Cocktail treatment, a promising strategy to treat acute cerebral ischemic stroke?

Li-jun Liang[1, #], Jin-ming Yang[1, #], Xin-chun Jin[2, *]

1 Children's Hospital of Shanxi Province, Taiyuan, Shanxi Province, China
2 Jiangsu Key Laboratory of Translational Research and Therapy for Neuro-Psycho-Diseases and Institute of Neuroscience, The Second Affiliated Hospital of Soochow University, Soochow University, Suzhou, Jiangsu Province, China
#These two authors contributed equally to this work.
*Correspondence to: Xin-chun Jin, M.D., xin-chunjin@gmail.com.
orcid: 0000-0002-5351-7914

Abstract

Up to now, over 1,000 experimental treatments found in cells and rodents have been difficult to translate to human ischemic stroke. Since ischemia and reperfusion, two separate stages of ischemic stroke, have different pathophysiological mechanisms leading to brain injury, a combination of protective agents targeting ischemia and reperfusion respectively may obtain substantially better results than a single agent. Normobaric hyperoxia (NBO) has been shown to exhibit neuro- and vaso-protective effects by improving tissue oxygenation when it is given during ischemia, however the effect of NBO would diminish when the duration of ischemia and reperfusion was extended. Therefore, during reperfusion drug treatment targeting inflammation, oxidative stress and free radical scavenger would be a useful adjuvant to extend the therapeutic window of tissue plasminogen activator, the only United States Food and Drug Administration (FDA) approved treatment for acute ischemic stroke. In this review, we discussed the neuro- and vaso-protective effects of NBO and recent finding of combining NBO with other drugs.

Key words: blood-brain barrier; combination therapy; normobaric hyperoxia; ischemic stroke; tissue plasminogen activator

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Introduction

Acute ischemic stroke happened when blood vessels were suddenly occluded by a thrombus or embolism followed by the immediate lack of oxygen and glucose to the brain (Lakhan et al., 2013). A major goal for the treatment of acute ischemic stroke is to promote arterial recanalization to salvage the ischemic penumbra (Ramos-Cabrer et al., 2011), which is a region of ischemic brain tissue with sufficient energy for short-term survival (Hakim, 1998). If ischemia persists, the penumbra will progress to permanent damage, primarily driven by ischemia-induced hypoxia and the subsequent energy failure (Dirnagl et al., 1999). This penumbra concept has inspired the development of two main categories of drugs to treat stroke in the acute phase: drugs interrupting cell death pathways (Reza Noorian et al., 2011) and drugs inducing reperfusion in the ischemic zone (Barreto and Alexandrov, 2012). Neuro-protection alone without restoration of tissue perfusion and vascular integrity may not be adequate for treatment of acute stroke (Zhang et al., 2012). However, when reperfusion is attained, additional irreversible damage can also develop following reperfusion due to a mechanism of reperfusion injury including inflammation, oxidative stress and excitatory injury (Schaller and Graf, 2004; Pan et al., 2007). Therefore, combination therapy targeting both ischemia- and reperfusion-induced nerve and blood vessel damage is needed.

Effect of normobaric hyperoxia (NBO) treatment on ischemic stroke

Improving tissue oxygenation has been studied for many years as a simplistic but plausible treatment strategy to reduce ischemic injury. NBO has been suggested as a practical

acute-phase treatment to slow down the onset of irreversible injury of the penumbra, thus potentially allowing for delayed or more effective treatment as it is readily available, safe and can be initiated promptly after stroke onset by paramedics (Kim et al., 2005; Henninger and Fisher, 2006; Liu et al., 2009a). When used as a monotherapy, NBO preserves the ischemic penumbra and reduces ischemic lesion volumes (Singhal et al., 2005; Chiu et al., 2006; Liu et al., 2006; Henninger et al., 2007; Shin et al., 2007; for review see Qi et al., 2013). The effect is attributed to the ability of NBO to improve energy metabolism in the ischemic penumbra, as evidenced by elevated interstitial oxygen partial pressure (Liu et al., 2006), increased cerebral blood flow and O_2 delivery (Shin et al., 2007), and reductions in acidosis and ATP deletion (Sun et al., 2011). More importantly, oxygen therapy does not increase production and damage induced by reactive oxygen species in focal cerebral ischemia (Sun et al., 2014). However, NBO-afforded early cerebral protection could diminish over time during reperfusion (Kim et al., 2005; Esposito et al., 2013), the mechanism of ischemia/reperfusion damage to the blood-brain barrier (BBB) may account for this phenomena (Aronowski et al., 1997).

