/or practice. This was done in order to demonstrate the therapeutic potential of this treatment. It is reasonable to expect that exploiting the HBOT in conjunction with other rehabilitation intervention can lead to even better results leading to optimal future practice. The current study paved the way for future investigations of this promising direction, which should be one of the aims of upcoming more elaborated clinical studies.

Current imaging technologies reveal that the stunned brain areas (regions of high anatomy-physiology mismatch) may persist for months and years after an acute brain event [11,12,13]. The changes in SPECT images after treatment demonstrate that the HBOT procedure led to reactivations of neuronal activity in the stunned areas. While SPECT imaging has limited spatial resolutions (in comparison, for example, to fMRI), the changes in activity were sufficiently robust to be clearly detected by the SPECT images. However, a future, more detailed study using fMRI (along with direct observations on animal model) will be able to provide additional valuable insights, in particular regarding the operative underlying mechanisms that activate the neuroplasticity (e.g. the putative role of glial cells). We note that patients were not selected based on their anatomical and functional brain imaging evaluation. It might be possible that the results would have been even better had the study included only patients with high SPECT/CT mismatch. This issue of the preferred population for HBOT should be further investigated in future clinical trials. We also note that in the current pioneering study aimed at "proof of concept", all patients underwent 40 HBOT sessions. Based on our current clinical experience, more sessions of HBOT may be

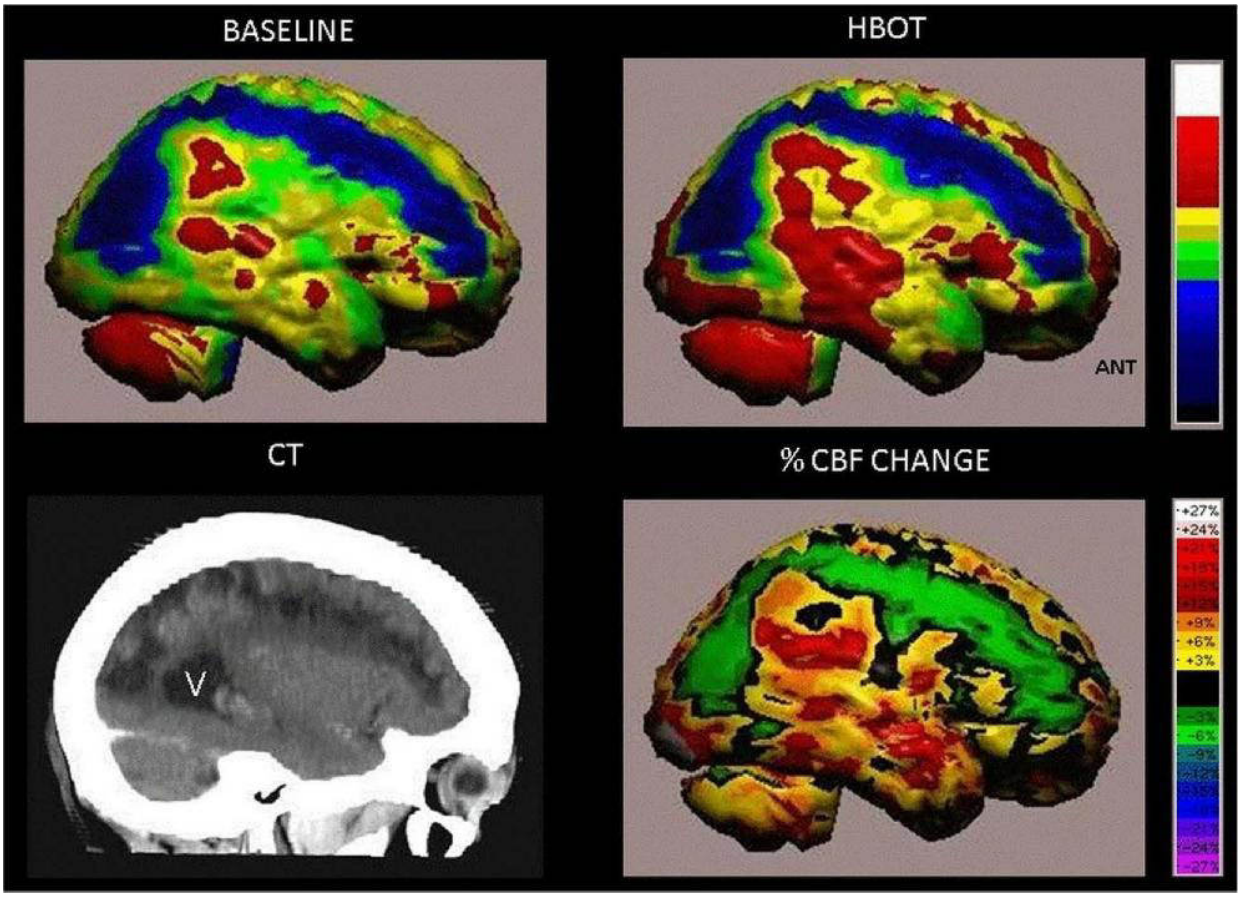

Figure 6. Volume rendered Brain SPECT perfusion maps of Example 3. The results are of a patient in the treated group suffering from left hemiparesis due to ischemic stroke that occurred 26 months prior to inclusion in the study. The brain perfusion maps (upper two images) show the infracted brain (deep blue color) involving the right antero-postero-lateral frontal, right superior-parietal and right parieto-occipital regions. Curved sagittal view in CT MIP reconstruction of the brain shows the anatomical stroke area (left lower image, V = posterior horn of right ventricle). The peri-infarct region show improved perfusion as demonstrated by HBOT image (right upper image). Quantitation of the cerebral blood flow (CBF) change (delta between baseline and HBOT) is demonstrated in the right lower image.
doi:10.1371/journal.pone.0053716.g006

needed, at least for some patients, in order to obtain the maximal improvement effect.

In any case, the observed reactivation of neuronal activity in the stunned areas imply that increasing the plasma oxygen concentration with hyperbaric oxygenation is a potent means of delivering to the brain sufficient oxygen for tissue repair: HBOT might initiate a cellular and vascular repair mechanism and improve cerebral vascular flow [8,13,16,17]. At the cellular level, HBOT can improve mitochondrial function (in both neurons and glial cells) and cellular metabolism; improve BBB and inflammatory reactions; reduce apoptosis; alleviate oxidative stress; increase levels of neurotrophins and nitric oxide, and up-regulate axon guidance agents [13,16,17,20]. Moreover, the effects of HBOT on neurons can be mediated indirectly by glial cells, including asrocytes [18]. HBOT may also promote neurogenesis of the endogenous neural stem cells [19]. The major limitation of the above-mentioned data is that it has been tested in different types of models and includes different protocols of HBOT. However, it is well noticed that there is at least one common denominator to all repair/regeneration mechanisms: they are all energy/oxygen dependent. It might be possible that HBOT enables the metabolic change simply by supplying the missing energy/oxygen needed for those regeneration processes.

To conclude, in this study we provide, for the first time, convincing results demonstrating that HBOT can induce significant neurological improvement in post stroke patients. The neurological improvements in a chronic late stage demonstrate that neuroplasticity can be operative and activated by HBOT even long after acute brain insult. Thus, the findings have important implications that can be of general relevance and interest in neurobiology. Although this study focused on stroke patients, the findings bear the promise that HBOT may serve as a valuable therapeutic practice in other neurological disorders exhibiting discrepancy between the anatomical and functional evaluation of the brain.

Supporting Information

Protocol S1 Clinical Study Protocol.
(DOCX)

Checklist S1 CONSORT 2010 checklist.
(DOCX)

Form S1 Informed consent form (English translation).
(PDF)

Text S1 Statistical, Randomization and Placebo Considerations.
(DOCX)

Text S2 Scatter plot analysis of the clinical scores. (DOCX)

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References

1. Teasell R (2009) Forward. An international perspective of stroke rehabilitation. Top Stroke Rehabil 16: v.
2. Prvu Bettger JA, Stineman MG (2007) Effectiveness of multidisciplinary rehabilitation services in postacute care: state-of-the-science. A review. Arch Phys Med Rehabil 88: 1526–1534.
3. Anderson DC, Bottini AG, Jagiella WM, Westphal B, Ford S, et al. (1991) A pilot study of hyperbaric oxygen in the treatment of human stroke. Stroke 22: 1137–1142.
4. Nighoghossian N, Trouillas P, Adeleine P, Salord F (1995) Hyperbaric oxygen in the treatment of acute ischemic stroke. A double-blind pilot study. Stroke; a journal of cerebral circulation 26: 1369–1372.
5. Rusyniak DE, Kirk MA, May JD, Kao LW, Brizendine EJ, et al. (2003) Hyperbaric oxygen therapy in acute ischemic stroke: results of the Hyperbaric Oxygen in Acute Ischemic Stroke Trial Pilot Study. Stroke; a journal of cerebral circulation 34: 571–574.
6. Vila JF, Balcarce PE, Abiusi GR, Dominguez RO, Pisarello JB (2005) Improvement in motor and cognitive impairment after hyperbaric oxygen therapy in a selected group of patients with cerebrovascular disease: a prospective single-blind controlled trial. Undersea & hyperbaric medicine: journal of the Undersea and Hyperbaric Medical Society, Inc 32: 341–349.
7. Imai K, Mori T, Izumoto H, Takabatake N, Kunieda T, et al. (2006) Hyperbaric oxygen combined with intravenous edaravone for treatment of acute embolic stroke: a pilot clinical trial. Neurologia medico-chirurgica 46: 373–378; discussion 378.
8. Harch PG, Andrews SR, Fogarty EF, Amen D, Pezzullo JC, et al. (2012) A phase I study of low-pressure hyperbaric oxygen therapy for blast-induced postconcussion syndrome and post-traumatic stress disorder. Journal of neurotrauma 29: 168–185.
9. Duncan PW, Goldstein LB, Matchar D, Divine GW, Feussner J (1992) Measurement of motor recovery after stroke. Outcome assessment and sample size requirements. Stroke; a journal of cerebral circulation 23: 1084–1089.
10. Astrup J, Symon L, Branston NM, Lassen NA (1977) Cortical evoked potential and extracellular K+ and H+ at critical levels of brain ischemia. Stroke 8: 51–57.
11. Fernandez-Bouzas A, Harmony T, Fernandez T, Silva-Pereyra J, Valdes P, et al. (2000) Sources of abnormal EEG activity in brain infarctions. Clin Electroencephalogr 31: 165–169.
12. Siddique MS, Fernandes HM, Wooldridge TD, Fenwick JD, Slomka P, et al. (2002) Reversible ischemia around intracerebral hemorrhage: a single-photon emission computerized tomography study. J Neurosurg 96: 736–741.
13. Neubauer RA, James P (1998) Cerebral oxygenation and the recoverable brain. Neurol Res 20 Suppl 1: S33–36.
14. Hossmann KA (2006) Pathophysiology and therapy of experimental stroke. Cell Mol Neurobiol 26: 1057–1083.
15. Culmsee C, Krieglstein J (2007) Ischaemic brain damage after stroke: new insights into efficient therapeutic strategies. International Symposium on Neurodegeneration and Neuroprotection. EMBO Rep 8: 129–133.
16. Golden ZL, Neubauer R, Golden CJ, Greene L, Marsh J, et al. (2002) Improvement in cerebral metabolism in chronic brain injury after hyperbaric oxygen therapy. Int J Neurosci 112: 119–131.
17. Zhang JH, Lo T, Mychaskiw G, Colohan A (2005) Mechanisms of hyperbaric oxygen and neuroprotection in stroke. Pathophysiology 12: 63–77.
18. Gunther A, Kuppers-Tiedt L, Schneider PM, Kunert I, Berrouschot J, et al. (2005) Reduced infarct volume and differential effects on glial cell activation after hyperbaric oxygen treatment in rat permanent focal cerebral ischaemia. Eur J Neurosci 21: 31893194.
19. Yang YJ, Wang XL, Yu XH, Wang X, Xie M, et al. (2008) Hyperbaric oxygen induces endogenous neural stem cells to proliferate and differentiate in hypoxicischemic brain damage in neonatal rats. Undersea Hyperb Med 35: 113129.
20. Calvert JW, Cahill J, Zhang JH (2007) Hyperbaric oxygen and cerebral physiology. Neurol Res 29: 132141.
21. Niklas A, Brock D, Schober R, Schulz A, Schneider D (2004) Continuous measurements of cerebral tissue oxygen pressure during hyperbaric oxygena-
tion–HBO effects on brain edema and necrosis after severe brain trauma in rabbits. J Neurol Sci 219: 77–82.
22. Reinert M, Barth A, Rothen HU, Schaller B, Takala J, et al. (2003) Effects of cerebral perfusion pressure and increased fraction of inspired oxygen on brain tissue oxygen, lactate and glucose in patients with severe head injury. Acta Neurochir (Wien) 145: 341–349; discussion 349–350.
23. Lo EH (2008) A new penumbra: transitioning from injury into repair after stroke. Nat Med 14: 497–500.
24. Kasner SE, Chalela JA, Luciano JM, Cucchiara BL, Raps EC, et al. (1999) Reliability and validity of estimating the NIH stroke scale score from medical records. Stroke 30: 1534–1537.
25. Brott T, Adams HP, Jr., Olinger CP, Marler JR, Barsan WG, et al. (1989) Measurements of acute cerebral infarction: a clinical examination scale. Stroke 20: 864–870.
26. Kol S, Ammar R, Weisz G, Melamed Y (1993) Hyperbaric oxygenation for arterial air embolism during cardiopulmonary bypass. The Annals of thoracic surgery 55: 401–403.
27. Jaszczak RJ, Chang LT, Stein NA, Moore FE (1979) Whole-body single-photon emission computed tomography using dual, large-field-of-view scintillation cameras. Physics in medicine and biology 24: 1123–1143.
28. Mahadeva S, Wee HL, Goh KL, Thumboo J (2009) The EQ–5D (Euroqol) is a valid generic instrument for measuring quality of life in patients with dyspepsia. BMC Gastroenterol 9: 20.
29. Muth CM, Radermacher P, Pittner A, Steinacker J, Schabana R, et al. (2003) Arterial blood gases during diving in elite apnea divers. International journal of sports medicine 24: 104–107.
30. James PB (2001) Hyperbaric oxygenation for cerebral palsy. Lancet 357: 2052– 2053.
31. Golding FC, Griffiths P, Hempleman HV, Paton WD, Walder DN (1960) Decompression sickness during construction of the Dartford Tunnel. British journal of industrial medicine 17: 167–180.
32. Austin D (1998) Gammow bag for acute mountain sickness. Lancet 351: 1815.
33. Goldbart AD, Cohen AD, Weitzman D, Tal A (2007) Effects of rehabilitation winter camps at the Dead Sea on European cystic fibrosis patients. The Israel Medical Association journal: IMAJ 9: 806–809.
34. Kramer MR, Springer C, Berkman N, Glazer M, Bublil M, et al. (1998) Rehabilitation of hypoxemic patients with COPD at low altitude at the Dead Sea, the lowest place on earth. Chest 113: 571–575.
35. Falk B, Nini A, Zigel L, Yahav Y, Aviram M, et al. (2006) Effect of low altitude at the Dead Sea on exercise capacity and cardiopulmonary response to exercise in cystic fibrosis patients with moderate to severe lung disease. Pediatric pulmonology 41: 234–241.
36. Abinader EG, Sharif D, Rauchfleich S, Pinzur S, Tanchilevitz A (1999) Effect of low altitude (Dead Sea location) on exercise performance and wall motion in patients with coronary artery disease. The American journal of cardiology 83: 250–251, A255.
37. Gabizon I, Shiyovich A, Novack V, Khalameizer V, Yosefy C, et al. (2011) Impact of descent and stay at a Dead Sea resort (low altitude) on patients with systolic congestive heart failure and an implantable cardioverter defibrillator. The Israel Medical Association journal: IMAJ 13: 402–407.
38. Rakic P (2002) Neurogenesis in adult primate neocortex: an evaluation of the evidence. Nature reviews Neuroscience 3: 65–71.
39. Zimerman M, Heise KF, Hoppe J, Cohen LG, Gerloff C, et al. (2012) Modulation of training by single-session transcranial direct current stimulation to the intact motor cortex enhances motor skill acquisition of the paretic hand. Stroke; a journal of cerebral circulation 43: 21852191.
40. Kattenstroth JC, Kalisch T, Peters S, Tegenthoff M, Dinse HR (2012) Longterm sensory stimulation therapy improves hand function and restores cortical responsiveness in patients with chronic cerebral lesions. Three single case studies. Frontiers in human neuroscience 6: 244.
41. Sterr A, Conforto AB (2012) Plasticity of adult sensorimotor system in severe brain infarcts: challenges and opportunities. Neural plasticity 2012: 970136.

