PERSPECTIVE

Intranasal insulin neuroprotection in ischemic stroke

Acute ischemic stroke (AIS) is a leading cause of death and long-term disability in the USA and worldwide. Significant advances in the last two decades have resulted in the introduction of intravenous tissue plasminogen activator and more recently catheter based endovascular interventions in selected patients (Berkhemer et al., 2015; Goyal et al., 2015). These interventions are applicable to a limited number of patients fulfilling specific criteria; therefore neuroprotection has attracted significant attention. Neuroprotection refers to strategies and interventions aiming to limit the extent of AIS-related injury and facilitate the naturally occurring regenerative mechanisms.

Acute ischemic injury triggers a series of events in a cellular and molecular level, resulting in energy failure and ultimately neuronal death: Inflammation, excitotoxicity, apoptosis, reactive oxygen and nitrogen species formation, mitochondrial failure have been implicated in the ischemic cascade (Lioutas et al., 2015).

In contrast to many other neuroprotective agents used in past clinical trials targeting specific single steps along the process, insulin's effects are pleiotropic: It suppresses pro-inflammatory transcription factors and might limit the detrimental effect of the inflammatory response (Garg et al., 2006). It produces an antithrombotic effect by decreasing the tissue factor and plasminogen activator inhibitor-1 levels and a vasodilatory effect by promoting activation of endothelial nitric oxide synthase; both actions could facilitate recruitment of collateral vessels and enhance the effect of thrombolysis, ultimately reducing the final infarct volume and improving long-term functional outcome (Huang et al., 2014). Insulin also favorably regulates cerebral energy homeostasis. In addition to the acute phase, insulin's effects extend to the subacute and chronic phase, exerting a potent antiapoptotic effect and promoting myelin and neurite regeneration, neurotransmission and functional connectivity of the brain (Duarte et al., 2012). The putative neuroprotective mechanisms of insulin in the ischemic cascade are summarized in **Figure 1**.

The intranasal route presents significant advantages: The absorption occurs mostly through paracellular transport and endocytosis, following the course of olfactory and trigeminal neurons that are present in the nasal cavity. This offers the significant advantage of bypassing the blood-brain barrier and achieving rapid, widespread central nervous system (CNS) penetration (detected in the CNS within 1 hour from administration). In animal experiments intranasal administration resulted in 100-fold higher CNS concentration compared to intravenous administration of equal insulin dose (Thorne et al., 2004). Although there is a predilection for higher concentrations in areas related to the trigeminal and olfactory pathways and there is a rostral-caudal transmission vector, there is evidence of more diffuse spread in various cerebral regions (Lochhead and Thorne, 2012).

Intranasal insulin has been used in healthy adult human subjects, diabetics and non-diabetics, patients with mild cognitive impairment (MCI) and Alzheimer's disease, focusing on safety, feasibility tolerability, neurologic and cognitive improvement. Intranasal insulin administration resulted in significant improvement in visuospatial memory, immediate and delayed recall, attention and verbal learning acutely and in the chronic phase, without any concerning side effects (Craft et al., 2012). In addition to clinical performance metrics, functional MRI and PET scan studies provided robust evidence suggesting improved vasoreactivity and cerebral perfusion in the gray and white matter, improved connectivity, slowing of cerebral hypometabolism. It is important to highlight that the effects were not limited to diabetics or MCI/Alzheimer's disease patients only, healthy adults benefitted in a similar way, indicating an overall positive effect of intranasal administered insulin in the cerebral function (Reger et al., 2008).

Intranasal administration of insulin has significant practical advantages: It is simple, fast, feasible, painless, uncomplicated and well-tolerated by patients. Therefore, it would not interfere with the rest of time-sensitive interventions during acute stroke, such as IV thrombolysis. If approved for treatment in acute stroke its simplicity will allow for rapid administration in the emergency department or even by the paramedical staff. The major safety concern would be the risk of hypoglycemia, which can be detrimental to human brain. However, this has not shown to be a problem in several human studies involving healthy and diabetic adults (Craft et al., 2012), as intranasal administration results in minimal systemic absorption and first-pass metabolism of insulin. Insulin actions on the CNS in fact favor energy homeostasis, as already described.