Effect of NBO on preservation of the BBB integrity and the extension of the thrombolytic time window

BBB damage and the strategy to protect vascular integrity

Over decade, monotherapy focusing on only neurons has not yielded promising results for human stroke. Expanding the research focus to include other cells and matrix may obtain a more comprehensive and realistic view of treatment of human brain injury (Lo et al., 2004).

The presence of a salvageable penumbra has been widely agreed as the premise for thrombolytic therapy (Foley et al., 2010), however, BBB integrity is considered to be central to the risks of vasogenic edema and hemorrhage transformation (HT), which are the most feared complication of thrombolytic therapy (Del Zoppo et al., 2009). BBB damage is a progressive process, with an initial injury resulting from ischemia (Simard et al., 2007; del Zoppo, 2013), aggravated by reperfusion (Jung et al., 2010). Due to direct contribution to edema and HT, reperfusion-associated BBB injury has been a topic of intensive investigation, which leads to the identification of several mechanisms accounting for reperfusion BBB injury, such as oxidative stress damage due to increased free radical generation (Liu and Rosenberg, 2005), inflammatory injury (Schofield et al., 2013), vascular activation and dysregulated extracellular proteolysis (Rosenberg and Yang, 2007; Jung et al., 2010; Moskowitz et al., 2010).

Ischemia-induced BBB damage in the early stroke stages is increasingly appreciated to negatively impact the safety and efficacy of thrombolytic therapy for ischemic stroke (Jin et al., 2014). In the early stage of acute stroke, ischemia-induced hypoxia and the subsequent bioenergetic failure induced irreversible damage to the penumbra, therefore, providing O_2 as soon as possible (through NBO treatment) to alleviate the ischemia induced brain injury and extend the time window of tPA treatment.

Tissue-type plasminogen activator (tPA) thrombolysis and HT

tPA dissolves blood clots to restore blood flow to the ischemic brain region and salvage the ischemic brain tissue (Wardlaw et al., 2012; Chapman et al., 2014). Until now, thrombolysis with tPA within 3 or 4.5 hours after symptom onset is the only FDA approved treatment for acute ischemic stroke (Hacke et al., 2008; Del Zoppo et al., 2009). However, brief therapeutic window and the high incidence of HT have profoundly constrained the clinical use of tPA in ischemic stroke patients.

Effect of NBO on extension of tPA thrombolytic window

Since NBO treatment during cerebral ischemia significantly alleviates BBB disruption and reduces edema formation (Liu et al., 2008; Liu et al., 2009b) and delaying thrombolytic therapy dramatically increases the risk of hemorrhage (Hatcher and Starr, 2011), it is rational to combine NBO with tPA to reduce thrombolysis reperfusion-associated complications (Liu et al., 2009a; Sun et al., 2011).

The potential of the combination NBO treatment with tPA thrombolysis was recognized several years ago (Henninger and Fisher, 2006; Fujiwara et al., 2009). A previous study has demonstrated that a combination treatment with NBO and delayed tPA treatment at 4.5 hours after stroke onset significantly reduces tPA-associated mortality, alleviates cerebral edema following hemorrhage, and decreases MMP-9 augmentation in a rat model of stroke (Liu et al., 2009a). It has been reported that a combination therapy of NBO and tPA does not increase hemorrhage volume at 10 hours or occurrence of confluent petechial hemorrhages at 24 hours in a rat model of thromboembolic stroke (Henninger et al., 2009). NBO can increase the safety of delayed tPA thrombolysis in stroke (Liu et al., 2009a), extend the time window for tPA, but not increase superoxide generation or MMP-9 in the brain (Kim et al., 2005). Therefore, co-administration of NBO to tPA is safe. NBO treatment shortly after ischemia onset and tPA therapy at a dealyed time point may represent a safe and effective strategy for acute stroke treatment (Henninger et al., 2009).

Recently, Liang et al. (2015) showed that early NBO treatment alleviated ischemic BBB damage and significantly improved the outcome of delayed tPA treatment, providing new evidence supporting NBO as an effective adjunctive therapy to expand the therapeutic time window of acute ischemic stroke for tPA.