Author Contributions

Conceived and designed the experiments: SE GF IK NG HG. Performed the experiments: SE GF YB JB MF. Analyzed the data: SE OV KK EB-J HG. Wrote the paper: SE EB-J HG. Performed the experiments (brain SPECT): OV. Performed the experiments (neurological evaluation part): IK. Performed the experiments (brain imaging part): HG.

Contribution from the Basic Sciences

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Neuroprotection by Oxygen in Acute Transient Focal Cerebral Ischemia Is Dose Dependent and Shows Superiority of Hyperbaric Oxygenation

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Key Words
Oxygen - Hyperbaric oxygenation - Normobaric oxygenation - Ischemia, transient

Abstract

The neuroprotective effect of oxygen after acute stroke in rats has been shown previously. However, the question of optimal dosing still remains unanswered. Thus, we investigated the use of oxygen at different concentrations by either normobaric oxygenation (NBO) or hyperbaric oxygenation (HBO) at different pressures in a model of transient ischemia/reperfusion in rats. Animals underwent 90 min of middle cerebral artery occlusion (MCAO) followed by 90 min of reperfusion before oxygen treatment. Oxygen was applied either by NBO (100% O_2 ; 1.0 absolute atmosphere, ATA) or HBO (100% O_2 ; 1.5, 2.0, 2.5 or 3.0 ATA) for 1 h. Primary endpoints were infarct volume and clinical outcome measured 24 h and 7 days following the MCAO. A statistically significant and long-lasting reduction in infarct volume was seen in the HBO 2.5 ATA and 3.0 ATA groups over a period of 7 days. The reduced infarct volume was accompanied with a statistically significant improvement in clinical outcome in the high-dose oxygen-treated groups. The presented data indicate that oxygen is a highly neuroprotective molecule in transient focal cerebral ischemia in rats, when applied early and at high doses. The effect is dose dependent and shows a superiority of HBO over NBO, when the primary endpoints infarct volume reduction and clinical outcome are analyzed. These data are important for the development of new acute stroke treatment studies in humans.

Introduction

The hallmarks of ischemic tissue damage, such as ischemic stroke, are oxygen and glucose deprivation leading to rapid ATP decrease and subsequent cell death [1]. Thus, early restoration of blood flow is one important acute treatment strategy to significantly decrease the extent of ischemic tissue damage [2].

In addition to early recanalization procedures, oxygen has been used as a therapeutic agent in a variety of experimental settings in myocardial ischemia [3] and in stroke studies on animals [4] . The use of oxygen as a therapeutic agent in ischemic stroke is often regarded as harmful, due to the concern that higher oxygen concentrations might increase the production of oxygen-derived free radicals. However, previous studies have shown con-

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clusively that the increase in free radicals is not correlated with the amount of oxygen delivered to the ischemic tissue [5, 6].

So far, 2 different ways of oxygen treatment have been described, normobaric oxygenation (NBO) and hyperbaric oxygenation (HBO). They differ with respect to the administered partial pressure of inspired oxygen. The arterial oxygen pressure (P_{aO2}) at sea level [760 mm Hg, 1 absolute atmosphere (ATA)] in ambient air ventilation (21% O_2) is 102 mm Hg. While the increase in the P_{aO2} of the blood is limited under NBO (100% O_2, 1 ATA) to 673 mm Hg, HBO can increase the oxygen transport capacity of the blood in dependence on the ambient pressure in a linear fashion. Thus, the P_{aO2} increases under 3-ATA treatment (100% O_2) to 2,193 mm Hg [7]. Naturally, HBO requires the use of a pressure chamber to achieve the increased ambient pressure.

NBO has been investigated in transient cerebral ischemia in rats. In a model of transient focal cerebral ischemia induced by middle cerebral artery occlusion (MCAO) for 2 h, Singhal et al. [8] reported a significant reduction in infarct volume if NBO was started as early as 15 or 30 min following the MCAO. Furthermore, the neuroprotective effects of NBO treatment during ischemia and reperfusion in transient MCAO have been demonstrated by a total infarct reduction of 70% [9]. Other studies using NBO showed significant clinical improvement and reduced infarct volume in transient MCAO in rats, with the largest reduction of infarct volume occurring when NBO was administered continuously during the MCAO and reperfusion [10].

HBO also attenuated the ischemic brain damage in a variety of ischemic models, mostly in rats [4, 11, 12]. Different stroke models (global ischemia, permanent focal ischemia or transient focal ischemia) in different species (gerbils [13, 14], dogs [15–17], cats [18], rabbits [19] and rats [20–24]) were used to study the effect of HBO. Most of the studies showed a beneficial effect of HBO, despite their heterogeneity. In a previous study, we investigated the optimal timing of HBO in transient MCAO in rats with respect to time windows of clinical settings, and could define a time window of less than 6 h following the MCAO as optimal for the treatment [22].

The neuroprotective effects of oxygen (NBO or HBO) in cerebral ischemia can be summarized as: (1) oxygen appears to be neuroprotective and shows maximum efficacy when administered early in the time course of cerebral ischemia; (2) neither NBO nor HBO appear to cause significant reperfusion injuries induced by oxygen-derived free radicals in a defined time window; (3) NBO is more feasible than HBO, because no pressure chamber is needed.

As already outlined in a recent review by Helms et al. [4], the optimal dose of oxygen treatment remains unclear from these reports. We therefore designed the present study in order to systematically evaluate the neuroprotective effects of oxygen in a model of transient MCAO, with a dose-escalating design, in rats. We defined infarct volume and clinical outcome as the primary end points of our study, to be determined at 24 h and 7 days following the MCAO. Since we have previously shown a time dependency for HBO in transient MCAO, we used the most efficient time point for the onset of oxygen therapy – 3 h following the MCAO [22].

Materials and Methods

Study Design

The local ethics committee approved all experimental protocols. One hundred and forty-nine male Sprague-Dawley rats were used (weight, 200 g; Charles River, Germany). They were kept under controlled conditions. Animals were randomly assigned to the following groups (n = 10 in each group) receiving either room air or oxygen in a dose-escalating design: A = control group, room air; B = NBO (100% O_2, 1 h); C = HBO 1.5 ATA (100% O_2, 1 h); D = HBO 2.0 ATA (100% O_2, 1 h); E = HBO 2.5 ATA (100% O_2, 1 h); F = HBO 3.0 ATA (100% O_2, 1 h). Oxygen treatment (NBO or HBO) was started 3 h after the MCAO for 1 h. The deeply anesthetized animals were sacrificed 24 h or 7 days after the onset of transient MCAO by cardiac perfusion for infarct volumetry (fig. 1).

Monitoring of Physiological Parameters

Physiological parameters (rectal temperature, arterial pH, P_{aCO2}, P_{aO2}, hemoglobin, hematocrit, glucose, potassium, sodium, calcium and chloride) were monitored in each group. Therefore, a polyethylene catheter (pp-50) was inserted through the femoral artery into the abdominal aorta under chloral hydrate anesthesia (400 mg/kg i.p.) 2 days before the MCAO. Blood samples were taken at different time points – (1) before the MCAO; (2) 30 min after the MCAO; (3) 30 min before the HBO treatment; (4) 60 min after the HBO treatment – and analyzed (Radiometer ABL 700, Copenhagen, DK).

Transient Middle Cerebral Artery Occlusion

Animals were anesthetized with chloral hydrate (400 mg/kg i.p.) for all surgical procedures. During surgery, each rat was allowed to breathe normally, and rectal temperature was maintained at 37 ° C with the use of a heating pad. The right middle cerebral artery was occluded for 90 min with subsequent reperfusion according to the method described by Longa et al. [25]. After 90 min, the filament was withdrawn to allow reperfusion. Animals that did not demonstrate a significant reduction of the regional cerebral blood flow (rCBF) during the MCAO or a rapid restoration of the laser Doppler signal during reperfusion were excluded from the study.

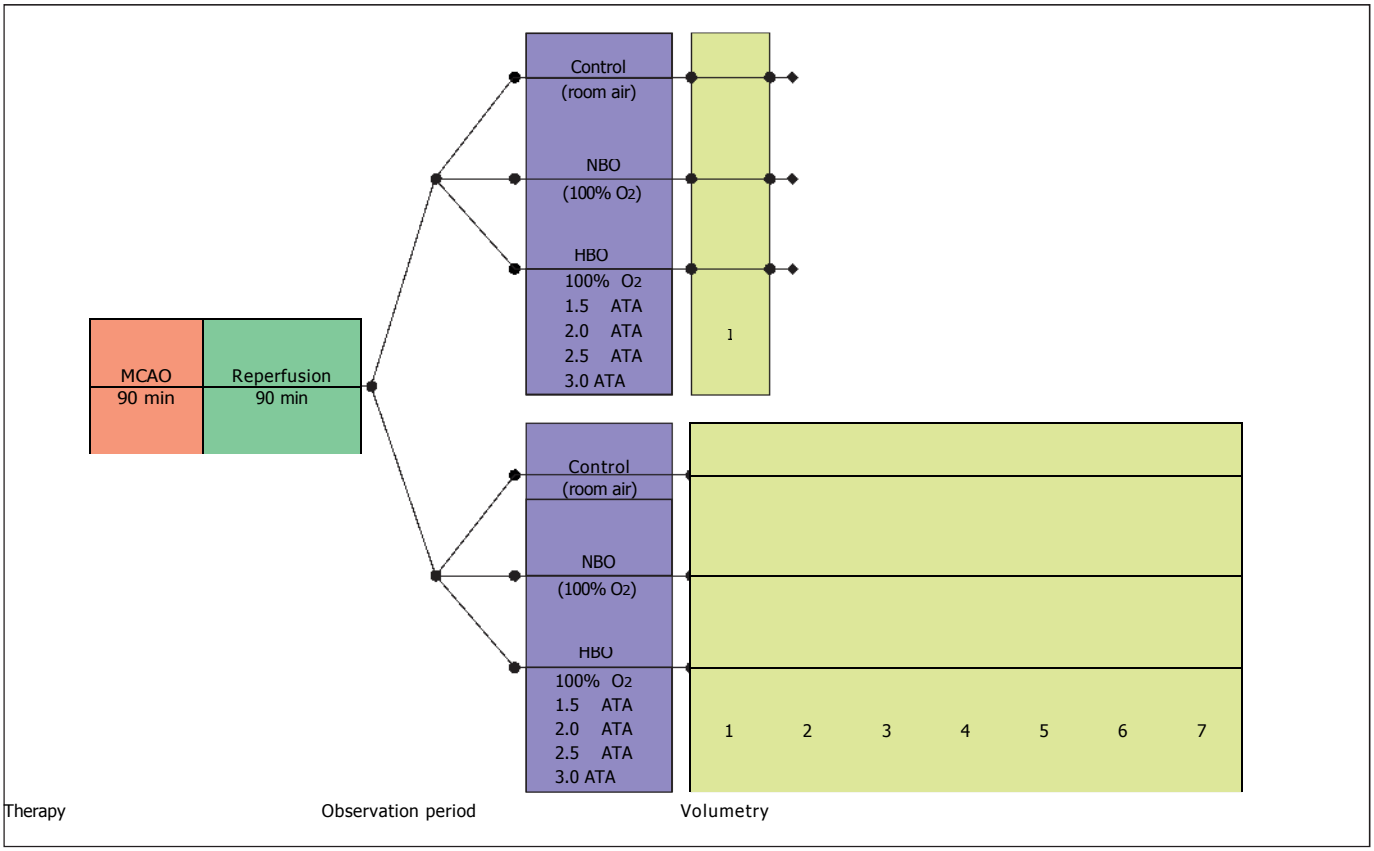


Fig. 1. Study design. All animals received 90 min of MCAO followed by 90 min of reperfusion prior to randomization into 24-hour or 7-day observation arms. Treatment lasted 1 h in all groups. An observation period followed, to assess clinical scores (Garcia and Bederson) and to detect putative complications. Numbers 1–7 indicate days of observation. Following the observation period, the animals were sacrificed by cardiac perfusion for histological infarct volumetry ( ).

Measurement ofthe rCBF

Laser Doppler flowmetry (Periflux System, Perimed, Sweden) was used to monitor the rCBF before, during and after the transient MCAO. Flow values were recorded every 10 min. The area selected for rCBF monitoring corresponded to the territory of the occluded middle cerebral artery.

Normobaric Oxygenation

Animals randomized for the NBO treatment were anesthetized and placed in an acrylic cage with a flexible ceiling in order to avoid pressure changes. The air inside the cage was replaced by pure oxygen and kept at 100% O_2 for 1 h (fig. 1).

Hyperbaric Oxygenation

The HBO was performed in an experimental pressure chamber. The spontaneously breathing animals were anesthetized to avoid movement. During the HBO administration, animals were observed through the transparent acrylic glass of the pressure chamber. The HBO was administered at different pressures, ranging from 1.5 to 3.0 ATA for 1 h with 100% oxygen (group C, 1.5 ATA; group D, 2.0 ATA; group E, 2.5 ATA; group F, 3.0 ATA), which was started 3 h following the MCAO (fig. 1). Arterial blood samples were obtained from a femoral artery catheter for blood gas analysis of the anesthetized animals during the HBO administration, without discontinuation of the HBO. Compression and decompression were achieved within 5 min. After the HBO, the femoral artery catheter was removed. The control group received the same dose of anesthesia at the same time points following the MCAO. Temperature inside the chambers and rectal temperatures were continuously monitored.

Infarct Volume Calculation

Twenty-four hours and 7 days after the MCAO, different rats from each group were deeply anesthetized with chloral hydrate (400 mg/kg) and perfused intracardially with the use of standard protocols. The entire brain was cut into coronal frozen sections (40 m). Every 20th slice of the brain was mounted on a glass slide and stained with cresyl violet. In total, 14 sections of each brain were stained and analyzed. Sections were digitalized using a scanner and analyzed by 2 blinded investigators using ImageJ (NIH, USA). To eliminate brain edema, the corrected infarct volume was calculated as described in detail by Schäbitz et al. [26].

