Co-administration of tissue plasminogen activator and hyperbaric oxygen in ischemic stroke: a continued promise for neuroprotection

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Abstract

Intravenous recombinant tissue-type plasminogen activator (r-tPA, alteplase) remains the recommended therapy for acute ischemic stroke. However, several factors are limiting its practical use. It makes it urgent for us to search more efficient strategies that can save the ischemic neurons, and safely extend the time window, while in the mean time reducing the detrimental effects for stroke thrombolysis. Hyperbaric oxygen therapy (HBOT) is considered to be potentially neuroprotective. Co-administration of r-tPA and HBOT has already been proved to be effective, safe and feasible in myocardial infarction. In this article, we would like to review whether HBOT has any beneficial effects on r-tPA thrombolysis. If there is, what is the underlying possible mechanisms and how to optimize for maximal effects?

INTRODUCTION

With the growing size and aging of the world's population, the global burden of ischemic stroke makes it now a severe public health concern.[1,2] Timely restoration of blood flow using thrombolytic therapy is the most effective management for saving those non-infarcted ischemic brain tissues. Intravenous recombinant tissue-type plasminogen activator (r-tPA, alteplase) is recommended for early thrombolysis within 4.5 hours of ischemic stroke onset. Right now, there are several factors limiting the practical use of intravenous alteplase. Firstly, there is a narrow time window. In the United States, about 22% of all ischemic stroke patients successfully present themselves to an emergency department within 3 hours; however, only 8% are eligible for alteplase treatment.[3] Further, the benefits of thrombolysis decrease over time. Recently, a meta-analysis that included 26 studies and 2,063 patients identified the overall incidence of partial or complete early recanalization ($\leq$ 3 hours after start of intravenous thrombolysis) was only 33%.[4] Besides, 34% of those patients who showed initial recanalization, were found middle cerebral artery (MCA) re-occlusion by transcranial Doppler ultrasound (TCD) within 2 hours of intravenous r-tPA therapy.[5] If the neurologic deterioration occurred within the first hour, it might be caused by a early recurrent ischemic stroke.[6] Thirdly, the complications increase

over time. In a prospective study, 52 out of 128 patients showed hemorrhagic transformation on 3-tesla MRI and susceptibility-weighted imaging, including 8 symptomatic cases, 7 days after thrombolytic therapy.[7]

The efforts of increasing the practical use of intravenous alteplase never stop. Aside from enhanced patient education and public awareness, extending the time window and reducing complications are the major concerns. A study showed that if alteplase was combined with argatroban, a direct thrombin inhibitor, given intravenously for 48 hours after initial alteplase treatment, the recanalization efficacy could be increased to 62%.[8] In preclinical animal experiments, the combination of annexin-A2 and alteplase dissolved clots more rapidly and completely.[9,10] A phase 3 trial (NCT01098981) is underway to confirm if adjunctive therapy with TCD sonothrombolysis yield better clinical outcome than alteplase alone.[11] In 2015, Liang and his colleagues[12] successfully expanded the therapeutic time window of r-tPA to 7 hours in MCA occlusion (MCAO) rats with early normobaric hyperoxia therapy (NBOT). Indeed, all these combined therapies request future exploration. We are still in the urgent need of searching more efficient strategies that can save the ischemic neurons, safely extend the time window, while in the mean time reducing the detrimental effects for stroke thrombolysis.[13]

Co-administration of R-tpa and Hyperbaric Oxygen Therapy (HBOT)

The r-tPA is physiologically synthesized by the vascular endothelial cells and released into the blood circulation. When combined with the fibrin, it initializes the enzymatic reaction and dissolves the fibrin scaffold in the clot.[14] However, r-tPA has also been reported causing vascular disruption with potential cerebral hemorrhage. The toxic effect of r-tPA is associated with activation of matrix metalloproteinases (MMPs).[15]

HBOT is the medical use of oxygen at a level higher than atmospheric pressure.[16] HBOT appears to be a potent way of oxygen delivery.[13,17,18] HBOT has been applied in the treatment of cerebral vascular diseases since the 1960s.[19,20] It is able to increase the oxygen partial pressure within the blood vessel and restore the oxygen supply after ischemic stroke.[21,22] It has been proven that HBOT successfully reduces the infarct size,[23,24] hemorrhagic transformation[25] and improves neurological function in animal models of ischemic stroke.[24] Thus it is considered to be potentially neuroprotective. As early as 2007, Singhal[13] concluded that HBOT might extend the time window and increase the efficiency of r-tPA thrombolysis after acute ischemic stroke.