Combination treatment with NBO

Until now, researchers have failed to translate over 1,000 experimental treatment methods in cells and rodents to therapy of humans (O'Collins et al., 2006). Since the monotherapy did not work as expected, combinational therapeutic approaches targeting different stages of acute stroke may be an ideal strategy (Rogalewski et al., 2006; Zhang et al., 2012).

NBO has been shown to effectively reduce tissue infarction and protect the BBB in animal ischemic stroke models (Singhal et al., 2002; Jin et al., 2013; Liang et al., 2015). The neuro- and vaso-protection make NBO a promising approach to expand the narrow time window of the reperfusion therapies for ischemic stroke (Henninger and Fisher, 2006; Liang et al., 2015). But its effect was diminished with extension of ischemia and reperfusion. Therefore, either combating inflammation, clearing the free radical, antagonizing glutamate, inhibiting the platelet accumulation, or reducing excessiveγ-aminobutyrie acid (GABA)-mediated tonic inhibition (ethonal treatment) will reduce the reperfusion-induced damage (Clarkson et al., 2010).

NBO plus minocycline

Inflammatory reactions occurring in the brain after ischemia may contribute to secondary damage to BBB and brain tissue (Wang et al., 2007). Minocycline, a tetracycline antibiotic, has been shown to protect against reperfusion-induced injury through its anti-inflammatory (Yrjanheikki et al., 1999), anti-apoptotic (Arvin et al., 2002; Friedlander, 2003), and BBB-protecting actions (Wang et al., 2002; Xu et al., 2004). Animal studies and early phase clinical trials showed that minocycline, even when administered at delayed time points, is neuroprotective (Xu et al., 2004; Hewlett and Corbett, 2006; Hayakawa et al., 2008; Fagan et al., 2010).

Jin and colleagues demonstrated that the combination of NBO and minocycline results in greater neuroprotective effects than each individual treatment in a rat model of transient focal cerebral ischemia, NBO plus minocycline effectively provides greater neuro- and vaso-protective effects than monotherapy by inhibiting matrix metalloproteinsase (MMP)-2/9-mediated occludin degradation and attenuation of caspase-dependent and independent apoptotic pathways (Jin et al., 2013).

NBO plus edaravone

Besides inflammation, reperfusion produces a lot of free radical and free radical could damage the brain (Liu and Rosenberg, 2005). An alternative strategy to reduce reperfusion induced injury is to clear the free radical. Edaravone, a potent scavenger of hydroxyl radicals, has been demonstrated to have beneficial effects when combined with NBO. Nonaka and colleagues showed that combination therapy of NBO plus edaravone showed more beneficial effects than monotherapy by preventing the neuronal damage after focal cerebral ischemia and reperfusion in mice. Their results showed significantly better neurological functions, less infarct volume as well as less TUNEL-positive cells in the ischemic boundary zone both in cortex and subcortex at 22 hours after reperfusion than the two monotherapy groups (Nonaka et al., 2008). These results suggest that combination therapy with NBO plus edaravone prevented the neuronal damage after focal cerebral ischemia and reperfusion in mice.

NBO plus melatonin

Melatonin is a potent free radical scavenger with excellent BBB permeability (Galano et al., 2014), which has been shown to protect brain against focal cerebral ischemia in both mice (Zou et al., 2006; Tai et al., 2010; Yang et al., 2014) and rats (Pei et al., 2003; Villapol et al., 2010; Jang et al., 2012). Therefore, melatonin may augment the survival-promoting action of NBO. Beker and colleagues have examined the effect of NBO treatment alone or in combination with melatonin after focal cerebral ischemia. They reported that combined NBO and melatonin synergistically reduces neuronal injury after mild focal cerebral ischemia induced by 30-minute MCAO. Combination therapy of NBO and melatonin regulated levels of phosphorylated Akt, antiapoptotic Bcl-xL, pro-apoptotic Bax and endothelial NO synthase, as well as reduced neuronal injury, neurological deficits, infarct volume and BBB permeability which has been found by NBO and particularly melatonin treatment alone (Beker et al., 2015).

