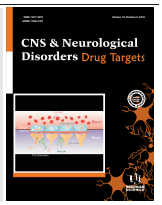
*CNS & Neurological Disorders - Drug Targets*, 2016, *Vol. 15, No. 9*





**Commentary**

**Hypothesis**

# Focused Ultrasound and NXY-059 in Experimental Cerebral Ischemia: A New Therapeutic Opportunity?

Burak Yulug[1*], Lütfü Hanoglu[1], Feysel Yalcin Yamaner[2], Ertugrul Kilic[3] and Wolf Rüdiger Schabitz[4]

[1]*Department of Neurology, University of Istanbul-Medipol, Istanbul, Turkey*

[2]*School of Engineering and Natural Sciences, University of Istanbul-Medipol, Istanbul, Turkey*

[3]*Department of Physiology, Brain Research Laboratory, University of Istanbul-Medipol, Istanbul, Turkey*

[4]*Department of Neurology, Bethel-EvKB, Bielefeld, Teaching Affiliate of University of Münster, Germany*

**Burak Yulug**

## THE HYPOTHESIS

Besides its central role in stroke pathogenesis, the blood-brain barrier (BBB) may be an important therapeutic target and mediator for the development of new treatment strategies. Here, we hypothesize that co-administration of microbubbles/ transcranial pulsed focused ultrasound and a well-known antioxidant agent (NXY-059) may increase the latter's neuroprotective effect by increasing its delivery into the target brain area. Previous negative clinical findings may have resulted from failure of NXY-059 to cross the BBB. A approach of microbubbles/transcranial pulsed focused ultrasound combined with NXY-059 may provide a novel therapeutic for stroke and serve as new model for protective treatment approaches in acute cerebral ischemia.

## BACKGROUND

### BBB Role in Stroke Pathogenesis

Stroke is the third leading cause of morbidity and mortality worldwide. Many deleterious cellular pathways have been proposed to explain the molecular pathogenesis of this clinically devastating disease [1, 2]. The pathophysiology of stroke is complex and involves not only calcium and glutamate-mediated excitotoxicity but also various inflammatory pathways, disturbance of ionic balance, increased production of free radicals and neuronal cell apoptosis [3-5]. Besides its critical role for ion homeostasis in the central nervous system, disturbance of BBB integrity plays a significant role in stroke pathogenesis [6-8]. In this respect, recent studies have established that loss of BBB integrity and secondary loss of ion regulation may lead to brain edema and subsequent brain damage after cerebral ischemia [7, 9, 10]. This suggests that stabilization of the BBB could be brain protective, although recent studies failed to confirm this [11-13]. Moreover, data show that cerebral ischemia-induced BBB disruption is increased by 24 hours after middle cerebral artery occlusion [14], thus providing only a short window for transport of macromolecular drugs into the infarcted brain [14, 15]. This therapeutic time-frame effectively limits treatment efficacy due to an inability to achieve a sufficiently high dose of drug in the target brain area [15]. Therapeutic agents are often difficult to administer to the brain due to BBB prevention of passage for systemically administered molecules and proteins [16-18]. Because of this pharmacological therapies have made limited progress, and much effort is now being directed to identify compounds that accumulate more efficaciously in the diseased brain [17-20].

Although preclinical studies have provided promising results for a number of neuroprotective agents (e.g., TAT-BCL) [21], many candidate drugs and therapeutic approaches to enhance drug delivery to the brain have failed in the clinic [11, 18, 22-24]. These treatment strategies may also increase the risk of systemic toxicity related to increasing drug dosage without achieving sufficient levels in the target brain area due to limited BBB permeability. These findings point to the need for therapeutic approaches which transiently open the BBB and extend the conventional therapeutic time window for delivering efficacious quantities of candidate neuroprotective drugs to the target brain region without increasing systemic dosage.