Co-administration of HBOT and R-tpa in Myocardial Infarction

To testify the feasibility of co-administration of HBOT and r-tPA, research was first done on experimental thrombogenic myocardial infarction dog in 1990.[26] Simultaneous HBOT plus r-tPA, 2 hours following experimental left anterior descending coronary artery occlusion, showed synergistic effects not only in the resolution of cardiac thrombosis, but also with restoration of enzyme activity. Then more animal studies proved their safety and feasibility with reduced infarct size,[27] rapid resolution of pain and ST-segment changes.[28-30] A small clinical trial even showed favorable effects on left ventricular systolic function in patients with myocardial infarction.[31]

Co-administration of HBOT and R-tpa in Ischemic Stroke

Based on the experiences of myocardial infarction, a few pre-clinical studies were initiated in ischemic stroke. The first study of early simultaneous treatment with r-tPA and HBOT was done in 1999 by a research group from Germany.[32] In an embolic ischemic stroke rat model, r-tPA (9 mg/kg) and HBOT (2.4 ATA (1 ATA = 100 kPa), 1 hour) were given intravenously 2 hours after stroke onset. The combination greatly improved the neurological deficits within 24 hours. Then they did more experiments to determine the timing of HBOT, using similar design and intervention. They found that the adjuvant HBOT (2.4 ATA, 1 hour) either started 45 minutes (sequential) or 2 hours (parallel) after stroke, significantly improved the functional outcome and reduced the infarct volume, than thrombolysis alone.[33,34]

Sun and his colleagues[25] started r-tPA (9 mg/kg) and HBOT (3 ATA, 60 minutes) 1 hour after thromboembolic MCAO. Either thrombin-induced thromboemboli (TT) or calcium-induced thromboemboli (CT) model was applied. Recanalization was only observed in TT model. In both models, the adjuvant HBOT decreased the blood-brain barrier (BBB) permeability and thus hemorrhagic transformation through attenuated activation of MMPs. Interestingly, HBOT significantly reduced infarct size in TT, but not in CT model. The effect of HBOT after thromboembolic ischemia might be associated with whether the recanalization was successful.

More recently, HBOT was found to increase r-tPA-induced thrombolysis *in vitro*. 1 μL r-tPA was added into the clot formed from 500 μL whole-blood drawn from Sprague-Dawley mature rats. The clot was then incubated in HBOT (2.5 ATA, 90 minutes) before it weighed again to assess the percentage of clot lysis. It turned out that HBOT

increased the thrombolytic effect of r-tPA by 64%, while NBOT only by 39%, as compared to controls.[35] There was also one case report showing a multimodality approach of intravenous and intra-arterial r-tPA thrombolysis repeated HBOT (2.0 ATA, 90 minutes, started 6 hours after stroke onset) and hypothermia improved the symptoms in spinal cord infarction.[36]

Long-term Effects of Co-administration of HBOT and R-tPA

Following experimental stroke, long-lasting inflammatory responses and neuronal loss were found. The long-term inflammatory responses are considered as important for repair and neuronal plasticity. In 2011, a research team from Germany initiated r-tPA (9 mg/kg) and HBOT (2.4 ATA, 60 minutes), simultaneously 2 hours after ischemia onset in MCAO rats. They found that after 28 days, the neuron loss was not ameliorated in the four ischemia-related regions. The results may be explained by the small number of study subjects. Besides, only 60-minute HBOT was applied. And the number of reduced NeuN-positive cells alone was not adequate for differentiation neuron loss from apoptosis and necrosis in those regions.[37] They also found that the administration of r-tPA with HBOT decreased the macrophage-like cell accumulation at days 14 and 28 post ischemia. Since the lack of a separate HBOT group in the study, it wants definitive proof to conclude that the decreased macrophage-like cell accumulation was related to the co-applied HBOT.[15]