NBO plus cilostazol

Cilostazol, a selective inhibitor of phosphodiesterase-3, has been reported to exert neuroprotection against acute brain injury after cerebral ischemia in rodents (Choi et al., 2002; Lee et al., 2003). Nonaka and colleagues demonstrated that after focal cerebral ischemia, acute and subacute lesion volumes were significantly reduced in the combination group but not in the two monotherapy groups. Compared to the monotherapy, the combination therapy increased endothelial nitric-oxide synthase (eNOS) activity in the lesion area after ischemia. Combination therapy with NBO plus cilostazol protected against focal cerebral ischemia/reperfusion injury in mice by improvement of regional cerebral blood flow (rCBF) after reperfusion, in part in association with eNOS activity (Nonaka et al., 2009).

NBO plus ethonal (EtOH)

EtOH consumption is inversely associated with the risk of ischemic stroke, suggesting a neuro-protection as a potential neuroprotectant for acute ischemic stroke (Wang

et al., 2012). Acute EtOH administration attenuated lactic acidosis in transient or permanent ischemic stroke. This EtOH-induced beneficial effect was potentiated by NBO therapy in permanent ischemia as shown by reduced cerebral monocarboxylate transporters and lactate levels by EtOH and NBO therapy in severe transient and permanent ischemic stroke (Geng et al., 2013a, b).

Acute EtOH treatment has been shown to reduce BBB dysfunction following ischemia/reperfusion injury (Zeng et al., 2012). Combination therapy with NBO and EtOH showed greater neuroprotection than monotherapy alone. Compared to stroke without treatment, monotherapy with NBO and/or EtOH demonstrated reductions in infarct volume and neurological deficits. The greatest neuro-protection was found in combination therapy group associated with the largest reductions in ADP/ATP ratios, reactive oxygen species levels, and nicotinamide adenine dinucleotide phosphate oxidase activity (Geng et al., 2013a, b).

Because both EtOH and NBO are readily available, inexpensive and easy to administer, their combination is attractive and could be implemented in the clinics shortly after stroke (Geng et al., 2013a, b).

NBO plus semifluorinated alkane (SFA)-containing emulsion (artificial oxygen carrier)

NBO preserves the ischemic penumbra and reduces ischemic lesion volume by improving tissue oxygenation (Singhal et al., 2005; Chiu et al., 2006; Liu et al., 2006; Henninger et al., 2007; Shin et al., 2007). The neuroprotective effects of NBO will be amplified if blood oxygen transport capacity could be enhanced. Seiffge and colleagues investigated the neuroprotective effects of increasing the blood oxygen transport capacity by applying an SFA-containing emulsion when transient focal ischemia rats received NBO treatment. Their results showed that oxygen delivery by an artificial oxygen carrier in conjunction with NBO is superior to NBO alone and combination treatment significantly reduced the infarction volume by alleviating the severity of hypoxia to a level sufficient to prevent cells from transition into irreversible damage. Administration of an SFA emulsion in conjunction with NBO is easily afforded in the pre-hospital phase. It may not only provide a significant neuroprotective effects but also expand the time window for ischemic stroke therapy (Seiffge et al., 2012).

Conclusion

Combination therapy with NBO plus drugs offers enhanced neuroprotective effects through cooperative intervention of both ischemia and reperfusion stages. As a result, NBO has the potential to not only expand the therapeutic time window for tPA administration, but also reduce subsequent reperfusion induced injury, thereby producing improved outcomes when combination therapy is used.

Abbreviations

BBB: blood-brain barrier; CBF: cerebral blood flow; eNOS: endothelial nitric-oxide synthase; FDA: Food and Drug Administration; HT: hemorrhage transformation; MCAO: middle cerebral artery occlusion; MMP-9: matrix metalloproteinase-9; NBO: normobaric hyperoxia; SFA: semifluorinated alkane; tPA: tissue plasminogen activator.

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Author contributions

LL, JY and XJ reviewed the NBO studies, XJ participated in the overall design of the review, LL, JY and XJ wrote the manuscript and XJ obtained the funding. All authors have read and approved the final version of the manuscript.

Conflicts of interest

The authors declare no competing financial interests.

References

Aronowski J, Strong R, Grotta JC (1997) Reperfusion injury: demonstration of brain damage produced by reperfusion after transient focal ischemia in rats. J Cereb Blood Flow Metab 17:1048-1056.