*Address correspondence to this author at the Department of Neurology, University of Istanbul-Medipol, Istanbul, Turkey; Tel/Fax: 0090 212 460 70 70; E-mail: burakyulug@gmail.com

1996-3181/16 $58.00+.00 

### A Brief Overview of the Neuroprotective Effect of NXY-059

NXY-059 is a nitrone compund with free radical scavenging activity shown to be neuroprotective in various animal models of stroke [25, 26]. These promising preclinical studies open an exciting window for further clinical neuroprotective studies [27, 28]. The SAINT I trial evaluating the neuroprotective effect of NXY-059 within 4 hours of stroke onset revealed that NXY-059 significantly reduced functional neurological outcome assessed by the modified Rankin Scale at 90 days [29]. Despite lack of additional improvement in primary outcome when combined with recombinant tissue plasmiogen activator, the same study surprisingly showed that hemorrhagic complications of tissue plasmiogen activator were reduced by NXY-059 [29, 30-34]. The explanation for the failure of NXY-059 might be that the compound failed to act beyond the BBB (endothelial protective effect only), in line with previous findings showing that NXY-059 has a poor BBB permeability [29, 30-37]. Unfortunately, the SAINT II trial revealed negative results in both primary and secondary outcomes [38]; the difference in outcome was mainly attributed to the non-homogeneous study population (different ischemic subtypes) [29-35]. Poor BBB permeability of NXY-059 may have contributed also to the negative trial outcome [33]. Considering the fact that NXY-059 is the only agent with proven clinical neuroprotective efficacy [29], it would be of some interest to test the neuroprotective effect of NXY-059 in combination with a therapeutic approach to overcome BBB impermeability, to clarify whether this agent exerts true parencymal free radical scavenger activity.

### The Role of Transcranial Pulsed Focused Ultrasound

Noninvasively applied transcranial pulsed focused ultrasound (FUS) may lead to local and reversible BBB disruption that allows for the transvascular delivery of macromolecules into the target brain region [39-52]. By using microbubbles administered systemically when applying FUS to a specific location, one can show that pulsed FUS produces an intense acoustic energy mechanically by cavitation, microstreaming and radiation forces, thereby increasing BBB permeability [39-52]. Magnetic resonance-guided FUS (MRgFUS)-mediated BBB disruption in small animals can be performed safely without significant brain damage by regulating various parameters including ultrasound sonication, microbubble dosage and ultrasound contrast agent [39-52].

Preclinical studies show that the transient increase in BBB permeability by MRgFUS is associated with improved delivery of chemotherapeutics and therapeutic antibodies (e.g. liposomal doxorubicin) to specific brain areas and can improve outcome [39-46, 52-55]. Investigations with animal models of brain tumors and Alzheimer disease confirm the beneficial effects of this delivery method [56, 57]. FUS may also enhance local drug delivery and improve the antitumor effect in brain tumors [52-54]. This is in line with another report that FUS exposure following Evans Blue injection significantly elevates the amount of extravasated dye in sonicated hepatoma [58]. Moreover, recent studies in Alzheimer disease models demonstrated that MRgFUS efficiently delivered the anti-amyloid β-peptide antibody BAM-10 from bloodstream to brain, thereby reducing amyloid β-peptide pathology [57]. This was also suggested by a study using FUS to enhance delivery of small fluorescent agents and large biological immunotherapeutics which led to improved outcome in a transgenic mouse model of Alzheimer disease [56]. These findings are consistent with a previous stem cell study demonstrating that the MRgFUS energy application to a specific brain region could direct stem cells from the blood to a target brain structure mediated by transient BBB opening [59]. These findings prompted efforts to enhance the poor BBB permeability of erythropoietin (EPO) to optimize its neuroprotective efficacy [47-60]. Wu *et al.* utilized microbubbles-FUS (MBs/FUS) for the localized delivery of EPO into the infracted rat brain and showed that EPO administration with MBs/FUS sonication after occlusion/reperfusion reduced infarct volume and improved neurobehavioural outcomes even the late post-stroke period (>5 hours) [47]. These findings suggest that the combination of EPO and MBs/FUS may produce a significant neuroprotective effect in both acute and chronic phases of experimental cerebral ischemia, further indicating that this delivery model provides an opportunity to overcome hurdles for drug delivery of large molecules or proteins to the brain.