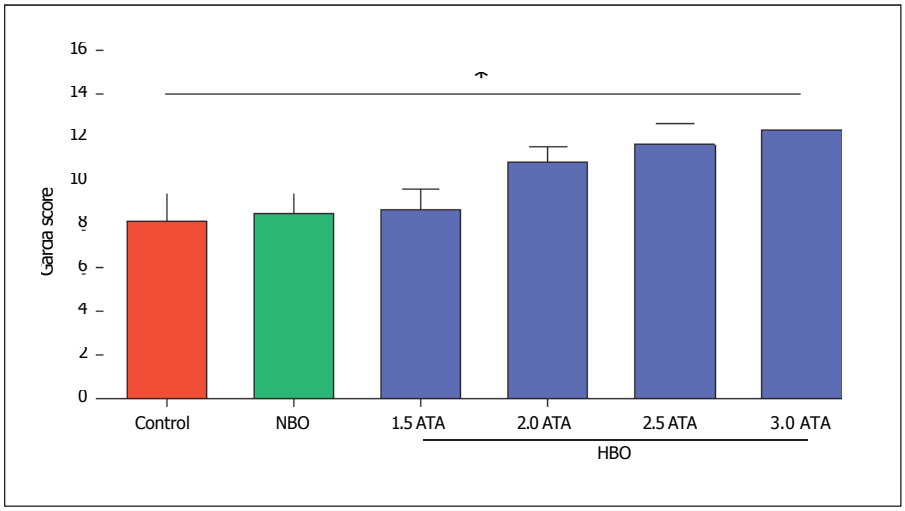


Fig. 2. Clinical outcome 24 h following the MCAO according to the grading system of Garcia. Clinical improvement (higher score) was seen in the high-dose treatment group F (3 ATA). n = 10 per group; mean ± SEM; ANOVA; * p < 0.05.

Garcia and Bederson Scores

Two neurological grading systems were used to assess the effects of ischemia. The first grading system was published by Bederson et al. in 1986 [27] . The grading system consists of a scale from 0 to 3: (0) rats with no observable deficit; (1) rats with decreased forelimb resistance to lateral push; (2) rats with forelimb flexion; (3) rats displaying circling behavior alongside the former symptoms.

The second system was introduced by Garcia et al. in 1995 [28]. It consists of 6 different criteria (spontaneous activity, symmetry in the movement of the 4 limbs, forepaw outstretching, climbing, body proprioception and response to vibrissae touch). The individual performance in each test was rated on a 0- to 3-point subscore. The sum of all 6 individual subscores was calculated within a range of 3–18. Thus, the score in healthy rats is 18. Two blinded investigators performed all tests.

Statistical Analysis

Statistical analysis was performed with the use of a 1-way ANOVA, and the post hoc Bonferroni test for pairwise comparisons if a significant difference was found. A probability value of $p < 0.05$ was considered significant. We conducted both nonlinear regressions and Spearman rank-sum correlations to test for a relationship between pressure and infarct size or clinical outcome. The tests were run on commercially available software (Prism 4.0c for Mac OS X, GraphPad Software Inc., San Diego, Calif., USA).

Results

Physiological Parameters

NBO and HBO increased P_{aO2} rapidly out of the range of detection; P_{aCO2} remained in a normal range over a 60- min exposure to HBO or NBO. All other physiologic parameters (particularly temperature and glucose) remained within the normal range and were not significantly different between the groups. The HBO did not alter the temperature inside the pressure chamber, nor did the HBO change the body temperature of the animals during the HBO. The mortality rate was equal before randomization into the treatment groups. Twelve animals that died prior to randomization were replaced. Another 6 animals died prior to study termination in the 24-hour observation arm, and 11 animals died prior to study termination in the 7-day observation arm and were replaced. There was no difference in mortality rates between the groups. The overall mortality rate in our study was 19.5%.

Regional Cerebral Blood Flow

In transient MCAO, an immediate decrease in the blood flow shows the correct position of the filament at the origin of the MCA. After the occlusion of the common carotid artery, rCBF dropped to 70.33 ± 8.1% in all groups. The MCAO caused a further decrease in rCBF, which fell to 16.36 ± 3.34%. The low blood flow was maintained at less than 20% of the pre-ischemic baseline level during the MCAO. After the withdrawal of the filament, the blood flow increased, followed by persistent hypoperfusion (about 70 ± 11%) until the end of the recording period. No significant difference in rCBF was seen between the control, NBO- or HBO-treated rats.

Neurological Evaluation

Clinical Scoring at 24 h after MCAO

Clinical deficits improved with the application of increased oxygen doses, whereas the control group showed

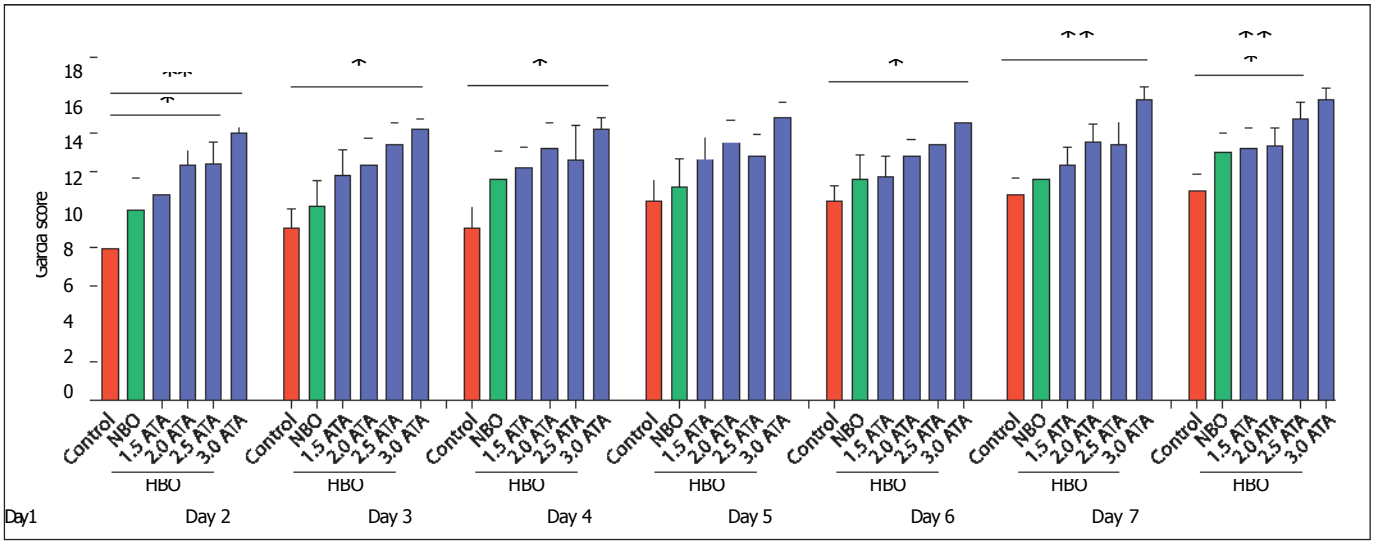


Fig. 3. Clinical evaluation over a period of 7 days following the MCAO, according to the grading system of Garcia. Clinical improvement (higher score) was seen in the high-dose treatment groups E (2.5 ATA) and F (3 ATA). n = 10 per group; mean ± SEM; ANOVA; * $p < 0.05$, ** $p < 0.01$.

severe deficits resulting in low Garcia and high Bederson scores. The control group (group A) reached a Garcia score of 8.2 ± 3.1. NBO-treated animals (group B) were scored at 8.5 ± 2.3. Among the HBO groups, the 3-ATA group (group F) showed the highest score of 12.3 ± 2.1, with a significant difference to the control ($p < 0.05$). The remaining HBO groups had scores of: 8.7 ± 2.3, group C; 10.8 ± 1.8, group D; 11.7 ± 2.4, group E. Neurological outcome improved with increasing chamber pressure. Significant differences ($p < 0.05$) were observed between the control group and the HBO 3-ATA group (fig. 2).

The Bederson score showed similar results in groups D, E and F; however, it failed to reach significance.

Clinical Scoring over 7 Days after MCAO

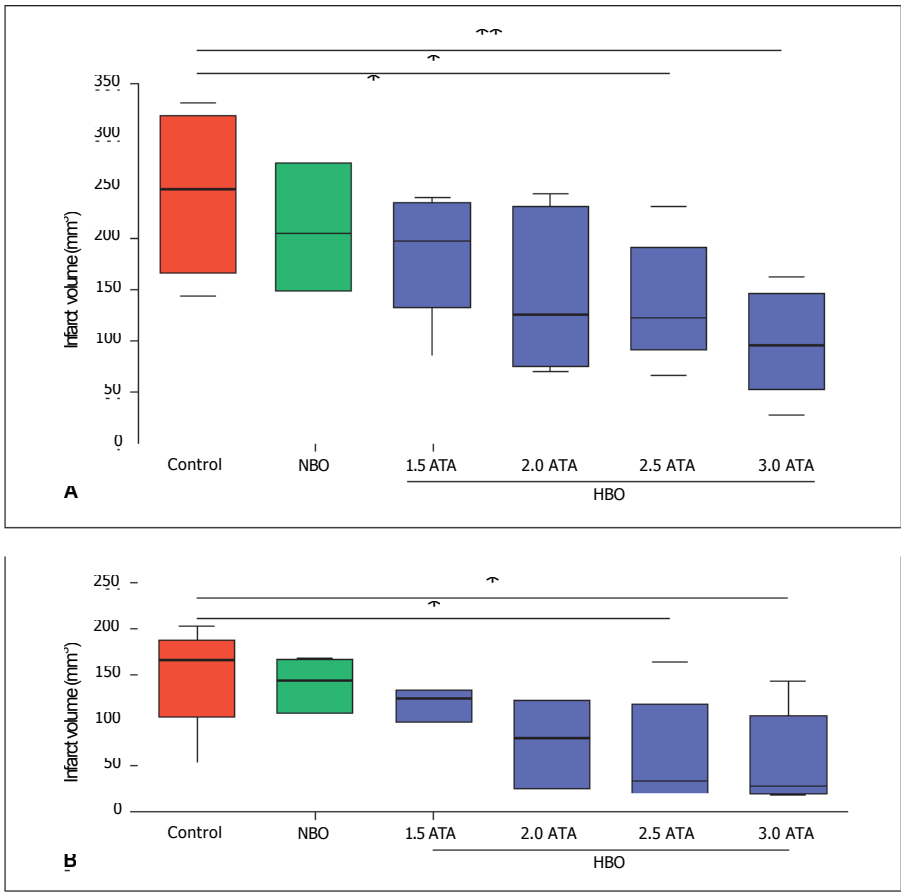
All animals improved with respect to their clinical deficits over time. In general, neurological outcome improved with increasing chamber pressure. Compared to control animals, only animals treated with HBO 2.5 ATA or HBO 3 ATA showed significant clinical improvement over a period of 7 days. As shown in figure 3 , animals from groups E and F showed a statistically significant attenuation of neurological deficits with the Garcia score. The control group developed from a score of 8.0 ± 1.0 on day 1 to 11.0 ± 0.9 on day 7. The NBO group scored 10.0 ± 1.6 on day 1 and 13.0 ± 1.0 on day 7. The HBO 1.5-ATA group showed a score of 10.8 ± 1.2 on day 1 and 13.2 ± 1.1 on day 7. HBO 2.0-ATA scores ranged from 12.3 ± 0.9 on day 1 to 13.3 ± 1.0 on day 7. The HBO 2.5-ATA group developed from 12.4 ± 1.1 on day 1 to 14.8 ± 0.9 on day 7. Finally, the HBO 3.0-ATA group had a baseline score of 14.0 ± 0.3 on day 1 and a final score of 15.8 ± 0.6 on day 7. Data are given as the mean ± SEM.

Assessment of the clinical development with Bederson scores showed comparable results: group F (HBO 3 ATA) showed a statistically significant improvement over 7 days. However, group E also improved, with a significant difference of $p < 0.05$, compared to the control group on day 7. In detail, the control group had a baseline score of 2.4 ± 0.3 on day 1 and improved to a score of 1.8 ± 0.2 on day 7. NBO-treated animals had a score of 2.2 ± 0.4 on day 1 and 1.6 ± 0.3 on day 7. HBO 1.5-ATA-treated animals had a score of 1.6 ± 0.25 on day 1 and 1.2 ± 0.2 on day 7. Data are given as the mean ± SEM.

Infarct Volumetry

Infarct Volumetry 24 h after MCAO

In all groups, infarct volume showed good reproducibility and displayed similar trends to those seen in the clinical scores. In the control group, total infarct volume was 244 ± 70 mm^3 . The average infarct volume from the NBO-treated group was 209 ± 62 mm^3 , which did not reach significance when compared to the control group. However, significant differences were detected in the HBO 2-ATA group (144 ± 77 mm^3; $p < 0.05$), the HBO 2.5-ATA group (135 ± 54 mm^3 ; $p < 0.05$) and the HBO



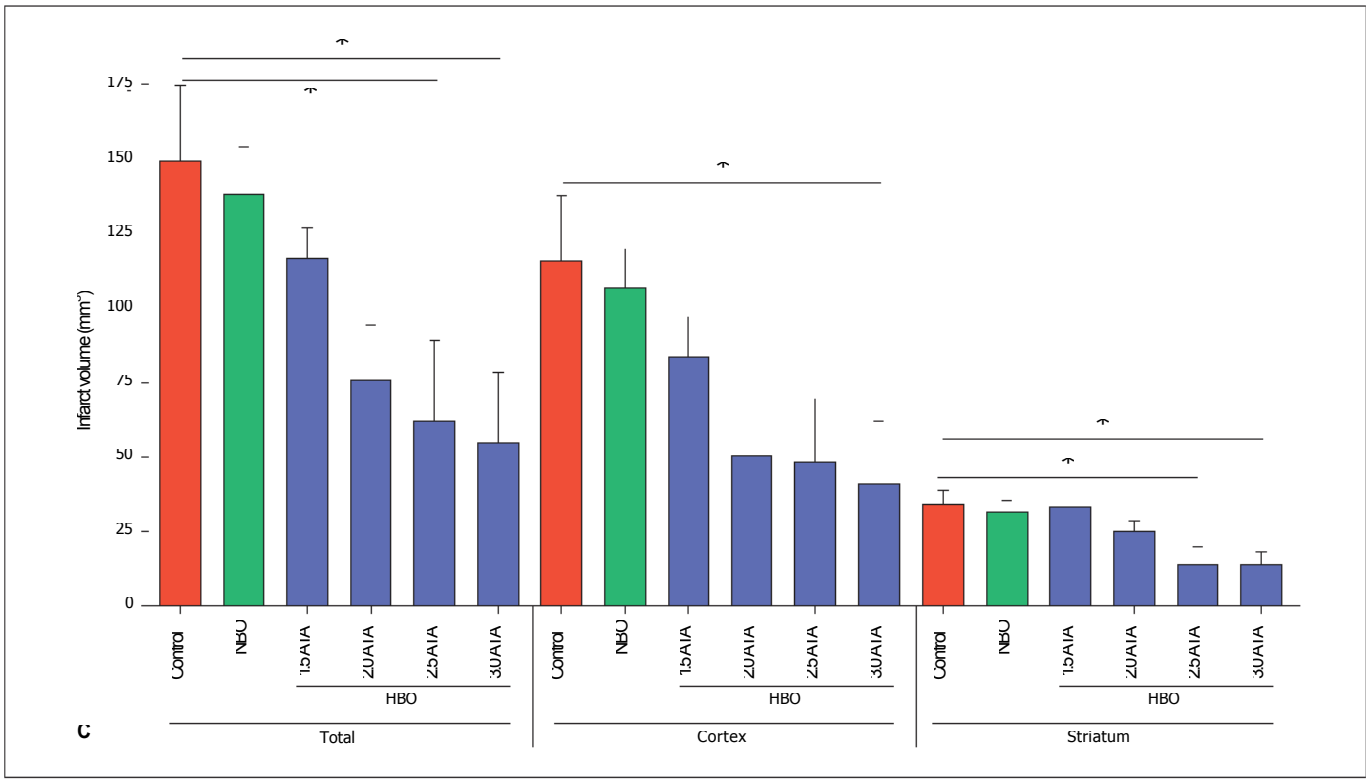


Fig. 4. Quantification of infarct volumes. Cryostat sections were stained with cresyl violet and infarct volumes were calculated after edema correction in a double-blind design at 24 h or 7 days after MCAO. Brain sections were digitalized and the infarct volumes calculated using Image J software. n = 10 per group; mean ± SEM; ANOVA; * p < 0.05, ** p < 0.01. **A** The 24-hour total infarct volumes are displayed. Increasing doses led to enhanced infarct volume reduction. **B** Infarct volumetry obtained 7 days following the MCAO. Total infarct volumes are displayed. The dose-dependent effect of infarct volume reduction was detectable over a period of 7 days. **C** Infarct volumetry obtained 7 days following the MCAO. Total (hemispheric), cortical and subcortical (striatal) infarct volumes are displayed separately. Volumes in both cortical and striatal infarct areas were reduced by HBO treatment.