Arvin KL, Han BH, Du Y, Lin SZ, Paul SM, Holtzman DM (2002) Minocycline markedly protects the neonatal brain against hypoxic-ischemic injury. Ann Neurol 52:54-61.

Barreto AD, Alexandrov AV (2012) Adjunctive and alternative approaches to current reperfusion therapy. Stroke 43:591-598.

Beker MC, Caglayan AB, Kelestemur T, Caglayan B, Yalcin E, Yulug B, Kilic U, Hermann DM, Kilic E (2015) Effects of normobaric oxygen and melatonin on reperfusion injury: role of cerebral microcirculation. Oncotarget 6:30604-30614.

Chapman SN, Mehndiratta P, Johansen MC, McMurry TL, Johnston KC, Southerland AM (2014) Current perspectives on the use of intravenous recombinant tissue plasminogen activator (tPA) for treatment of acute ischemic stroke. Vasc Health Risk Manag 10:75-87.

Chiu EH, Liu CS, Tan TY, Chang KC (2006) Venturi mask adjuvant oxygen therapy in severe acute ischemic stroke. Arch Neurol 63:741-744.

Choi JM, Shin HK, Kim KY, Lee JH, Hong KW (2002) Neuroprotective effect of cilostazol against focal cerebral ischemia via antiapoptotic action in rats. J Pharmacol Exp Ther 300:787-793.

Clarkson AN, Huang BS, Macisaac SE, Mody I, Carmichael ST (2010) Reducing excessive GABA-mediated tonic inhibition promotes functional recovery after stroke. Nature 468:305-309.

del Zoppo GJ (2013) Plasminogen activators and ischemic stroke: conditions for acute delivery. Semin Thromb Hemost 39:406-425.

Del Zoppo GJ, Saver JL, Jauch EC, Adams HP Jr, American Heart Association Stroke Council (2009) Expansion of the time window for treatment of acute ischemic stroke with intravenous tissue plasminogen activator: a science advisory from the American Heart Association/American Stroke Association. Stroke 40:2945-2948.

Dirnagl U, Iadecola C, Moskowitz MA (1999) Pathobiology of ischaemic stroke: an integrated view. Trends Neurosci 22:391-397.

Esposito E, Mandeville ET, Hayakawa K, Singhal AB, Lo EH (2013) Effects of normobaric oxygen on the progression of focal cerebral ischemia in rats. Exp Neurol 249:33-38.

Fagan SC, Waller JL, Nichols FT, Edwards DJ, Pettigrew LC, Clark WM, Hall CE, Switzer JA, Ergul A, Hess DC (2010) Minocycline to improve neurologic outcome in stroke (MINOS): a dose-finding study. Stroke 41:2283-2287.

Foley LM, Hitchens TK, Barbe B, Zhang F, Ho C, Rao GR, Nemoto EM (2010) Quantitative temporal profiles of penumbra and infarction during permanent middle cerebral artery occlusion in Rats. Transl Stroke Res 1:220-229.

Friedlander RM (2003) Apoptosis and caspases in neurodegenerative diseases. N Engl J Med 348:1365-1375.

Fujiwara N, Murata Y, Arai K, Egi Y, Lu J, Wu O, Singhal AB, Lo EH (2009) Combination therapy with normobaric oxygen (NBO) plus thrombolysis in experimental ischemic stroke. BMC Neurosci 10:79.

Galano A, Medina ME, Tan DX, Reiter RJ (2015) Melatonin and its metabolites as copper chelating agents and their role in inhibiting oxidative stress: a physicochemical analysis. J Pineal Res 58:107-116.

Geng X, Parmar S, Li X, Peng C, Ji X, Chakraborty T, Li WA, Du H, Tan X, Ling F, Guthikonda M, Rafols JA, Ding Y (2013a) Reduced apoptosis by combining normobaric oxygenation with ethanol in transient ischemic stroke. Brain Res 1531:17-24.

Geng X, Fu P, Ji X, Peng C, Fredrickson V, Sy C, Meng R, Ling F, Du H, Tan X, Huttemann M, Guthikonda M, Ding Y (2013b) Synergetic neuroprotection of normobaric oxygenation and ethanol in ischemic stroke through improved oxidative mechanism. Stroke 44:1418-1425.