### Rationale for the Hypothesis

The methods may involve the use of a cerebral ischemia model in mice and analysis of downstream cell survival and death mechanisms in the ischemic brain while the effects of various ultrasound parameters on the efficacy of NXY-059 extravasation can be studied. The rationale for combining NXY-059 with MBs/FUS is to overcome the BBB in acute cerebral ischemia and to optimize NXY-059 delivery to the brain.

### Evaluation of the Hypothesis

Based on previous findings showing that FUS is more effective after Evans Blue application, we will apply MBs/FUS in a three-vessel occlusion model in mice and inject microbubbles as bolus before targeted sonication. As suggested by previous studies, pulsed sonication will be applied with specific parameters (i.e., duration of each sonication, peak negative pressure, burst length, repetition frequency). A three-vessel occlusion model provides a more consistent cortical injury compared to the middle cerebral artery occlusion model and is more suitable for evaluation of BBB opening with MBs/FUS. Quantification of NXY-059 entering the brain tissue can be investigated via ELISA and infarct volume, with neurological status of the mice being analyzed 24 hours after ischemia. The underlying mechanisms of neuroprotective effect of microbubbles/focused ultrasound will be evaluated via immunohistochemical staining in the acute and sub-acute periods after three-vessel occlusion.

## CONCLUSION

Based on the findings discussed here, we hypothesize that FUS presents a treatment opportunity to enhance delivery of candidate neuroprotectants to the ischemic brain. In terms of potential clinical application, FUS transducers should be combined with magnetic resonance imaging in order to guide the FUS transducer to achieve a more precisely sonication. Furthermore, our preclinical results support the potential administration of NXY-059 and MBs/FUS combined with various reperfusion treatment strategies (i.e. endovascular, tissue plasminogen activator, stent retrieval) in addition to the conventional therapeutic approaches in stroke patients.