Proposed Mechanisms: HBOT Combined with R-tPA

The follows are the proposed mechanisms why and how HBOT might add the beneficial effects of r-tPA in thrombogenic-induced ischemic stroke (**Figure 1**). (1) HBOT has long been demonstrated to be able to dissolve

thrombosis by gas bubbles in decompression sickness.[38] Then it was speculated that such a mechanical thrombolytic effect might as well be effective for atherosclerotic thrombosis. (2) Secondly, HBOT has been recommended in the treatment of central retinal artery occlusion.[39] Learning from the eyes, we know when 100% oxygen is breathed under hyperbaric conditions, at 3 ATA for example, the oxygen dissolved in the plasma rises from 0.31 vol% to 6 vol%.[40] Then enough plasma dissolved oxygen is available to meet metabolic needs of the infracted brain areas and earn more time for r-tPA. (3) Thirdly, study showed HBOT could up-regulate the endogenous production of r-tPA by inhibiting the plasminogen activator inhibitor-1 (PAI-1) activity which suppresses r-tPA secretion into the body.[41] (4) Further, it is known to all that delayed thrombolytic therapy dramatically increases the risk of hemorrhage because of the disrupted BBB.[42] NBOT has been proven to alleviate ischemic BBB damage and significantly improve outcome when r-tPA was given at 4.5 hours[43] or even 7 hours[12] after stroke onset. HBOT as well, significantly protect BBB integrity during cerebral ischemia *via* the suppression of MMPs, to reduce the reperfusion damage after recanalization.[44,45]

Key Points of Efficient Co-administration of HBOT and R-tPA
Dose of HBOT

There is still no comprehensive study to compare the different HBOT ATA and duration, let alone in the settings of r-tPA co-administration. Many research used only one HBOT session of 60 minutes at a certain ATA. As early as 2003, Rogatsky et al.[46] put forward that, in order to guarantee the neuroprotective efficacy of HBOT in clinical and experimental acute ischemic stroke (AIS), the dose of HBOT (defined as HBOT ATA, multiplied by hour of a

Proposed mechanism of HBOT combined r-tPA
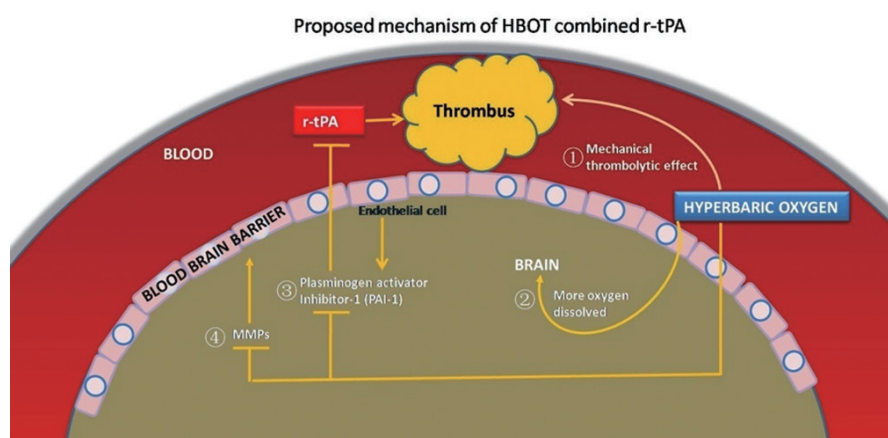
Note: Numbers ① to ④ correlate to each listed mechanism, respectively. ① HBOT has long been demonstrated to be able to dissolve thrombosis by gas bubbles in decompression sickness. ② HBOT has been recommended in the treatment of central retinal artery occlusion. ③ Study showed HBOT could up-regulate the endogenous production of r-tPA by inhibiting the plasminogen activator Inhibitor-1 (PAI-1) activity which suppresses r-tPA secretion into the body. ④ It is known to all that delayed thrombolytic therapy dramatically increases the risk of hemorrhage because of the disrupted blood-brain barrier. HBOT: Hyperbaric oxygen therapy; r-tPA: tissue-type plasminogen activator; MMPs: matrix metalloproteinases.