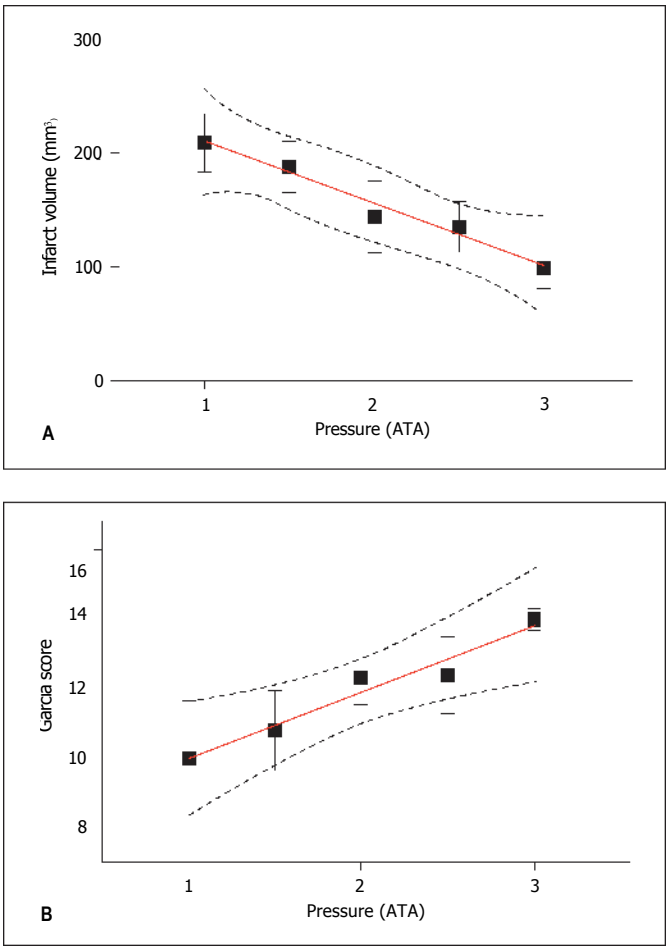


Fig. 5. Regression analysis. The results are given as means ± SEM (n = 10). The dashed lines display the 95% confidence interval. **A** Total infarct volumes were taken as the dependent variable, and pressure from NBO to HBO 3.0 ATA was taken as the independent variable. Regression analysis revealed a highly significant linear correlation of the 2 parameters. The Spearman correlation coefficient was +1.0; p = 0.0083. **B** Clinical outcome as measured by the Garcia score and ambient pressure was analyzed. The regression analysis revealed a highly significant linear correlation of the 2 parameters. The Spearman correlation coefficient was +1.0; p = 0.0083.

3-ATA group (98 ± 45 mm^3; p < 0.01). Again, the most significant reduction of infarct volume was achieved in the HBO 3-ATA group (fig. 4 A).

Infarct Volumetry 7 Days after MCAO

Histological analysis of the brain sections obtained 7 days after the MCAO confirmed the neuroprotective effect of high-dose oxygen administration by HBO. Total infarct volume of the control animals (150 ± 25 mm 3) was reduced in the HBO groups, by increasing oxygen doses, to a minimum of 55 ± 24 mm^3 in the HBO 3-ATA group. Specifically, NBO-treated animals had an average infarct volume of 128 ± 16 mm ^{3} , the HBO 1.5-ATA group had an average infarct volume of 116 ± 10 mm^3, the HBO-2.0 group had an average of 76 ± 19 mm^3 and HBO 2.5 ATA had an average of 62 ± 27 mm^3 (mean ± SEM). Statistically significant reduced infarct volumes were found only in the high-dose oxygen-treatment groups HBO 2.5 and HBO 3.0 ATA (fig. 4 B).

Figure 4C shows a subanalysis of total, cortical and striatal infarct volumes. Group A had cortical and striatal infarct volumes of 116 ± 22 and 34 ± 5 mm ^{3}, respectively, while group B had a 107 ± 13-mm^3 cortical and a 32 ± 4-mm^3 striatal infarct volume. The largest reduction in infarct volume was seen in the high-dose treated groups: group E (cortical infarct volume 48 ± 21 mm^3, striatal infarct volume 14 ± 6 mm^3) and group F (cortical infarct volume 41 ± 21 mm^3 , striatal infarct volume 14 ± 4 mm ^{3}). Data are given as means ± SEM.

Regression Analysis

A regression analysis was performed in order to analyze a possible correlation between the oxygen dose and the reduction of total infarct volume. The independent value x was pressure (measured in absolute atmospheres) and the dependent value y was infarct volume (measured in cubic millimeters). A linear correlation between infarct volume and applied pressure could be detected (p < 0.0083). The Spearman correlation coefficient was +1.0 (fig. 5 A). In a second step, clinical outcome data using the Garcia score and oxygen dose were tested for linear regression. Again, a linear correlation between the Garcia score and applied pressure could be detected (p < 0.0083). The Spearman correlation coefficient was +1.0 (fig. 5 B).

Discussion

Possible future treatment of human stroke with HBO needs solid data from animal models to select the best possible conditions. Timing and dosage of HBO are first questions asked when it comes to selection of the optimal conditions for such treatments. In our previous study, we defined an optimal time window for starting HBO treatment as 3 h following MCAO [22] . The present study was aimed at testing the hypothesis of a putative dose response effect of oxygen therapy in acute transient ischemic stroke in rats, while keeping the time point following the MCAO fixed at 3 h. Therefore, animals were treat-

ed with different doses of oxygen, such as NBO and HBO, up to 3 ATA. The primary end points of the study were defined as infarct volume and clinical outcome at 24 h and 7 days following the MCAO.

To the best of our knowledge, this is the first study systematically analyzing the neuroprotective effects of oxygen in acute transient focal ischemic stroke in a dose-escalating design. Our data indicate a strong neuroprotective effect of oxygen therapy in this ischemia/reperfusion model. The infarct volumes, measured histologically, showed a clear trend towards a dose-dependent infarct-volume reduction at 24 h and at 7 days following the MCAO. Statistically significant reductions of infarct volumes were only seen in the high-dose treatment groups starting at 2.0, 2.5 and 3.0 ATA. Histological analysis of infarct volumes at 7 days showed a significant reduction in infarct volumes for the 2.5- and 3.0-ATA groups. We did not use hyperbaric air as a control due to the phenomenon of decompression sickness. Decompression sickness injury of the brain caused by small nitrogen bubbles is a well-known complication, for example in divers. Nitrogen microbubble formation in the CNS may alter the cerebral circulation, cause multiple strokes, endothelial dysfunction and blood-brain barrier opening [7] . In our opinion, the latter effects would have clearly impaired the quality of our study. To avoid decompression sickness, we would have needed to change the hyperbaric protocol in order to include defined decompression stops in the hyperbaric air group. This would have severely limited the comparability of the groups.

In our study, HBO-induced infarct volume reduction was not limited to cortical areas, as usually seen in ischemia studies with neuroprotective substances, but showed also a robust effect in the infarct core. In contrast, NBO did not cause any significant infarct volume reduction. Our data confirm the strong neuroprotective effect of early HBO treatment, as previously published by our group and others [22, 29] . Furthermore, our data are supported by findings of Veltkamp et al. [30] , who recently analyzed the effects of NBO and HBO (3.0 ATA) in a model of permanent and transient ischemia in mice. They reported larger infarct volume reductions in HBO-treated animals compared with NBO. The neuroprotective effect of oxygen was more prominent in transient focal ischemia than in permanent focal ischemia. However, in the latter study cerebral ischemia in mice was performed by 2 different methods – coagulation and suture model – which makes a direct comparison more difficult [30].

Earlier reports have shown neuroprotective effects of NBO in experimental stroke. Kim et al. [31] reported decreased infarct volumes in rats following transient ischemia treated with NBO. In a previous study, the same group showed a reduced infarct volume and MRI diffusion abnormalities in NBO-treated animals [8]. These data appear to be at variance with our data, since NBO failed to reduce infarct volume significantly in our study. However, it should be emphasized that Kim et al. [31] and Singhal et al. [8, 9] started NBO therapy 5 and 15 min following the MCAO, respectively, whereas in our experiments oxygen treatment started 3 h following the MCAO for a period of 1 h. Thus, we interpret the lack of efficacy of NBO in our model to be due to differences in the experimental design. We performed a regression analysis of the measured total infarct volume and the applied pressure of oxygen therapy. The results indicate a linear correlation between the oxygen dose applied and the infarct volume, which suggests a neuroprotective effect of oxygen in our model.

The clinical improvement and oxygen dose were significantly correlated in a linear fashion, which further underlines the efficacy of early high-dose oxygen treatment in our model. Our study shows statistically significant clinical improvement in the high-dose-treated groups at 24 h. This clinical improvement was a robust finding, since it could be followed over a period of 7 days. The presented data show a strong and long-lasting neuroprotective effect of HBO in acute experimental stroke, which suggests superiority of HBO compared to NBO. Furthermore, our data suggest an important role for high-dose oxygen therapy in the acute phase of ischemic stroke therapy in rats. In other models of cerebral ischemia, such as global ischemia after cardiac arrest, oxygen itself appears to have a disadvantageous effect upon oxidative energy metabolism in the brain, as recently published by Richards et al. [32] . Due to these fundamental differences in transient global versus focal ischemia models, we suggest careful interpretation of such oxygen treatment studies in acute ischemic events. Thus, each pathogenetic condition for ischemic injury must be investigated carefully for the underlying mechanisms of oxygen-induced neuroprotection, and its potentially harmful effects in animal models, prior to the start of clinical trials. At present, in combination with our previous study [22] , we have demonstrated that early high-dose oxygen therapy appears to be beneficial for a long-lasting infarct volume reduction and clinical outcome in rats.

References

1 Lipton P: Ischemic cell death in brain neurons. Physiol Rev 1999; 79: 1431–1568.
2 Stingele R, Eschenfelder CC, Zeller J: Therapy of acute stroke. Haemostaseologie 2006; 26: 316–325.
3 Yogaratnam JZ, Laden G, Madden LA, Seymour AM, Guvendik L, Cowen M, Greenman J, Cale A, Griffin S: Hyperbaric oxygen: a new drug in myocardial revascularization and protection? Cardiovasc Revasc Med 2006; 7: 146–154.
4 Helms AK, Whelan HT, Torbey MT: Hyperbaric oxygen therapy of cerebral ischemia. Cerebrovasc Dis 2005; 20: 417–426.
5 Sunami K, Takeda Y, Hashimoto M, Hirakawa M: Hyperbaric oxygen reduces infarct volume in rats by increasing oxygen supply to the ischemic periphery. Crit Care Med 2000; 28: 2831–2836.
6 Agardh CD, Zhang H, Smith ML, Siesjo BK: Free radical production and ischemic brain damage: influence of postischemic oxygen tension. Int J Dev Neurosci 1991; 9: 127–138.
7 Jain KK: Physical, physiological and biochemical aspects of hyperbaric oxygenation; in Jain KK (ed): Textbook of Hyperbaric Medicine. Göttingen, Hogrefe & Huber, 2004, pp 10–20.
8 Singhal AB, Dijkhuizen RM, Rosen BR, Lo EH: Normobaric hyperoxia reduces MRI diffusion abnormalities and infarct size in experimental stroke. Neurology 2002; 58: 945–952.
9 Singhal AB, Wang X, Sumii T, Mori T, Lo EH: Effects of normobaric hyperoxia in a rat model of focal cerebral ischemia-reperfusion. J Cereb Blood Flow Metab 2002; 22: 861–868.
10 Flynn EP, Auer RN: Eubaric hyperoxemia and experimental cerebral infarction. Ann Neurol 2002; 52: 566–572.
11 Berrouschot J, Schwab S, Schneider D, Hacke W: Hyperbaric oxygen therapy (HBO) after acute focal cerebral ischemia. Nervenarzt 1998; 69: 1037–1044.
12 Zhang JH, Lo T, Mychaskiw G, Colohan A: Mechanisms of hyperbaric oxygen and neuroprotection in stroke. Pathophysiology 2005; 12: 63–77.
13 Weinstein PR, Hameroff SR, Johnson PC, Anderson GG: Effect of hyperbaric oxygen therapy or dimethyl sulfoxide on cerebral ischemia in unanesthetized gerbils. Neurosurgery 1986; 18: 528–532.
14 Burt JT, Kapp JP, Smith RR: Hyperbaric oxygen and cerebral infarction in the gerbil. Surg Neurol 1987; 28: 265–268.
15 Ruiz E, Brunette DD, Robinson EP, Tomlinson MJ, Lange J, Wieland MJ, Sherman R: Cerebral resuscitation after cardiac arrest using hetastarch hemodilution, hyperbaric oxygenation and magnesium ion. Resuscitation 1986; 14: 213–223.
16 Takahashi M, Iwatsuki N, Ono K, Tajima T, Akama M, Koga Y: Hyperbaric oxygen therapy accelerates neurologic recovery after 15- minute complete global cerebral ischemia in dogs. Crit Care Med 1992; 20: 1588–1594.
17 Iwatsuki N, Takahashi M, Ono K, Tajima T: Hyperbaric oxygen combined with nicardipine administration accelerates neurologic recovery after cerebral ischemia in a canine model. Crit Care Med 1994; 22: 858–863.
18 Weinstein PR, Anderson GG, Telles DA: Results of hyperbaric oxygen therapy during temporary middle cerebral artery occlusion in unanesthetized cats. Neurosurgery 1987; 20: 518–524.
19 Mink RB, Dutka AJ: Hyperbaric oxygen after global cerebral ischemia in rabbits reduces brain vascular permeability and blood flow. Stroke 1995; 26: 2307–2312.
20 Shiokawa O, Fujishima M, Yanai T, Ibayashi S, Ueda K, Yagi H: Hyperbaric oxygen therapy in experimentally induced acute cerebral ischemia. Undersea Biomed Res 1986; 13: 337–344.
21 Kawamura S, Yasui N, Shirasawa M, Fukasawa H: Therapeutic effects of hyperbaric oxygenation on acute focal cerebral ischemia in rats. Surg Neurol 1990; 34: 101–106.
22 Lou M, Eschenfelder CC, Herdegen T, Brecht S, Deuschl G: Therapeutic window for use of hyperbaric oxygenation in focal transient ischemia in rats. Stroke 2004; 35: 578–583.
23 Veltkamp R, Siebing DA, Heiland S, Schoenffeldt-Varas P, Veltkamp C, Schwaninger M, Schwab S: Hyperbaric oxygen induces rapid protection against focal cerebral ischemia. Brain Res 2005; 1037: 134–138.
24 Veltkamp R, Siebing DA, Sun L, Heiland S, Bieber K, Marti HH, Nagel S, Schwab S, Schwaninger M: Hyperbaric oxygen reduces blood-brain barrier damage and edema after transient focal cerebral ischemia. Stroke 2005; 36: 1679–1683.
25 Longa EZ, Weinstein PR, Carlson S, Cummins R: Reversible middle cerebral artery occlusion without craniectomy in rats. Stroke 1989; 20: 84–91.
26 Schäbitz WR, Sommer C, Zoder W, Kiessling M, Schwaninger M, Schwab S: Intravenous brain-derived neurotrophic factor reduces infarct size and counterregulates bax and bcl-2 expression after temporary focal cerebral ischemia. Stroke 2000; 31: 2212–2217.
27 Bederson JB, Pitts LH, Tsuji M, Nishimura MC, Davis RL, Bartkowski H: Rat middle cerebral artery occlusion: evaluation of the model and development of a neurologic examination. Stroke 1986; 17: 472–476.
28 Garcia JH, Wagner S, Liu KF, Hu XJ: Neurological deficit and extent of neuronal necrosis attributable to middle cerebral artery occlusion in rats: statistical validation. Stroke 1995; 26: 627–634, discussion 635.
29 Badr AE, Yin W, Mychaskiw G, Zhang JH: Dual effect of HBO on cerebral infarction in MCAO rats. Am J Physiol Regul Integr Comp Physiol 2001; 280:R766–R770.
30 Veltkamp R, Sun L, Herrmann O, Wolferts G, Hagmann S, Siebing DA, Marti HH, Veltkamp C, Schwaninger M: Oxygen therapy in permanent brain ischemia: potential and limitations. Brain Res 2006; 1107: 185–191.
31 Kim HY, Singhal AB, Lo EH: Normobaric hyperoxia extends the reperfusion window in focal cerebral ischemia. Ann Neurol 2005; 57: 571–575.
32 Richards EM, Fiskum G, Rosenthal RE, Hopkins I, McKenna MC: Hyperoxic reperfusion after global ischemia decreases hippocampal energy metabolism. Stroke 2007; 38: 1578–1584.