## REFERENCES


[1] Rothwell PM. The high cost of nonfunding stroke research: a comparison with heart disease and cancer. Lancet 2001; 357: 1612-6.
[2] Dirnagl U, Iadecola C, Moskowitz MA. Pathobiology of ischemic stroke: an integrated view. Trends Neurosci 1999; 22: 391-7.
[3] Woodruff TM, Thundyil J, Tang SC, Sobey CG, Taylor SM, Arumugam TV. Pathophysiology, treatment, and animal and cellular models of human ischemic stroke. Mol Neurodegener 2011; 6: 11.
[4] Thompson BJ, Ronaldson PT. Drug delivery to the ischemic brain. Adv Pharmacol 2014; 71: 165-202.
[5] Yulug B, Yildiz A, Güzel O, Kilic E, Schäbitz WR, Kilic E. Risperidone attenuates brain damage after focal cerebral ischemia *in vivo*. Brain Res Bull 2006; 69(6): 656-9.
[6] Brown RC, Davis TP. Calcium modulation of adherens and tight junction function: a potential mechanism for blood-brain barrier disruption after stroke. Stroke 2002; 33: 1706-11.
[7] Albayrak S, Zhao Q, Siesjo BK, Smith ML. Effect of transient focal ischemia on blood-brain barrier permeability in the rat: correlation to cell injury. Acta Neuropathol 1997; 94: 158-63.
[8] Reese TS, Karnovsky MJ. Fine structural localization of a blood-brain barrier to exogenous peroxidase. J Cell Biol 1967; 34: 207-17.
[9] Ronaldson PT, Davis TP. Blood-brain barrier integrity and glial support: mechanisms that can be targeted for novel therapeutic approaches in stroke. Curr Pharm Des 2012; 18(25): 3624-44.
[10] Betz AL, Keep RF, Beer ME, Ren XD. Blood-brain barrier permeability and brain concentration of sodium, potassium, and chloride during focal ischemia. J Cereb Blood Flow Metab 1994; 14: 29-37.
[11] O'Collins VE, Macleod MR, Donnan GA, Horky LL, van der Worp BH, Howells DW. 1,026 experimental treatments in acute stroke. Ann Neurol 2006; 59: 467-77.
[12] Stroke Therapy Academic Industry Roundtable. Recommendations for standards regarding preclinical neuroprotective and restorative drug development. Stroke 1999; 30: 2752-8.
[13] ElAli A, Doeppner TR, Zechariah A, Hermann DM. Increased blood-brain barrier permeability and brain edema after focal cerebral ischemia induced by hyperlipidemia: role of lipid peroxidation and calpain-1/2, matrix metalloproteinase-2/9, and RhoA overactivation. Stroke 2011; 42: 3238-44.
[14] Menzies SA, Betz AL, Hoff JT. Contributions of ions and albumin to the formation and resolution of ischemic brain edema. J Neurosurg 1993; 78: 257-66.
[15] Upadhyay RK. Drug delivery systems, CNS protection, and the blood brain barrier. Biomed Res Int 2014; 2014: 869269.
[16] Pardridge WM. Drug and gene delivery to the brain: the vascular route. Neuron 2002; 36: 555-8.
[17] Begley DJ. Delivery of therapeutic agents to the central nervous system: the problems and the possibilities. Pharmacol Ther 2004; 104: 29-45.
[18] Kilic E, Spudich A, Kilic U, *et al.* ABCC1: a gateway for pharmacological compounds to the ischaemic brain. Brain 2008; 131: 2679-89.
[19] Hermann DM, Kilic E, Spudich A, Kra¨mer SD, Wunderli-Allenspach H, Bassetti CL. Role of drug efflux carriers in the healthy and diseased brain. Ann Neurol 2006; 60: 489-98.
[20] Loscher W, Potschka H. Drug resistance in brain diseases and the role of drug efflux transporters. Nat Rev Neurosci 2005; 6: 591-602.