Hacke W, Kaste M, Bluhmki E, Brozman M, Davalos A, Guidetti D, Larrue V, Lees KR, Medeghri Z, Machnig T, Schneider D, von Kummer R, Wahlgren N, Toni D (2008) Thrombolysis with alteplase 3 to 4.5 hours after acute ischemic stroke. N Engl J Med 359:1317-1329.

Hakim AM (1998) Ischemic penumbra: the therapeutic window. Neurology 51:S44-46.

Hatcher MA, Starr JA (2011) Role of tissue plasminogen activator in acute ischemic stroke. Ann Pharmacother 45:364-371.

Hayakawa K, Mishima K, Nozako M, Hazekawa M, Mishima S, Fujioka M, Orito K, Egashira N, Iwasaki K, Fujiwara M (2008) Delayed treatment with minocycline ameliorates neurologic impairment through activated microglia expressing a high-mobility group box1-inhibiting mechanism. Stroke 39:951-958.

Henninger N, Fisher M (2006) Normobaric hyperoxia - a promising approach to expand the time window for acute stroke treatment. Cerebrovasc Dis 21:134-136.

Henninger N, Bouley J, Nelligan JM, Sicard KM, Fisher M (2007) Normobaric hyperoxia delays perfusion/diffusion mismatch evolution, reduces infarct volume, and differentially affects neuronal cell death pathways after suture middle cerebral artery occlusion in rats. J Cereb Blood Flow Metab 27:1632-1642.

Henninger N, Bratane BT, Bastan B, Bouley J, Fisher M (2009) Normobaric hyperoxia and delayed tPA treatment in a rat embolic stroke model. J Cereb Blood Flow Metab 29:119-129.

Hewlett KA, Corbett D (2006) Delayed minocycline treatment reduces long-term functional deficits and histological injury in a rodent model of focal ischemia. Neuroscience 141:27-33.

Jang JW, Lee JK, Lee MC, Piao MS, Kim SH, Kim HS (2012) Melatonin reduced the elevated matrix metalloproteinase-9 level in a rat photothrombotic stroke model. J Neurol Sci 323:221-227.

Jin X, Liu J, Liu W (2014) Early ischemic blood brain barrier damage: a potential indicator for hemorrhagic transformation following tissue plasminogen activator (tPA) thrombolysis? Curr Neurovasc Res 11:254-262.

Jin X, Liu J, Liu KJ, Rosenberg GA, Yang Y, Liu W (2013) Normobaric hyperoxia combined with minocycline provides greater neuroprotection than either alone in transient focal cerebral ischemia. Exp Neurol 240:9-16.

Jung JE, Kim GS, Chen H, Maier CM, Narasimhan P, Song YS, Niizuma K, Katsu M, Okami N, Yoshioka H, Sakata H, Goeders CE, Chan PH (2010) Reperfusion and neurovascular dysfunction in stroke: from basic mechanisms to potential strategies for neuroprotection. Mol Neurobiol 41:172-179.

Kim HY, Singhal AB, Lo EH (2005) Normobaric hyperoxia extends the reperfusion window in focal cerebral ischemia. Ann Neurol 57:571-575.

Lakhan SE, Kirchgessner A, Tepper D, Leonard A (2013) Matrix metalloproteinases and blood-brain barrier disruption in acute ischemic stroke. Front Neurol 4:32.

Lee JH, Lee YK, Ishikawa M, Koga K, Fukunaga M, Miyakoda G, Mori T, Hosokawa T, Hong KW (2003) Cilostazol reduces brain lesion induced by focal cerebral ischemia in rats--an MRI study. Brain Res 994:91-98.

Liang J, Qi Z, Liu W, Wang P, Shi W, Dong W, Ji X, Luo Y, Liu KJ (2015) Normobaric hyperoxia slows blood-brain barrier damage and expands the therapeutic time window for tissue-type plasminogen activator treatment in cerebral ischemia. Stroke 46:1344-1351.

Jian Liu K, Rosenberg GA (2005) Matrix metalloproteinases and free radicals in cerebral ischemia. Free Radic Biol Med 39:71-80.

Liu S, Liu W, Ding W, Miyake M, Rosenberg GA, Liu KJ (2006) Electron paramagnetic resonance-guided normobaric hyperoxia treatment protects the brain by maintaining penumbral oxygenation in a rat model of transient focal cerebral ischemia. J Cereb Blood Flow Metab 26:1274-1284.