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Hyperbaric oxygen in the treatment of acute ischemic stroke: an unsettled issue

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Abstract

Therapy for acute ischemic stroke can be approached in two basic ways: first, by an attempt to restore or improve blood flow in an occluded vascular territory and, second, via therapy directed at the cellular and metabolic targets. As local anoxia and energy failure are the initiating cellular stage in ischemia, the inhalation of oxygen at increased atmospheric pressures might be effective. Treatment of acute focal cerebral ischemia with hyperbaric oxygen (HBO) has been reported in animals and humans. In general, the results of research in animals have suggested a promising role for the use of HBO. More than 400 cases of human ischemic stroke treated with HBO have been reported. In about half of the cases, improvement in status has been claimed on clinical or electroencephalographic grounds. In fact, the effectiveness of HBO in most disease processes other than carbon monoxide poisoning and decompression sickness is a subject of major ongoing debate. This short review will attempt: (1) to recall some early experiments involving HBO in the treatment of acute ischemia; (2) to point out some conflicting results regarding the role of HBO on cellular and metabolic disorders; and (3) to determine the possibility of a future role for HBO therapy in acute ischemic stroke. © 1997 Elsevier Science B.V.

Keywords: Ischemic stroke; Hyperbaric oxygen; Efficiency

1. Introduction

Although ischemic stroke is a leading cause of death and disability, its past management was often marked by feelings of hopelessness. This sense resulted, in part, from the lack of any therapy that was clearly established as useful in reversing the neurological effects of stroke. Therapy for acute ischemic stroke can be approached in two basic ways (Fisher, 1995). First, an attempt can be made to restore or improve blood flow in an occluded vascular territory and, second, therapies can be directed at the cellular and metabolic disturbances of focal brain ischemia. These two interventional strategies are certainly not mutually exclusive and interactions are easily anticipated. Within this scope, consideration of hyperbaric oxygen (HBO) treatment for ischemia of the brain derives from the belief that this treatment might salvage the still viable, though nonfunctioning tissue surrounding the infarcted area (Astrup et al., 1981; Hakim, 1987). As local anoxia and energy failure are the initiating cellular stage in ischemia, the inhalation of oxygen at increased atmospheric pressures might produce a marked elevation in arterial blood oxygen partial pressure and content. The resultant increase in oxygen delivery to the tissues might prolong functional activity during severe ischemia which is characterized according to positron emission tomography (Sette et al., 1989) by depression of oxygen consumption and CBF decrease (stage 3 of ischemia).

Treatment of acute and subacute focal cerebral ischemia with hyperbaric oxygen (HBO) has been reported in animals (Corkill et al., 1985; Burt et al., 1987; Weinstein et

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al., 1987; Reitan et al., 1990) and humans (Ingvar and Lassen, 1965; Saltzman et al., 1966; Mogami et al., 1969; Hart and Thompson, 1971; Holbach et al., 1976; Neubauer and End, 1980; Kapp, 1981). In general, the results of research in animals have suggested a promising role for the use of HBO. More than 400 cases of human ischemic stroke treated with HBO have been reported. In about half of these cases, improvement in status has been claimed on clinical or electroencephalographic grounds. In fact, the effectiveness of HBO in most disease processes other than carbon monoxide poisoning and decompression sickness (Grim et al., 1990; Gabb and Robbin, 1987) is the subject of major ongoing debate. Therefore, the overall clinical impact has been modest and, for some, disappointing (Anderson et al., 1991). This short review will attempt to recall the past interest for the use of HBO in acute stroke and then to determine whether HBO might be still a competitive therapy for this devastating neurological disorder.

2. HBO, a glance at early animal experiments

Boerema et al. (1960) noted that hyperbaric oxygen (HBO) permits biological fluids depleted of erythrocytes or hemoglobin to deliver sufficient oxygen to the tissues to sustain life and neurological function. However, conflicting results about the effectiveness of HBO in cerebral ischemia have been reported from various experimental studies. The role of HBO therapy in ischemia has been assessed in dogs (Smith et al., 1961). Temporary occlusion of both carotid and vertebral arteries was performed, and the abnormalities on the electroencephalogram, with the animals exposed either to room air or to oxygen at 1500 mmHg partial pressure, were noted. No flattening of the electroencephalogram occurred in the pressure chamber up to periods of 0.5 h compared to flattening within 30 s in room air. This observation supported the view that, at high pressure, sufficient oxygen may be 'stored' in the brain to oppose the anoxia induced by occluding the vessels. Illingworth et al. (1961) have suggested that HBO might exert a protective effect under moderate hypothermia. This protective effect of hyperbaric oxygenation in cerebral anoxia in hypothermic dogs has also been demonstrated by Whalen et al. (1966). In this study the animals undergoing hyperbaric oxygenation demonstrated a 109% increase in the time required for the appearance of the electroencephalographic abnormalities following interruption of the cerebral circulation. In spite of the above evidence, Jacobson and Lawson (1963) were unable to detect comparable protection in their experimental occlusion of the middle cerebral artery associated with a moderate hypothermia (26–27°C). Moreover, the animals exposed to a continual high PO_2 showed evidence of pulmonary oxygen toxicity.

3. HBO and cerebral blood flow (CBF), a persisten controversy

CBF regulation might be influenced by changes oxygen content. Lambertsen et al. (1953) have demo strated that OHB at 3.5 ATA caused a 25% reduction total CBF, measured by the nitrous oxide inhalati method in normal awake man. Since there was a reducti in arterial PCO_2 during OHB administration, the reducti in flow was ascribed to hypocapnia. In anaesthetized do Jacobson et al. (1963) found that OHB at 2 ATA cause 21% reduction in CBF, measured by Krypton-85 b clearance. Despite constant arterial PCO_2, HBO can redu CBF by direct cerebral vasoconstriction, although br tissue PCO_2 may be a controlling factor (Miller et 1970). A theoretically beneficial effect of this decrease been suggested by clinical CBF studies. This latter co be related to the inverse steal phenomenon described Lassen and Palvögyi (1968), in which constriction vessels in normal brain may increase blood flow unresponsive areas. Kawamura et al. (1990) have observ that vasoconstriction due to hyperoxia induces a decre in CBF and cerebral blood volume in rats, thus resulting a decrease of intracranial pressure. Therefore the presum efficiency of HBO for brain edema may be related to dual effects of available oxygen increase and the cereb blood volume reduction. Accordingly, despite a lo CBF, an oxygen supply increase and a reduction of intracranial pressure can be expected to improve cereb ischemia. Conversely, after complete cerebral ischemia dogs (Yatsuzuka et al., 1989), HBO therapy was associated to a decrease of CBF, because of the lack vascular response to high PaO_2. These authors a observed a reduction in intracranial pressure, which usu ly increases after ischemia and leads to the development reperfusion failure.

HBO may also perturb parameters such as hematoc level, which influences whole blood oxygen conte Hematocrit and fibrinogen level may influence transcran Doppler blood flow velocities through their effects whole blood and plasma viscosity, respectively (Brass al., 1988; Ameriso et al., 1990). Some studies suggest t arterial oxygen content is a fundamental CBF determina perhaps more important than blood viscosity (Macko et 1993). Macko et al. (1993) have shown, in man, significant inverse relation between arterial oxygen cont and interindividual middle cerebral artery blood fl velocity. Therefore HBO might be useful in increasing arterial oxygen content.

An increase in hematocrit caused by exposure to H has been reported (Takahashi, 1969). This fact might another limitation for the use of HBO therapy for crocirculatory obstruction, though an increase in blo viscosity might be expected (Putnum et al., 1967). Ho ever, in dogs treated by HBO after global cerebral

chemia, Takahashi et al. (1992) did not find an increase of hematocrit.

4. HBO and glucose metabolism

The human brain does not completely oxidize blood glucose, but glycolyses a small part (7–8%) that is delivered into the venous blood as lactate and pyruvate. Different inspiratory oxygen pressures might have an influence on cerebral glucose metabolism. High oxygen pressures initially cause a disturbance of the oxidative glucose metabolism of the brain (Haugaard, 1965; Williams and Haugaard, 1970). Since the non-oxidative metabolic pathway of glucose, glycolysis, is relatively resistant to oxygen toxicity, it may be stimulated when the oxidative metabolic pathway, particularly the processes involved in ATP production, are already inhibited by higher oxygen pressures (Williams and Haugaard, 1970).

The experiment of Holbach et al. (1977), in humans who had ischemic stroke or head injury, showed that the increase of cerebral glycolysis occurring at an inspiratory oxygen pressure of 2.0 ATA was related to cerebral oxygen toxicity. Conversely, when oxygen pressure was lowered to 1.5 ATA, a balanced cerebral glucose metabolism was observed, as revealed by the pertinent glucose oxidation quotient values. This finding indicates an improved or sufficient brain oxygenation and energy production. Moreover, patients who were exposed to an inspiratory oxygen pressure of 1.5 ATA showed significantly better neurological results than those exposed to 2.0 ATA.

5. Does HBO promote reperfusion injury through the production of free radicals?

Several investigators have also suggested that the application of HBO after cerebral ischemia may be detrimental to neurological recovery, since HBO has been shown to increase the amount of free radicals generated in tissues known for favouring reperfusion injury (Jerret et al., 1973; Noda et al., 1983). Indeed, in the reoxygenation phase, increased intracellular calcium ion levels trigger the generation of superoxide radicals, lipid peroxidation by phospholipase A2 activation, and the production of vasoactive mediators from arachidonic acid, all of which aggravate post-ischemic cell damage, so-called reoxygenation injury. Therefore, the possibility of generating oxygen free radicals and lipid peroxides by hyperbaric oxygenation is the major concern in clinical application of HBO. Mickel et al. (1987) have shown that exposure of mongolian gerbils to a 100% oxygen atmosphere after 15 min of global brain ischemia resulted in a marked increase in the production of pentane, an in vivo product of lipid peroxidation. Much less pentane production occurred in animals subjected global brain ischemia that were then exposed to an atmosphere and also in animals exposed to a 100% oxyg atmosphere without ischemia. Conversely, as suggested Mink and Dutka (1995), treatment with HBO after chemia in rabbits increased the amount of oxygen fr radicals in the brain, but this latter fact was not associat with an increase of lipid peroxidation or with a reducti in neurophysiological recovery. According to this stud the additional free radicals generated by HBO exposu were sufficiently scavenged by endogenous antioxidan such as reduced glutathione. Along the same lines, plasr lipid peroxide concentration, measured by chemilumine cence analysis, did not increase during HBO in patier with ischemic brain damage (Nakagawa et al., 1984).

HBO might also decrease intracellular sodium ion leve and normalize intracellular potassium ion concentrations red blood cells in patients with hypoxic encephalopat (Fujita and Kitani, 1978). Thus, HBO therapy mig ameliorate the impaired function of ion pumps in t ischemic cell membranes. If HBO may prevent the pr duction of these biologically active substances, rather th facilitate them, this therapy might contribute to the r covery of the damaged cells.

6. A future for HBO in human stroke?

During the 1960s, there was widespread use of h perbaric treatment for myocardial infarction and stro (Grim et al., 1990). Because of its almost global applic tion to a wide variety of diseases, HBO therapy lends itse easily to medical adventurism (a therapy in search diseases). Therefore, enthusiasm waned after poor resu of some clinical trials (Gabb and Robbin, 1987). Neverth less, animals studies and greater clinical experience ov the last two decades have produced a set of indications f which HBO therapy might appear beneficial. Thou ischemic stroke may provide a good rationale for the u of HBO, stroke is not a commonly accepted indication delineated by the Undersea and Hyperbaric Society (198 the professional association of physicians administeri HBO therapy. This mistrust for HBO therapy might related to the lack of controlled prospective analysis in t early post-ischemic period, and also to persistent que tioning of its mechanisms and potential harmful effects

Indeed, acute ischemic stroke treatment trials may ha a narrow time window in which potential beneficial effe can result. Though the period of time that HBO can utilized to delay the onset of cerebral ischemia is limit by the onset of oxygen toxicity, the threshold of oxyg concentration and the duration of exposure needed produce damages have yet to be determined.