[21] Kilic E, Dietz GP, Hermann DM, Bähr M. Intravenous TAT-Bcl-Xl is protective after middle cerebral artery occlusion in mice. Ann Neurol 2002; 52: 617-22.
[22] Hanson LR, Frey WH 2nd. Intranasal delivery bypasses the blood-brain barrier to target therapeutic agents to the central nervous system and treat neurodegenerative disease. BMC Neurosci. 2008 Dec 10; 9 Suppl 3:S5.
[23] Hermann DM, Bassetti CL. Implications of ATP-binding cassette transporters for brain pharmacotherapies. Trends Pharmacol Sci 2007; 28: 128-34.
[24] Ehrenreich H, Weissenborn K, Prange H, *et al.* Recombinant human erythropoietin in the treatment of acute ischemic stroke. Stroke 2009; 40(12): e647-56.
[25] Kuroda S, Tsuchidate R, Smith ML, Maples KR, Siesjo BK. Neuroprotective effects of a novel nitrone, NXY-059, after transient focal cerebral ischemia in the rat. J Cereb Blood Flow Metab 1999; 19: 778-87.
[26] Sydserff SG, Borelli AR, Green AR, Cross AJ. Effect of NXY-059 on infarct volume after transient or permanent middle cerebral artery occlusion in the rat; studies on dose, plasma concentration and therapeutic time window. Br J Pharmacol 2002; 135: 103-12.
[27] Lapchak PA. Fast neuroprotection (fast-NPRX) for acute ischemic stroke victims: the time for treatment is now. Transl Stroke Res 2013; 4(6): 704-9.
[28] Green AR, Ashwood T, Odergren T, Jackson DM. Nitrones as neuroprotective agents in cerebral ischemia, with particular reference to NXY-059. Pharmacol Ther 2003; 100(3): 195-214.
[29] Lees KR, Zivin JA, Ashwood T, *et al.* Stroke-Acute Ischemic NXY Treatment (SAINT I) Trial Investigators. NXY-057 for acute ischemic stroke. N Eng J Med 2006; 354: 588-600.
[30] Lees KR, Davalos A, Davis SM, *et al.* Additional outcomes and subgroup analyses of NXY-059 for acute ischemic stroke in the SAINT I trial. Stroke 2006; 37: 2970-8.
[31] Koziol JA, Feng AC. On the analysis and interpretation of outcome measures in stroke clinical trials: Lessons from the saint i study of NXY-059 for acute ischemic stroke. Stroke 2006; 37: 2644-7.
[32] Saver JL. Clinical impact of NXY-059 demonstrated in the saint I trial: Derivation of number needed to treat for benefit over entire range of functional disability. Stroke 2007; 38: 1515-8.
[33] Fisher M, Lees K, Papadakis M, Buchan AM. NXY-059: Brain or vessel protection. Stroke 2006; 37: 2189-90.
[34] Shirley R, Ord EN, Work LM. Oxidative Stress and the Use of Antioxidants in Stroke. Antioxidants 2014; 3: 472-501.
[35] Savitz SI, Schäbitz WR. A Critique of SAINT II. wishful thinking, dashed hopes, and the future of neuroprotection for acute stroke. Stroke 2008; 39: 1389-91.
[36] Gao X, Qian J, Zheng S, Changyi Y, Zhang J, Ju S, Zhu J, Li C. Overcoming the blood-brain barrier for delivering drugs into the brain by using adenosine receptor nanoagonist. ACS Nano 2014 Apr 22;8(4): 3678-89.
[37] Dehouck MP, Cecchelli R, Richard GA, Renftel M, Lundquist S. *In vitro* blood-brain barrier permeability and cerebral endothelial cell uptake of the neuroprotective nitrone compound NXY-059 in normoxic, hypoxic and ischemic conditions. Brain Res 2002; 955: 229-35.
[38] Shuaib A, Lees KR, Lyden P, *et al.* NXY-059 for the treatment of acute ischemic stroke. N Engl J Med 2007; 357: 562-71.