Liu W, Hendren J, Qin XJ, Liu KJ (2009) Normobaric hyperoxia reduces the neurovascular complications associated with delayed tissue plasminogen activator treatment in a rat model of focal cerebral ischemia. Stroke 40:2526-2531.

Liu W, Hendren J, Qin XJ, Shen J, Liu KJ (2009) Normobaric hyperoxia attenuates early blood-brain barrier disruption by inhibiting MMP-9-mediated occludin degradation in focal cerebral ischemia. J Neurochem 108:811-820.

Liu W, Sood R, Chen Q, Sakoglu U, Hendren J, Cetin O, Miyake M, Liu KJ (2008) Normobaric hyperoxia inhibits NADPH oxidase-mediated matrix metalloproteinase-9 induction in cerebral microvessels in experimental stroke. J Neurochem 107:1196-1205.

Lo EH, Broderick JP, Moskowitz MA (2004) tPA and proteolysis in the neurovascular unit. Stroke 35:354-356.

Moskowitz MA, Lo EH, Iadecola C (2010) The science of stroke: mechanisms in search of treatments. Neuron 67:181-198.

Nonaka Y, Shimazawa M, Yoshimura S, Iwama T, Hara H (2008) Combination effects of normobaric hyperoxia and edaravone on focal cerebral ischemia-induced neuronal damage in mice. Neurosci Lett 441:224-228.

Nonaka Y, Koumura A, Hyakkoku K, Shimazawa M, Yoshimura S, Iwama T, Hara H (2009) Combination treatment with normobaric hyperoxia and cilostazol protects mice against focal cerebral ischemia-induced neuronal damage better than each treatment alone. J Pharmacol Exp Ther 330:13-22.

O'Collins VE, Macleod MR, Donnan GA, Horky LL, van der Worp BH, Howells DW (2006) 1,026 experimental treatments in acute stroke. Ann Neurol 59:467-477.

Pan J, Konstas AA, Bateman B, Ortolano GA, Pile-Spellman J (2007) Reperfusion injury following cerebral ischemia: pathophysiology, MR imaging, and potential therapies. Neuroradiology 49:93-102.

Pei Z, Fung PC, Cheung RT (2003) Melatonin reduces nitric oxide level during ischemia but not blood-brain barrier breakdown during reperfusion in a rat middle cerebral artery occlusion stroke model. J Pineal Res 34:110-118.

Qi Z, Liu W, Luo Y, Ji X, Liu KJ (2013) Normobaric hyperoxia-based neuroprotective therapies in ischemic stroke. Med Gas Res 3:2.

Ramos-Cabrer P, Campos F, Sobrino T, Castillo J (2011) Targeting the ischemic penumbra. Stroke 42:S7-11.

Reza Noorian A, Nogueira R, Gupta R (2011) Neuroprotection in acute ischemic stroke. J Neurosurg Sci 55:127-138.

Rogalewski A, Schneider A, Ringelstein EB, Schabitz WR (2006) Toward a multimodal neuroprotective treatment of stroke. Stroke 37:1129-1136.

Rosenberg GA, Yang Y (2007) Vasogenic edema due to tight junction disruption by matrix metalloproteinases in cerebral ischemia. Neurosurg Focus 22:E4.

Schaller B, Graf R (2004) Cerebral ischemia and reperfusion: the pathophysiologic concept as a basis for clinical therapy. J Cereb Blood Flow Metab 24:351-371.

Schofield ZV, Woodruff TM, Halai R, Wu MC, Cooper MA (2013) Neutrophils-a key component of ischemia-reperfusion injury. Shock 40:463-470.

Seiffge DJ, Lapina NE, Tsagogiorgas C, Theisinger B, Henning RH, Schilling L (2012) Improvement of oxygen supply by an artificial carrier in combination with normobaric oxygenation decreases the volume of tissue hypoxia and tissue damage from transient focal cerebral ischemia. Exp Neurol 237:18-25.

Shin HK, Dunn AK, Jones PB, Boas DA, Lo EH, Moskowitz MA, Ayata C (2007) Normobaric hyperoxia improves cerebral blood flow and oxygenation, and inhibits peri-infarct depolarizations in experimental focal ischaemia. Brain 130:1631-1642.