The major criticism to most HBO studies has been t lack of controlled prospective analysis in the early po

ischemic period. Recently, Anderson et al. (1991) administered HBO or air in a double-blind protocol to 39 patients with ischemic cerebral infarction; the average time from onset of the symptoms until the first dive was 51.8 h. The study was not completed, as a trend favoring the air-treated patients was observed. Moreover, most patients experienced the usual complications of HBO therapy resulting from barometric pressure changes or oxygen toxicity. Conversely, we did not share the same conclusion in our HBO double-blind study, in which patients were included within 18±3.2 h in the air group versus 19±2.7 h in the HBO group (Nighoghossian et al., 1995). This discrepancy raises some controversy. Although the small number of patients in each group in our study precludes any conclusion regarding the deleterious potential of HBO, we did not observe major side effects. A trend favoring HBO was noted at 1 year, but we did not find a significant difference in outcome between groups according to the pre- and post-therapeutic differences after the 1-year follow-up.

On theoretical grounds, the ideal candidate circumstances would be focal, incomplete ischemia with potential for reperfusion by clot lysis or recruitment of collaterals. It might be speculated that the patients most likely to respond favorably to HBO therapy are those who have infarcts related to large vessel thrombosis and surrounded by ischemic penumbra (Kapp, 1981). In support of this are reports claiming a favorable transient or, less often, permanent response to HBO in cases selected for demonstrated carotid occlusion (Holbach et al., 1976; Kapp, 1981). As there are definite time limits on the viability of marginally perfused cells, early application of HBO might be crucial, like any treatment of evolving brain infarction (Sterman et al., 1987; Barsan et al., 1989). As two double-blind pilot studies failed to give any definitive response concerning the use of HBO therapy, the clinical application of HBO to ischemic disease remains to be fully exploited. Therefore, a large double-blind study might be required in the future. Based on experimental data, HBO at 1.5 ATA during 1 h might be proposed, as neurotoxicity is rare with low pressure and short duration (Davis et al., 1988). If HBO treatment is safe and effective, it could be added to thrombolytic therapy which has recently shown its efficiency in restoring cerebral blood flow. (The National Institute of Neurological Disorders and Stroke rt-PA Stroke Study Group, 1995).

References

Ameriso, S.F., Paganini-Hill, A., Meiscliman, H.J. and Fisher, M. (1990) Correlates of middle cerebral artery blood flow velocity in the elderly. Stroke, 21: 1579–1583.

Anderson, D.C., Bottini, A.G., Jagiella, W.M., Westphal, B., Ford, S., Rockswold, G.L. and Loewenson, R.B. (1991) A pilot study of hyperbaric oxygen in the treatment of human stroke. Stroke, 22: 1137–1142.

Astrup, J., Siesjö, B.K. and Symon, L. (1981) Thresholds in cerebral ischemia: the ischemic penumbra. Stroke, 12: 723–725.

Barsan, W.G., Brott, T.G., Olinger, C.P. and Marler, J.R. (1989) Early treatment for acute ischemic stroke (Editorial). Ann. Intern. Med., 111: 449–451.

Boerema, I., Meyne, N.G., Brummelkamp, W.K., Bouma, S., Mensch, M.H., Kammermans, F., Sternhanf, M. and Van Aaldern, W. (1960) Life without blood: a study of influence of high atmospheric pressure and hypothermia on dilution of blood. Cardiovasc Surg., 1: 133–146.

Brass, L.M., Pavlakis, S.G., De Vivo, D., Piomelli, S. and Mohr, J.P. (1988) Döppler measurement of the middle cerebral artery: effect of hematocrit. Stroke, 19: 1466–1469.

Burt, J.T., Kapp, J.P. and Smith, R.R. (1987) Hyperbaric oxygen and cerebral infarction in the gerbil. Surg. Neurol., 28: 265–268.

Corkill, G., Van Housen, K., Hein, L. and Reitan, J. (1985) Videodensitometric estimation of the protective effect of hyperbaric oxygen in the ischemic gerbil brain. Surg. Neurol., 24: 206–210.

Davis, J.C., Dunn, J.M. and Heimbach, R.D. (1988) Hyperbaric medicine: patient selection, treatment procedures, and side effects. In: J.C. Davis and T.K. Hunt (Eds.), Problem Wounds: The Role of Oxygen. Elsevier Science Publishing Co., New York, NY, pp. 225–235.

Fisher, M. (1995) Potentially effective therapies for acute ischemic stroke. Eur. Neurol., 35: 3–7.

Fujita, T. and Kitani, Y. (1978) Intracellular electrolytes in erythrocytes during and after shok. Relation to impaired consciousness. J. Trauma, 18: 345–354.

Gabb, G. and Robbin, E.D. (1987) Hyperbaric oxygen: a therapy in search of diseases. Chest, 92: 1074–1082.

Grim, P.S., Gottlieb, L.J. and Boddie, A. (1990) Hyperbaric oxygen therapy. J. Am. Med. Assoc., 263: 2216–2220.

Hakim, A.M. (1987) The cerebral ischemic penumbra. Can. J. Neurol. Sci., 14: 557–559.

Hart, G.B. and Thompson, R.E. (1971) The treatment of cerebral ischemia with hyperbaric oxygen (OHP). Stroke, 2: 247–250.

Haugaard, N. (1965) Poisoning of cellular reactions by oxygen. Symposium on hyperbaric oxygenation. Ann. NY Acad. Sci., 117: 736–744.

Holbach, K.H., Wassmann, H. and Hohelüchter, K.L. (1976) Reversibility of the chronic post-stroke state. Stroke, 7: 296–300.

Holbach, K.H., Caroli, A. and Wassmann (1977) Cerebral energy metabolism in patients with brain lesions at normo and hyperbaric oxygen pressures. J. Neurol., 217: 17–30.

Illingworth, C.F.W., Smith, G., Lawson, D.D., Ledingham, I.McA., Sharp, G.R. and Griffiths, J.C. (1961) Surgical and physiological observation in an experimental pressure chamber. Br. J. Surg., 49: 222–227.

Ingvar, H.D. and Lassen, N.A. (1965) Treatment of focal cerebral ischemia with hyperbaric oxygen. Acta Neurol. Scand., 41: 92–95.

Jacobson, I. and Lawson, D.D. (1963) The effect of hyperbaric oxygen on experimental cerebral infarction in the dog with preliminary correlations of cerebral blood flow at 2 atmospheres of oxygen. J. Neurosurg., 20: 849–859.

Jacobson, I., Harper, A.M. and McDowall, D.G. (1963) The effects of oxygen under pressure on cerebral blood flow and cerebral venous oxygen tension. Lancet, ii: 549.

Jerret, S.A., Jefferson, D. and Mengel, C.E. (1973) Seizures, H2O2 formation and lipid peroxydes in brain during exposure to oxygen under high pressure. Aerospace Med., 44: 40–44.

Kapp, J.P. (1981) Neurological response to hyperbaric oxygen: a criteria for cerebral revascularisation. Surg. Neurol., 15: 43–46.

Kawamura, S., Yasui, N., Shirasawa, M. and Fukasawa, H. (1990) Therapeutic effects of hyperbaric oxygenation on acute focal cerebral ischemia in rats. Surg. Neurol., 34: 101–106.

Lambertsen, C.J., Kough, R.H., Cooper, D.Y., Emmel, G.L., Loeschke, H.H. and Schmidt, C.F. (1953) Oxygen toxicity: effects in man of oxygen inhalation at 1 and 3.5 atmospheres upon blood gas transport, cerebral circulation and cerebral metabolism. J. Appl. Physiol., 5: 471–486.

Lassen, N.A. and Palvögyi, R. (1968) Cerebral steal during hypercapnia and the inverse reaction during hypocapnia observed by the 133-Xenon technique in man. Scand. J. Clin. Lab. Invest., Suppl. 102, XIIID.

Macko, R.F., Ameriso, S.F., Akmal, M., Paganini-Hill, A., Mohler, J.G., Massry, S.G., Meiselman, H.J. and Fisher, M. (1993) Arterial oxygen content and age are determinants of middle cerebral artery blood flow velocity. Stroke, 24: 1025–1028.

Mickel, H.S., Vaishnav, Y.N., Kempski, O., Von Lubitz, D., Weiss, J.F. and Feuerstein, G. (1987) Breathing 100% oxygen after global brain ischemia in mongolian gerbils results in increased lipid peroxydation and increased mortality. Stroke, 18: 426–430.

Miller, J.D., Ledingham, I.McA. and Jennett, W.B. (1970) Effects of hyperbaric oxygen on intracranial pressure and cerebral blood flow in experimental cerebral oedema. J. Neurol. Neurosurg. Psychiatry, 33: 745–755.

Mink, R.B. and Dutka, A.J. (1995) Hyperbaric oxygen after global cerebral ischemia in rabbits does not promote brain lipid peroxydation. Crit. Care Med., 23: 1398–1404.

Mogami, H., Hayakawa, T., Kanai, N., Kuroda, R., Yamada, R., Ikeda, T., Katsurada, K. and Sugimoto, T. (1969) Clinical application of hyperbaric oxygenation in the treatment of acute cerebral damage. J. Neurosurg., 31: 636–643.

Nakagawa, Y., Tsuru, M. and Kawahigashi, H. (1984) Oxygenation at high pressure (OHP) for cerebral ischemia (in Japenese). Hokkaido J. Med. Sci., 59: 397–411.

Neubauer, R.A. and End, E. (1980) Hyperbaric oxygenation as an adjunct therapy in strokes due to thrombosis: a review of 122 patients. Stroke, 11: 297–300.

Nighoghossian, N., Trouillas, P., Adeleine, P. and Salord, F. (1995) Hyperbaric oxygen in the treatment of acute ischemic stroke, a double blind study. Stroke, 26: 1369–1372.

Noda, Y., McGeer, P.L. and McGeer, E.G. (1983) Lipid peroxyde distribution in brain and the effect of hyperbaric oxygen. J. Neurochem., 40: 1329–1332.

Putnum, T.C., Kevy, S.V. and Replogle, R.L. (1967) Surg. Gynecol. Obstet., 124: 547–552.

Reitan, J.A., Kien, N.D., Thorup, S. and Corkill, G. (1990) Hyperbaric oxygen increases survival following carotid ligation in gerbils. Stroke, 21: 119–123,

Saltzman, H.A., Anderson, B., Whalen, R.E., Heyman, A. and Sieker, H.O. (1966) Hyperbaric oxygen therapy of acute cerebral insufficiency. In: I.W. Brown and B.G. Cox (Eds.), Proceeding Third International Conference on Hyperbaric Medicine. Academy of Sciences, Washington, DC, pp. 440–446.

Sette, G., Baron, J.C., Mazoyer, B., Levasseur, M., Pappata, Crouzel, C. (1989) Local brain haemodynamics and oxygen lism in cerebrovascular disease. Positron emission tomography 112: 931–951.

Smith, G., Lawson, D.D., Renfrew, S., Ledingham, I. and Shar (1961) Preservation of cerebral cortical activity by breathing ox two atmospheres of pressure during cerebral ischemia. Surg. C Obstet., 113: 13–16.

Sterman, A.B., Furlan, A.J., Pessin, M., Kase, C., Caplan, Williams, G. (1987) Acute stroke therapy trials: an introdu recurring design issues. Stroke, 18: 524–527.

Takahashi, K. (1969) Experimental studies on hyperbaric oxyg combined with hypothermia (in Japanese with English a Tohoku Igaku Z., 79: 20–31.

Takahashi, M., Iwatsuki, N., Ono, K., Tajima, T., Akama, M. an Y. (1992) Hyperbaric oxygen therapy accelerates neurologic r after 15-minute complete global cerebral ischemia in dogs. Cr Med., 20: 1588–1594.

The National Institute of Neurological Disorders and Stroke rt-PA Study Group (1995) Tissue plasminogen activator for acute i stroke. N. Engl. J. Med., 333: 1581–1587.

Undersea and Hyperbaric Medical Society (1986) Hyperbaric Therapy: A Committee Report. Undersea and Hyperbaric Society, Bethesda, MD.

Yatsuzuka, H., Shiwaku, Y. and Kondo, M. (1989) Effect of hy oxygen therapy on the ischemic brain injury induced by 18 mi complete cerebral ischemia in dogs (in Japanese with Eng stract). Nihon Kokiatsu Kankyo Igaku Kaishi, 24: 83–90.

Weinstein, P.R., Anderson, G.G. and Telles, D.A. (1987) Re hyperbaric oxygen therapy during temporary middle cerebra occlusion in unanesthetized cats. Neurosurgery, 20: 511–524.

Whalen, R.E., Heyman, A., Saltzman, H. and Durham, N.C. (19 protective effect of hyperbaric oxygenation in cerebral anoxi Neurol., 14: 15–20.

Williams, C.D. and Haugaard, N. (1970) toxic effects of oxy cerebral metabolism. J. Neurochem., 17: 709–720.

Nighoghossian N, Trouillas P, Adeleine P, Salord F.: Hyperbaric oxygen in the treatment of acute ischemic stroke. A double-blind pilot study. Stroke. 1995 Aug;26(8):1369-72

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BACKGROUND AND PURPOSE:
The effects of hyperbaric oxygen (HBO) therapy on humans are uncertain. Our study aims first to outline the practical aspects and the safety of HBO treatment and then to evaluate the effect of HBO on long-term disability.
METHODS:
Patients who experienced middle cerebral artery occlusion and were seen within 24 hours of onset were randomized to receive either active (HBO) or sham (air) treatment. The HBO patients were exposed daily to 40 minutes at 1.5 atmospheres absolute for a total of 10 dives. We used the Orgogozo scale to establish a pretreatment functional level. Changes in the Orgogozo scale score at 6 months and 1 year after therapy were used to assess the therapeutic efficacy of HBO. In addition, we used the Rankin scale and our own 10-point scale to assess long term-disability at 6 months and 1 year. Two sample t tests and 95% confidence intervals were used to compare the mean differences between the two treatment groups. Student's two-tailed test was used to compare the differences between pretherapeutic and posttherapeutic scores at 6 months and 1 year in the two treatment groups.
RESULTS:
Over the 3 years of study enrollment, **34 patients were randomized**, 17 to hyperbaric treatment with air and 17 to hyperbaric treatment with 100% oxygen. There was no significant difference at inclusion between groups regarding age, time from stroke onset to randomization, and Orgogozo scale scores. Neurological deterioration occurred during the first week in 4 patients in the sham group, 3 of whom died; this worsening was clearly related to the ischemic damage. Treatment was also discontinued for 3 patients inthe HBO group who experienced myocardial infarction, a worsening related to the ischemic process, and claustrophobia. Therefore, 27 patients (13 in the sham group and 14 in the HBO group) completed a full course of therapy. The mean score of the HBO group was significantly better on the Orgogozo scale at 1 year ($P < .02$). However, the difference at 1 year between pretherapeutic and posttherapeutic scores was not significantly different in the two groups ($P < .16$). Moreover, no statistically significant improvement was observed in the HBO group at 6 months and 1 year according to Rankin score ($P < .78$) and our own 10-point scale ($P < .50$).
CONCLUSIONS:
Although the small number of patients in each group precludes any conclusion regarding the potential deleterious effect of HBO, we did not observe the major side effects usually related to HBO. Accordingly, it can be assumed that hyperbaric oxygen might be safe. We hypothesize that HBO might improve outcome after stroke, as we detected an outcome trend favoring HBO therapy. A large randomized trial might be required to address the efficacy of this therapy.