[39] Yang FY, Lee PY. Efficiency of drug delivery enhanced by acoustic pressure during blood-brain barrier disruption induced by focused ultrasound. Int J Nanomed 2012; 7: 2573-82.
[40] McDannold N, Vykhodtseva N, Hynynen K. Effects of acoustic parameters and ultrasound contrast agent dose on focused-ultrasound induced blood-brain barrier disruption. Ultrasound Med Biol 2008; 34: 930-7.
[41] Yang FY, Wang HE, Lin GL, *et al.* Micro-spect/ct-based pharmacokinetic analysis of 99mtc-diethylenetriaminepentaacetic acid in rats with blood-brain barrier disruption induced by focused ultrasound. J Nucl Med 2011; 52: 478-84.
[42] Yang FY, Horng SC, Lin YS, Kao YH. Association between contrast- enhanced mr images and blood-brain barrier disruption following transcranial focused ultrasound. J Magn Reson Imaging 2010; 32: 593-9.
[43] Yang FY, Lin YS, Kang KH, Chao TK. Reversible blood-brain barrier disruption by repeated transcranial focused ultrasound allows enhanced extravasation. J Control Release 2011; 150: 111-6.
[44] Yang FY, Liu SH, Ho FM, Chang CH. Effect of ultrasound contrast agent dose on the duration of focused-ultrasound-induced blood-brain barrier disruption. J Acoust Soc Am 2009; 126: 3344-9.
[45] Kroll RA, Neuwelt EA. Outwitting the blood-brain barrier for therapeutic purposes: Osmotic opening and other means. Neurosurgery 1998; 42: 1083-99.
[46] Choi JJ, Selert K, Gao Z, Samiotaki G, Baseri B, Konofagou EE. Noninvasive and localized blood-brain barrier disruption using focused ultrasound can be achieved at short pulse lengths and low pulse repetition frequencies. J Cereb Blood Flow Metab 2011; 31: 725-37.
[47] Wu SK, Yang MT, Kang KH, *et al.* Targeted delivery of erythropoietin by transcranial focused ultrasound for neuroprotection against ischemia/reperfusion-induced neuronal injury: a long-term and short-term study. PLoS One 2014; 28: 9(2).
[48] Hynynen K, McDannold N, Sheikov NA, Jolesz FA, Vykhodtseva N. Local and reversible blood-brain barrier disruption by noninvasive focused ultrasound at frequencies suitable for trans-skull sonications. Neuroimage 2005; 24: 12-20.
[49] Hynynen K, McDannold N, Vykhodtseva N, Jolesz FA. Noninvasive MR imaging-guided focal opening of the blood-brain barrier in rabbits. Radiology 2001; 220: 640-6.
[50] Sheikov N, McDannold N, Vykhodtseva N, Jolesz F, Hynynen K. Cellular mechanisms of the blood-brain barrier opening induced by ultrasound in presence of microbubbles. Ultrasound Med Biol 2004; 30: 979-89.
[51] Weng JC, Wu SK, Lin WL, Tseng WY. Detecting blood-brain barrier disruption within minimal hemorrhage following transcranial focused ultrasound: a correlation study with contrast-enhanced MRI. Magn Reson Med 2011; 65: 802-11.
[52] Yang FY, Teng MC, Lu M, *et al.*Treating glioblastoma multiforme with selective high-dose liposomal doxorubicin chemotherapy induced by repeated focused ultrasound. Int J Nanomed 2012; 7: 965-74.
[53] Yang FY, Wang HE, Lin GL, Lin HH, Wong TT. Evaluation of the increase in permeability of the blood-brain barrier during tumor progression after pulsed focused ultrasound. Int J Nanomed 2012; 7: 723-30.
[54] Yang FY, Wong TT, Teng MC, *et al.* Focused ultrasound and interleukin-4 receptor-targeted liposomal doxorubicin for enhanced targeted drug delivery and antitumor effect in glioblastoma multiforme. J Control Release 2012; 3: 652.
[55] Treat LH, Mc Dannold N, Vykhodtseva N, *et al.* Targeted delivery of doxorubicin to the rat brain at therapeutic levels using MRI-guided focused ultrasound. Int J Cancer 2007; 121: 901-7.
[56] Raymond SB, Treat LH, Dewey JD, McDannold NJ, Hynynen K, Bacskai BJ. Ultrasound enhanced delivery of molecular imaging and therapeutic agents in Alzheimer's disease mouse models. PLoS One 2008; 14: 3(5).
[57] Jordão JF, Ayala-Grosso CA, Markham K, *et al.* Antibodies targeted to the brain with image-guided focused ultrasound reduces amyloid-beta plaque load in the TgCRND8 mouse model of Alzheimer's disease. PLoS One 2010; 11: 5(5).
[58] Bekeredjian R, Kroll R, Fein E, *et al.* Ultrasound targeted microbubble destruction increases capillary permeability in hepatomas. Ultrasound Med Biol 2007; 33: 1592-8.
[59] Burgess A, Ayala-Grosso CA, Ganguly M, Jordão JF, Aubert I, Hynynen K. Targeted delivery of neural stem cells to the brain using MRI-guided focused ultrasound to disrupt the blood-brain barrier. PLoS One 2011; 6(11): e27877.
[60] Minnerup J, Wersching H, Schäbitz WR. EPO for stroke therapy - Is there a future for further clinical development? Exp Transl Stroke Med 2010; 12: 2(1): 10.