Figure 1: The proposed mechanisms how HBOT might add to the beneficial effects of r-tPA in thrombogenic-induced ischemic stroke.

single exposure and total number of treatments) must be maximally optimized. After analyzing retrospectively 265 patients in different hyperbaric centers, they concluded the efficacy of HBOT was closely correlated with higher level of HBOT dose. Applying at least 30–32 doses of HBOT may provide the maximum (100%) possible effects. In other words, the HBOT dose in the published literatures is unlikely to be sufficient.

Time window of HBOT

The therapeutic time window for AIS is generally believed to be started as soon as possible.[47-49] Research showed that the oxygen therapy was neuroprotective when started either during ischemia,[50,51] as early as 10 minutes,[52] 25 minutes,[53] 40 minutes,[54,55] 60 minutes,[56] 90 minutes,[57] or 180 minutes after MCAO.[58-62] Our previous study proved that delayed HBOT 48 hours after permanent MCAO (pMCAO) can still convey neuroprotection and restorative cell proliferation.[63] HBOT has recently been shown to be effective when started 2–5 days after ischemic stroke onset,[64,65] or even induces neuroplasticity in the chronic stage.[66] A retrospective statistical analysis proved HBOT initiated within the first 3 hours post-stroke the most promise for efficacy. Pre-r-tPA administration of HBOT may also be a useful challenge to assess the improvement of r-tPA therapy.[38,67] All these time points need to be tested in the context of r-tPA co-administration.

Safety in humans

In both the pilot study and randomized multicenter trial of acute myocardial infarction patients, HBOT combined with r-tPA thrombolysis was feasible and safe. Sixty-six patients with inferior acute myocardial infarction and forty-six patients with anterior acute myocardial infarction, were randomized to treatment with HBOT combined with either r-tPA or streptokinase (STK), or r-tPA or STK alone. There were two deaths in the control and one in those treated with HBOT. The HBOT resulted in more rapid resolution of pain and ST segment changes.[28,30]

Feasibility

Scientists from the Wake Forest, School of Medicine, North Carolina, USA have designed and built a prototype hyperbaric oxygen ambulance for stroke patients, where HBOT can be initiated before arriving at the hospital.[38] As soon as it completes, it will be used in conjunction with ambulance-based telemedicine techniques,[68] a CT scanner operable in the HBO environment of our mobile chamber equipped ambulance, and in clinical trials to test the safety and validity, eventually facilitate ambulance based r-tPA administration with FDA time window.

HBOT *vs*. NBOT

Only one study compared the effects of NBOT and HBOT in experimental stroke.[34] The animals were assigned to MCAO control, NBOT, HBOT, r-tPA or HBOT + r-tPA group. Either NBOT (1 hour) or HBOT (2.4 ATA, 1 hour) was initiated 2 hours after ischemia onset. They found significant functional improvement in the NBOT, r-tPA and HBOT + r-tPA group, but not in the HBOT group. However, HBOT did tend to stabilize BBB and reduce MMP activation. Moreover, its concomitant treatment with r-tPA also provided early functional improvement. It is a pity that the study failed to add a group of NBOT + r-tPA. As a result, further studies are required to identify the beneficial effects of both NBOT and HBOT, in the setting of interactions with r-tPA, especially the optimized dose and time window of HBOT.

SUMMARY

In embolic ischemic stroke models, HBOT is able to add to the effects of r-tPA thrombolysis with improved neurological functions. The underlying mechanisms may be associated with the mechanical effects, increased percentage of oxygen dissolved in the plasma, the up-regulated level of endogenous r-tPA and stabilization of BBB. The maximal effects can be achieved by optimizing the dose and time window of HBOT. HBOT is still a promising strategy to treat acute cerebral ischemic stroke. Future directions include experiments on different models of ischemic cerebrovascular diseases,[69] cocktail treatment[14,70] with NBOT,[71] well designed clinical trials and more mechanism studies.

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