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For a large number of patients with stroke no therapeutic option can be offered, even after approval of thrombolytic therapy for treatment of acute ischemic stroke in the US. In cerebral ischemia local anoxia and energy failure lead to further cellular damage and finally to complete stroke. All therapeutic concepts try to salvage structurally intact tissue which is at risk for irreversible damage (so-called penumbra). Hyperbaric oxygen (HBO) treatment has been reported in animal models of cerebral ischemia, and in a few clinical reports. **In general, the results of these studies have been promising.** This review focuses on the clinical perspective of HBO therapy and summarizes both the clinical and experimental data available on HBO therapy following ischemic stroke.

Fischer BR, Palkovic S, Holling M, Wölfer J, Wassmann H.: Rationale of hyperbaric oxygenation in cerebral vascular insult. Curr Vasc Pharmacol. 2010 Jan;8(1):35-43

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Cerebrovascular diseases and especially ischemic stroke are a leading cause of death. They occur mostly due to an insufficient oxygen (O2) supply to the central neural tissue as a result of thromboembolic events and/or obstructive vessel disease. The primary damage of the brain tissue cannot be restored. However, adequate therapy could minimize secondary impairment of brain tissue and restore neuronal function in the so-called "penumbra region". Apart from reopening occluded vessels, additional O2 supply is essential for survival of malfunctioning neural tissue. Breathing of 100% O2 under hyperbaric conditions, hyperbaric oxygenation (HBO), is the only method to increase the O2 concentration in tissue with impaired blood supply. Experimental as well as clinical studies have reported a positive effect of HBO therapy. Survival rate has increased under HBO therapy and neurological outcome has improved. The optimal levels of pressure as well as duration and numbers of HBO sessions need to be specified to avoid undesirable effects. Unfortunately, many questions remain unanswered before routinely recommending HBO as additional therapy in clinical practice. In this review we consider the (patho)physiological background of HBO-therapy, the latest results of experimental and clinical studies and stress the evidence in patients with cerebrovascular disease.

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HYPERBARIC OXYGEN THERAPY (HBO2) IN THE TREATMENT OF ACUTE HYPOXIC ENCEPHALOPATHIES

Sanchez EC, Schmitz G, Nochetto M, Medina A, Suarez A, Gomez A, Uribe R. Hospital Angeles Del Pedregal, Mexico D.F., Mexico.

BACKGROUND: Acute hypoxic encephalopathies are frequent, important and devastating lesions. The acute treatment may improve the outcome of patients. Hyperbaric oxygenation has been proposed to help salvage tissue when used early.

Purpose: To determine the value of HBO2 in the treatment of acute hypoxic encephalopathies.

MATERIALS AND METHODS: A MEDLINE search, using as keywords hyperbaric oxygen/oxygenation and brain/head/neurological encephalopathies, was conducted to review the clinical literature regarding this subject.

RESULTS: A total of 1709 articles were retrieved, from these in humans: 11 double blind, 15 prospective controlled and 24 prospective. An evidenced based medicine approach (AHA) was used to analyze these articles.

CONCLUSIONS: Hyperbaric oxygenation appears to be beneficial in the acute treatment of hypoxic encephalopathies. Even with the vast favorable results published, adequate scientific articles based on evidence need to be produced to prove its real value in the treatment of acute hypoxic encephalopathies.

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Research Article

Long Course Hyperbaric Oxygen Stimulates Neurogenesis and Attenuates Inflammation after Ischemic Stroke

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Several studies have provided evidence with regard to the neuroprotection benefits of hyperbaric oxygen (HBO) therapy in cases of stroke, and HBO also promotes bone marrow stem cells (BMSCs) proliferation and mobilization. This study investigates the influence of HBO therapy on the migration of BMSCs, neurogenesis, gliosis, and infiammation after stroke. Rats that sustained transient middle cerebral artery occlusion (MCAO) were treated with HBO three weeks or two days. The results were examined using a behavior test (modified neurological severity score, mNSS) and immunostaining to evaluate the effects of HBO therapy on migration of BMSCs, neurogenesis, and gliosis, and expression of neurotrophic factors was also evaluated. There was a lower mNSS score in the three-week HBO group when compared with the two-day HBO group. Mobilization of BMSCs to an ischemic area was more improved in long course HBO treatments, suggesting the duration of therapy is crucial for promoting the homing of BMSCs to ischemic brain by HBO therapies. HBO also can stimulate expression of trophic factors and improve neurogenesis and gliosis. These effects may help in neuronal repair after ischemic stroke, and increasing the course of HBO therapy might enhance therapeutic effects on ischemic stroke.

1. Introduction

Ischemic stroke is characterized by the interruption of blood flow and oxygen to brain tissues [1]. During focal ischemia, tissue surrounding the ischemic core is called penumbra, which is still viable and is a possible target to be rescued [2]. The only effective treatment approved in clinical practice at present is early thrombolytic therapy and reperfusion. However, most patients with ischemic stroke failed to receive proper management in time. Stroke remains an important cause of death and disability for humans and stroke therapy remains an important health issue today.

Hyperbaric oxygen (HBO) has been used as a primary or adjunctive stroke therapy over years. Mechanism of the neuroprotection of HBO treatment after ischemia was thought to be mediated by improving oxygen supply [3]. A good body of evidence suggests that HBO treatment is neuroprotective. HBO treatment can decrease infarction volume on MRI examination and improve neurological outcome [4]. Hyperbaric oxygen was also found to decrease ischemia-reperfusion injury induced by neutrophil [5]. Researchers have also demonstrated that exposure to HBO will cause rapid mobilization of bone marrow stem cells in humans, and the number of bone marrow stem cells (BMSCs) remains elevated in peripheral blood during the course of HBO treatments [6].

BMSCs transplantation in rats has been shown to improve outcome ofvarious neuronal diseases, such as ischemic stroke [7], spinal cord injury [8], and traumatic brain injury [9]. However, the "homing" of BMSCs is very important in regenerative therapy. In this study, we tested the hypothesis that HBO could promote the mobilization and migration of

BMSCs to ischemic brain, attenuating ischemic injury, and improving functional recovery. We evaluated the therapeutic effects of HBO on transient middle cerebral artery occlusion (MCAO). Further we tested the hypothesis that BMSCs would improve neurogenesis, gliosis, neurotrophic factor (brain-derived neurotrophic factor, BDNF; nerve growth factor, NGF; and glial-derived neurotrophic factor, GDNF) level, and the expression of MPO (presenting neutrophil activity).

2. Materials and Methods

2.1. Animal. Adult male Sprague-Dawley rats weighing 270 g to 320 g were used in these experiments. They were on a 12-hour light/dark cycle and allowed free access to food and water. All experimental procedures followed the instructions of the Taiwan National Science Council and were approved by the National Cheng Kung University Animal Care and Use Committee with every effort being made to minimize discomfort during the surgery and recovery period. After MCAO insult or Sham procedure, the rats were subjected to hyperbaric oxygen (HBO) therapy or the normal oxygen condition according to group destination. The rats were randomly assigned to one of four groups: (1) Sham group: rats received surgical procedure but without MCAO; (2) nontreatment group: rats sustained MCAO but without HBO treatments; (3) HBO2ds: rats sustained MCAO and received two days HBO treatment course, sacrificed on day 21; (4) HBO3wks: rats sustained MCAO, and received 15-days HBO (5 days/week) course, sacrificed on day 21. The flow charts are represented in Figure 1. Food and water were freely available ad libitum throughout the experimental course.

2.2. Middle Cerebral Artery Occlusion. Rats were anesthetized with intraperitoneal (ip) injection of ketamine (2 mg/100 g). Transient middle cerebral artery occlusion [10–12] was induced using the method of intraluminal vascular occlusion. Temperature was continuously monitored with a rectal probe and maintained at 37.0°C with a thermostatically controlled heating pad. Briefly, the right common carotid artery, external carotid artery (ECA), and internal carotid artery (ICA) were exposed. A 4–0 monofilament nylon suture, with its tip rounded by heating near a flame, was advanced from the ECA into the lumen of the ICA until it blocked the origin of the MCA. One hour after MCAO, reperfusion was achieved by withdrawal of the suture until the tip cleared the lumen of the ECA.

2.3. HBO Therapy. HBO sessions were conducted as the following procedures. Each rat was put into HBO chamber after reperfusion, then was treated with 100% oxygen at 253 kPa (2.5 atm) for 90 mins. The chamber filled with pure oxygen (100%) was pressurized to 253 kPa at a rate of 51 kPa/min for 90 mins and was terminated at the decompression rate of 20 kPa/min.

2.4. Assessing Cerebral Infarction and Functional Outcome. Functional outcome was evaluated using modified neurological severity score (mNSS) [13]. The rats were evaluated by several tests, such as raising rat by tail, placing rat on floor, and beam balance walking, then all test scores were added into the mNSS score (as shown in Table 1). The mNSS evaluations were performed before MCAO and at 1, 7, 14, and 21 days after MCAO. In addition, the triphenyltetrazolium chloride (TTC) staining [14] was used to check the brain infarction size. The colorless TTC is reduced to a red formazan product by dehydrogenases, which are most abundant in mitochondria [15]. Rats were sacrificed at day 3 and day 21. Due to TTC staining is a function test of dehydrogenase enzyme activity and is usually used for early histochemical diagnosis of infraction [16]. Therefore, rats were sacrificed at day 3 to check infraction change by TTC. After documenting mNSS score till day 21, rats were all sacrificed on day 21, then to do immunochemical staining. Under deep anesthesia (Sodium pentobarbital, 100 mg/kg, ip) rats were perfused intracardially with saline. The brain tissue was then removed, immersed in cold saline for 5 min, and sliced into 2.0 mm sections. The brain slices were incubated in 2% TTC dissolved in Phosphate buffered saline (PBS) for 30 min at 37°C and then transferred to 5% formaldehyde solution for fixation.

2.5. BrdU Labeling. BrdU (Roche), a thymidine analogue that is incorporated into the DNA of dividing cells during S phase, was used for mitotic labeling. The labeling protocol followed those previously described [17]. A cumulative labeling method was used to examine the population of proliferative cells, with the rat receiving daily intraperitoneal injections of 50 mg/kg BrdU for 15 consecutive days, starting on the first day of HBO. The $BrdU^+$ cells in both hemispheres of the hippocampus and cortex were digitally counted with the use of a 20x objective lens with a laser scanning confocal microscope (LSM510; Carl Zeiss MicroImaging, Inc.) via a computer imaging analysis system (Imaging Research). For each animal, 40 coronal sections (each 12 ym thick) throughout the hippocampus and cortex were analyzed. The image analysis was also used to examine the distributions of $BrdU^+$ cells with Neu-N and GFAP.

2.6. Immunohistochemistry. Animals were allowed to survive for 21 days after MCAO, and at that time animals were sacrificed with urethane (1.5 g/kg, IP). Rat brains were flxed by transcardial perfusion with saline, followed by perfusion and immersion in 4% paraformaldehyde. The brains were removed and then immersed in PBS with 15% and 30% sucrose overnight. The indirect lesion area (the intact area of the ipsilateral hemisphere) was subtracted from the area of the contralateral hemisphere and was calculated. The lesion volume is presented as a volume percentage of the lesion compared with the contralateral hemisphere.

Single or double immunofluorescence staining was used to identify cells derived from BMSCs. For staining, adjacent 12 ym thick sections were consecutively (1) 2 mol/L HCl-incubated for 30 minutes, (2) rinsed with 0.1 mol/L boric acid (pH 8.5) at room temperature for 10 minutes, (3) incubated overnight with primary antibodies in PBS containing 0.5% normal donkey serum at 4°C, and (4) incubated at room temperature for 1 hour with secondary antibodies.

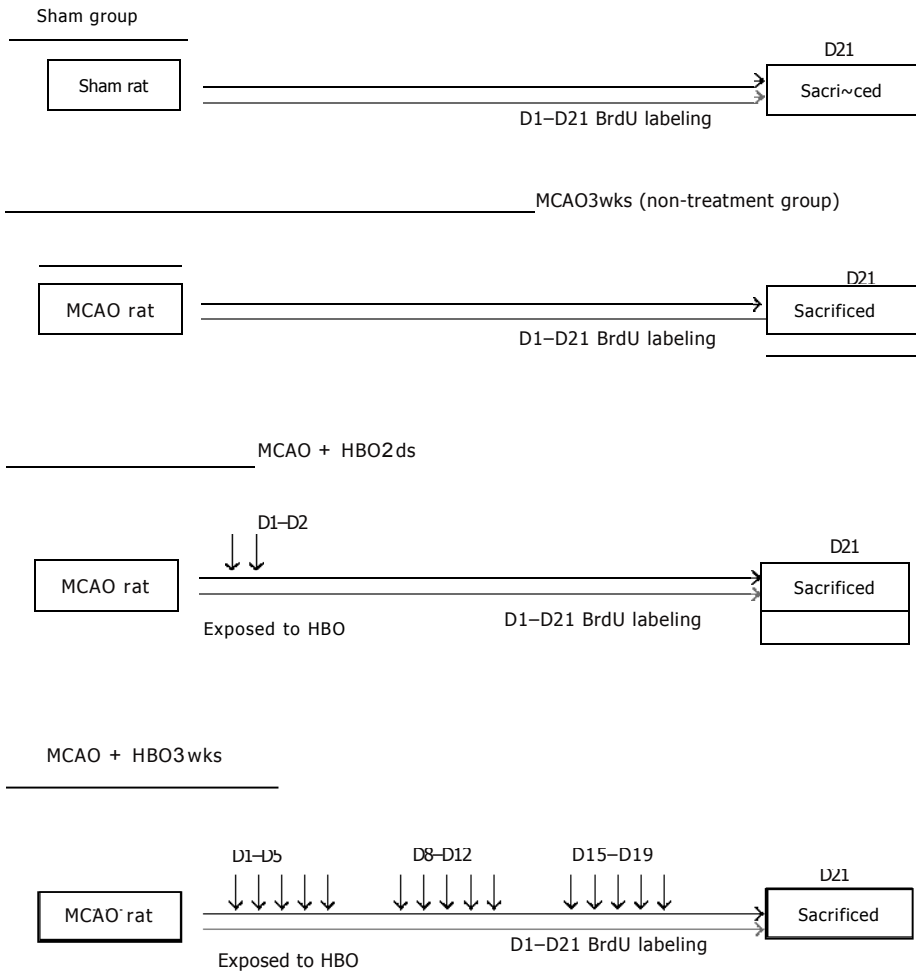


FiGuRE 1: Demonstration of HBO treatment protocol in different groups.

The antibodies were, sequentially, rat monoclonal anti-BrdU (abcam, 1:200), rat monoclonal anti-CD34 (Biolegend, 1:200), mouse monoclonal anti-NeuN (Milipore, 1:200), rabbit polyclonal anti-GFAP (Milipore, 1:1000), rabbit polyclone anti-Myeloperoxidase (abcam, 1:100), DAPI (Sigma, 1:1000), Alexa Flour 488-conjugated goat anti-mouse antibodies, Alexa Flour 488-conjugated donkey anti-rabbit antibodies, Alexa Flour 488-conjugated donkey anti-mouse antibodies, and the Alexa Flour 594-conjugated donkey anti-rat antibodies (Invitrogen). The sections without BrdU were then incubated with DAPI and coverslipped the mounting medium (fluorescent mounting medium; Dako).