Simard JM, Kent TA, Chen M, Tarasov KV, Gerzanich V (2007) Brain oedema in focal ischaemia: molecular pathophysiology and theoretical implications. Lancet Neurol 6:258-268.

Singhal AB, Wang X, Sumii T, Mori T, Lo EH (2002) Effects of normobaric hyperoxia in a rat model of focal cerebral ischemia-reperfusion. J Cereb Blood Flow Metab 22:861-868.

Singhal AB, Benner T, Roccatagliata L, Koroshetz WJ, Schaefer PW, Lo EH, Buonanno FS, Gonzalez RG, Sorensen AG (2005) A pilot study of normobaric oxygen therapy in acute ischemic stroke. Stroke 36:797-802.

Sun L, Wolferts G, Veltkamp R (2014) Oxygen therapy does not increase production and damage induced by reactive oxygen species in focal cerebral ischemia. Neurosci Lett 577:1-5.

Sun L, Strelow H, Mies G, Veltkamp R (2011) Oxygen therapy improves energy metabolism in focal cerebral ischemia. Brain Res 1415:103-108.

Tai SH, Chen HY, Lee EJ, Chen TY, Lin HW, Hung YC, Huang SY, Chen YH, Lee WT, Wu TS (2010) Melatonin inhibits post-ischemic matrix metalloproteinase-9 (MMP-9) activation via dual modulation of plasminogen/plasmin system and endogenous MMP inhibitor in mice subjected to transient focal cerebral ischemia. J Pineal Res 49:332-341.

Villapol S, Fau S, Renolleau S, Biran V, Charriaut-Marlangue C, Baud O (2011) Melatonin promotes myelination by decreasing white matter inflammation after neonatal stroke. Pediatr Res 69:51-55.

Wang CX, Yang T, Noor R, Shuaib A (2002) Delayed minocycline but not delayed mild hypothermia protects against embolic stroke. BMC Neurol 2:2.

Wang F, Wang Y, Geng X, Asmaro K, Peng C, Sullivan JM, Ding JY, Ji X, Ding Y (2012) Neuroprotective effect of acute ethanol administration in a rat with transient cerebral ischemia. Stroke 43:205-210.

Wang Q, Tang XN, Yenari MA (2007) The inflammatory response in stroke. J Neuroimmunol 184:53-68.

Wardlaw JM, Murray V, Berge E, del Zoppo G, Sandercock P, Lindley RL, Cohen G (2012) Recombinant tissue plasminogen activator for acute ischaemic stroke: an updated systematic review and meta-analysis. Lancet 379:2364-2372.

Xu L, Fagan SC, Waller JL, Edwards D, Borlongan CV, Zheng J, Hill WD, Feuerstein G, Hess DC (2004) Low dose intravenous minocycline is neuroprotective after middle cerebral artery occlusion-reperfusion in rats. BMC Neurol 4:7.

Yang Y, Jiang S, Dong Y, Fan C, Zhao L, Yang X, Li J, Di S, Yue L, Liang G, Reiter RJ, Qu Y (2015) Melatonin prevents cell death and mitochondrial dysfunction via a SIRT1-dependent mechanism during ischemic-stroke in mice. J Pineal Res 58:61-70.

Yrjanheikki J, Tikka T, Keinanen R, Goldsteins G, Chan PH, Koistinaho J (1999) A tetracycline derivative, minocycline, reduces inflammation and protects against focal cerebral ischemia with a wide therapeutic window. Proc Natl Acad Sci U S A 96:13496-13500.

Zeng X, Asmaro K, Ren C, Gao M, Peng C, Ding JY, Fredrickson V, Ji X, Ding Y (2012) Acute ethanol treatment reduces blood-brain barrier dysfunction following ischemia/reperfusion injury. Brain Res 1437:127-133.

Zhang L, Zhang ZG, Chopp M (2012) The neurovascular unit and combination treatment strategies for stroke. Trends Pharmacol Sci 33:415-422.

Zou LY, Cheung RT, Liu S, Li G, Huang L (2006) Melatonin reduces infarction volume in a photothrombotic stroke model in the wild-type but not cyclooxygenase-1-gene knockout mice. J Pineal Res 41:150-156.