2.7. Laser Scanning Confocal Microscopy and Cytometry. Colocalization of BrdU with neuronal marker was conducted by laser scanning confocal microscopy (LSCM) with the use of a Bio-Rad MRC 1024 (argon and krypton) laser scanning confocal imaging system mounted onto a Zeiss microscope (Bio-Rad). For immunofluorescence double-labeled coronal sections, green (FITC for NeuN and GFAP) and red cyanine-5.18 (Cy5 for BrdU) fluorochromes on the sections were excited by a laser beam at 488 nm and 647 nm; emissions were sequentially acquired with 2 separate photomultiplier tubes through 522 nm and 680 nm emission filters, respectively. Interested cells were counted with tissue cytometry using TissueQuest software.

2.8. Reverse Transcription PCR. Hippocampi from Sham, MCAO, HBO2ds, and HBO3wks were dissected. Total RNAs were isolated using TRIzol reagent (Invitrogen) according to manufacturer's instructions. Reverse transcription of equal amounts of total RNA were carried out using Superscript II First-Strand Synthesis kit (Invitrogen) according to the manufacturer's instructions. Obtained cDNA were amplified using the following primers: for BDNF, 5'-CAGTGGACATGTCCGGTGGGACGGTC-3' and 3'-TTC-TTGGCAACGGCAACAAACCACAAC-5'; for GDNF, 5'-AGGGGCAAAAATCGGGGGTG-3' and 3'-GCATGC-ATCCACGACTCGGA-5'; and for GAPDH, 5'-GACCCC-TTCATTGACCTCAAC-3' and 3'-TCTTACTCCTTGGAG-GCCATG-5'.

2.9. Statistical Analysis. Results are expressed as the mean ± SE for three or more independent experiments. To compare data, we used the ANOVA test. A value of $P < 0.05$ was considered to be statistically significant.

TABLE 1: Detail description of the items forming the modified neurological severity score (mNSS).

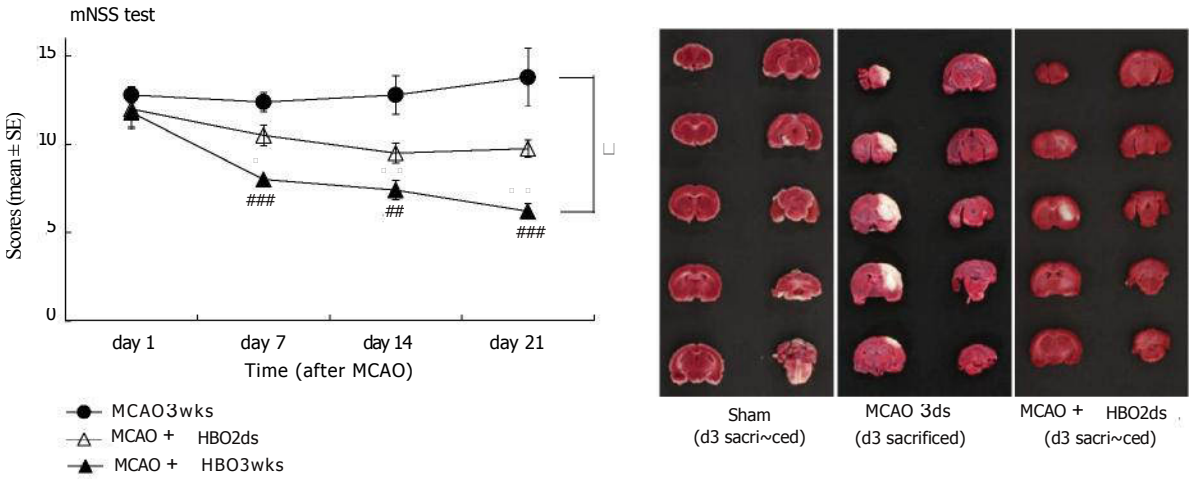
Motor tests	
Raising rat by tail (normal = 0, maximum = 3)	(3)
Flexion of forelimb	1
Flexion of hindlimb	1
Head moved >10 degree limb vertical axis within 30s	1
Placing rat on floor (normal = 0, maximum = 3)	(3)
Normal walk	0
Inability to walk straight	1
Circling toward the paretic side	2
Falling down to paretic side	3
Sensory tests (normal = 0, maximum = 2)	(2)
Placing test (visual and tactile test)	1
Proprioceptive test (deep sensation, pushing paw against table edge to stimulate limb muscles)	1
Beam balance tests (normal = 0, maximum = 6)	(6)
Balance with steady posture	0
Grasps side of beam	1
Huging beam and 1 limb falling down from beam	2
Huging beam and 2 limbs falling down from beam, or spins on beam (>60 s)	3
Attempting to balance on beam but falling off (>40 s)	4
Attempting to balance on beam but falling off (>20 s)	5
Falling off; no attempt to balance or hang on the beam (<20 s)	6
Reflex absence and abnormal movements (normal = 0, maximum = 4)	(4)
Pinna reflex (head shaken when auditory meatus is touched)	1
Corneal reflex (eyes blink when cornea is lightly touched with cotton)	1
Startle reflex (motor response to brief noise from clapping hands)	1
Seizures, myoclonus, myodystony	1
Maximum points	(18)

One point is given for an absent reflex tested or for the animal's inability to perform a task: 1–6 mild injury, 7–12 moderate injury, and 13–18 severe injury

3. Results

3.1. HBO Improved Functional Outcome and Decreased Infarction Size. HBO therapy outcomes in rats were evaluated using the modified neurological severity score (mNSS). A lower score indicated rats had less neurological defects from MCAO, presented with more improved outcome by HBO therapy. Behavior tests showed rats had significantly improved functional outcome when receiving longer repetitive HBO therapy ($P < 0.001$). Despite there only being two days of HBO therapy in the HBO2ds group, rats still showed functional improvement in the following days, with expressing declining curve of mNSS, until day 14 ($P < 0.01$). And the declining curve of mNSS in the HBO3wks group became more obvious after day 14 ($P < 0.01$) (Figure 2(a)). TTC staining showed the ischemic area on the rat's brain tissue. More white color change over brain tissue was found in the MCAO group, which was correlated with more ischemic injury, compared with other groups (Figure 2(b)). Behavior tests were evaluated with mNSS: MCAO3wks, rats sustained MCAO, but without HBO therapy; HBO2ds, rats sustained MCAO, but with two days HBO therapy; HBO3wks, rats sustained MCAO, but with repetitive HBO therapy for three weeks. The infarcted area showed TTC staining (white color) was prominent in the MCAO group, but decreased in HBO treated groups. This indicated that HBO might attenuate cerebral ischemic injury in rats (Figure 2).

3.2. HBO Improved BMSCs Migration to Brain. CD34-DAPI double staining showed the presentation of BMSCs in brain tissue. The number of CD34-DAPI double staining cells was higher in the HBO3wks group, as compared with the shame group, MCAO3wks group, and HBO2ds group (Figure 3(a)). This indicated that rats would recruit BMSCs to brain after acute stroke injury, without HBO therapy. However, the recruited amount of BMSCs was not enough. HBO therapy promotes migration of BMSCs to brain after focal ischemic injury. Longer duration and repetitive HBO would enhance increased BMSCs migration (Figures 3(b) and 3(c)). Migrated BMSC, presenting with double staining of CD34 and DAPI, were found in the MCAO3wks group, HBO2ds group, and



(a) (b)

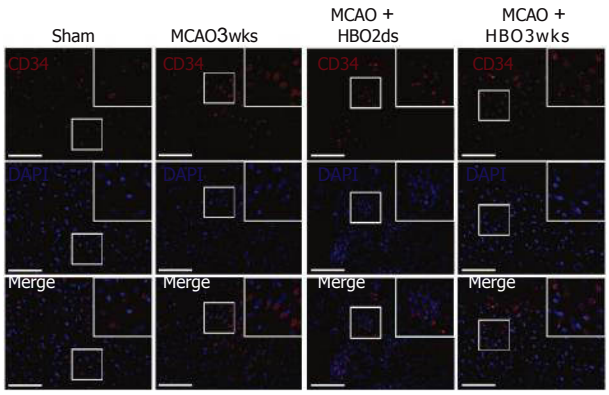
FiGuRE 2: Long course HBO improved functional outcome and decreased infarction size. (a) Behavior tests showed HBO significantly improved function outcome with dose-dependent effect. The mNSS in groups received HBO therapies is significantly less than that in control group ($^{***}P < 0.001$). The declining curve of mNSS in the HBO3wks group became more obviously after day 14. (b) Infarcted area shown on TTC staining (white color) was prominent in the MCAO group but decreased in HBO treated groups.

HBO3wks group. And there were increased numbers of purple cells (double staining with CD34, red color, and DAPI, blue color) in the HBO3wks group. Representative images of tissue cytometry using TissueQuest software are shown in Figure 3(b). CD-34 positive cells and DAPI positive cells were counted and the signal intensity was quantified. The number of double staining cells with CD34 and DAPI was 14.16% in the Sham group, 17.09% in the MCAO3wks group, 21.99% in the HBO2ds group, and 39.4% in the HBO3wks group, respectively. The amount of double positive cells with CD34 and DAPI in the ischemic boundary was recorded as the percentage of CD34 positive cells in all cells. Data were presented as mean± standard error of the mean (SEM). The difference was significant as compared with MCAO3wks group and HBO3wks group ($P < 0.05$). The difference was even more significant as compared with Sham group and HBO3wks group ($P < 0.01$).

3.3. HBO Increased MCAO-Induced Neurogenesis. BrdU and Neu-N double staining cells showed the number of new proliferation of neuronal cells in the perilesioned cortex and hippocampus (Figures 4(a) and 4(b)). The BrdU positive cells presented with a red color and the Neu-N positive cells were presenting with a green color. The double staining of BrdU and NeuN cells would present with a yellow color. There were more yellow-colored cells (indicating new proliferated neuronal cells) found in the HBO2ds group and HBO3wks group. However, the number of double staining cells (yellow color) was higher in the HBO3wks group. This feature indicated HBO improved neurogenesis, but longer and repetitive HBO course would induce a greater degree of neurogenesis.

BrdU-NeuN double staining showed that there were significantly more newly formed neurons (cells with yellow color) in the ischemic boundary area of hippocampus (Figure 4(a)) and perilesioned cortex (Figure 4(b)). There were more BrdU and NeuN double staining cells in the HBO3wk group. Figure 4(c) showed the representative image of tissue cytometry using TissueQuest software. BrdU positive cells and Neu-N positive cells were counted and the signal intensity was quantified. The number of double staining cells with BrdU and Neu-N was 10.43% in the Sham group, 15.63% in MCAO3wks group, 22.81% in HBO2ds group, and 34.95% in HBO3wks group, individually. The amount of double staining positive cells with BrdU and Neu-N in the ischemic boundary was recorded as the percentage of BrdU positive cells in all cells. Data were presented as mean± standard error of the mean (SEM). The difference was significant, as compared with the MCAO3wks group and HBO3wks group ($P < 0.01$). There was still a difference between the HBO2ds group and HBO3wks group ($P < 0.05$). The difference was more significant, as compared with the Sham group and HBO3wks group ($P < 0.001$).

3.4. HBO Increased MCAO-Induced Gliosis. BrdU and GFAP double staining cells showed the number of new proliferations of glial cells in the dentate gyrus and perilesioned cortex (Figures 5(a) and 5(b)). The BrdU positive cells were presented with red color and the GFAP positive cells were presented as a green color. The double staining of BrdU and GFAP cells would present as a yellow color. There were yellow-colored cells (indicating new proliferated glial cells) found in the MCAO3wks group (24.61%), HBO2ds group (22%), and HBO3wks (30.93%) group. However, the number of double staining cells (yellow color) seemed higher in the HBO3wks group. This suggests that longer and repetitive HBO course would induce a greater degree of gliosis. Demonstrated figures of BrdU-GFAP double staining cells showed that there were more newly forming or reactive glia in



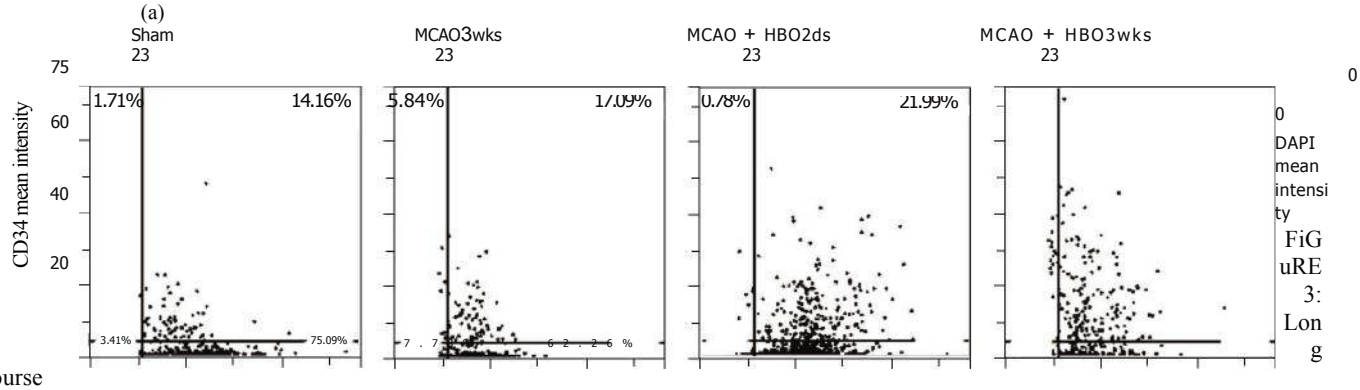
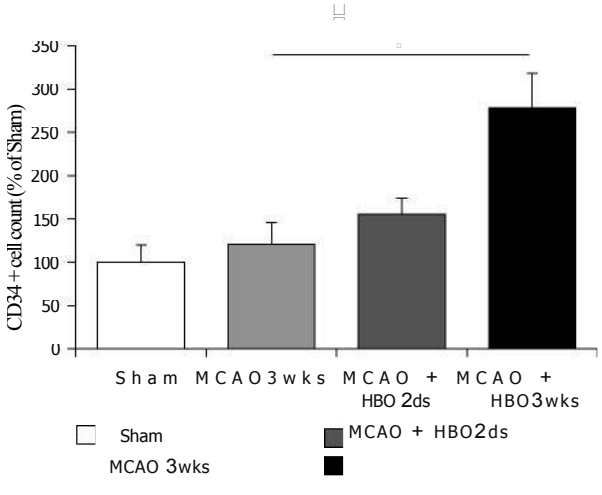


FiGuRE 3: Long course HBO improved BMSCs migration to brain. (a) Demonstration of CD34-DAPI double staining showed presentation of BMSCs after brain ischemia. (b) Representative image of tissue cytometry using TissueQuest software. CD-34 positive cells and DAPI positive cells were (t



(c

counted andthe signal intensity was quantified. (c) The amount of double positive cells with CD34 and DAPI in the ischemic boundary was recorded as the percentage ofCD34 positive cells in all cells. The difference was significant, as compared with MCAO3wks group and HBO3wks group ($^{*}p < 0.05$). The difference was more significant, as compared with Sham group and HBO3wks group ($^{**}p < 0.01$).

the ischemic boundary area (dentate gyrus (Figure 5(a)) and cortex (Figure 5(b))). And there were more double staining cells (yellow colored cells) in the HBO3wks group. The amount of BrdU and GFAP double staining positive cells in the ischemic boundary was recorded as the percentage of BrdU positive cells in all cells. Data were presented as mean ± standard error of the mean (SEM). Despite there being more double staining cells found in the HBO3wks group, there was

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