Neuroprotection has attracted significant interest and numerous agents have been tested in large scale human trials. Despite robust theoretical background and promising in vivo (animal) experimental data, none of these trials resulted in unequivocal success and there is no neuroprotectant approved for use in acute stroke. N-methyl-D-aspartate (NMDA) receptor antagonists, free radical scavengers, cellular membrane stabilizers, monoclonal antibodies all had neutral or, at times negative effects (Lioutas et al., 2015). The reasons for the series of translational failures have concerned academic trialists and the pharmaceutical industry likewise and resulted in a series of recommendations on how to optimize the process of developing an agent with neuroprotective properties and minimizing premature advancement to futile, resource-demanding trials (Albers et al., 2011). Specific qualities that a neuroprotective agent should ideally possess include:

• A pleiotropic mechanism of action. It has been felt that the failure of many of the above mentioned agents to provide a functional benefit was at least in part due to their very specific target. However, the ischemic cascade includes a series of different events and it is unlikely that blocking on step along the process would significantly limit the damage. In a conceptual level, hypothermia is considered an ideal paradigm of a plurifunctional agent, although it should be noted that its benefit in cerebral ischemia has not been proven. Insulin's actions in the CNS indeed are not limited to a singular mechanism; in the contrary it has an overarching effect, blocking multiple detrimental processes in the acute and subacute phase and augmenting neuroplasticity mechanisms.

• Rapid, preferably pre-hospital, administration. Recent large scale acute stroke trials have showed that "in the field" treatment is feasible for the appropriate agents. Intranasal insulin delivery is very simple, straightforward and easily applicable without the need for sophisticated equipment or special personnel training. Some if insulin's properties, namely its vasodilatory and antithrombotic effect might prove particularly beneficial in penumbral salvage and extending the time window upon which reperfusion therapies will prove beneficial. Moreover, its safety profile across several age groups, underlying neurologic and other comorbid conditions is reassuring, not necessitating narrow screening or specific pre-administration laboratory or imaging testing. This will need to be confirmed in a stroke population.

• Selective cerebral delivery. Intranasal administration has the benefit of bypassing the blood brain barrier and enter the CNS rapidly and achieving significantly higher (up to 100-fold in animals) CNS concentrations compared to intravenous administration.

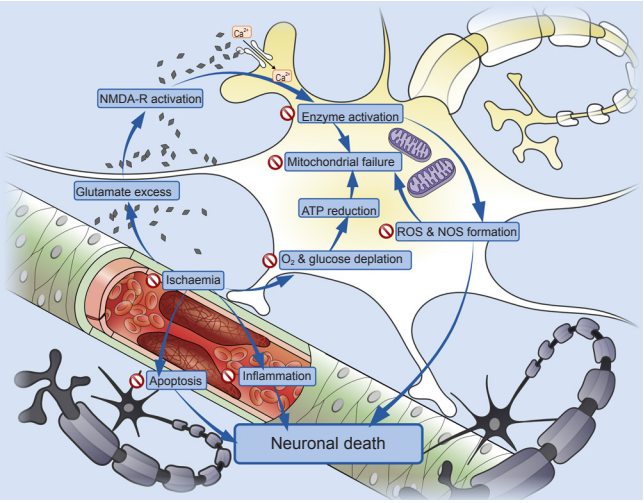

Figure 1 Mechanisms implicated in the ischemic cascade and potential targets for insulin in response to acute ischemia. ***"Stop" signs indicate the steps of the ischemic cascade where insulin can intervene to limit the extent of ischemic damage.***
Image reprinted with permission from Springer, License No. 3670991012466, issued July 16, 2015.

Besides the significant practical and theoretical advantages, there are areas of uncertainty that merit clarification before advancing to a large scale trial:

Additional data from animal stroke models, following randomized, blinded, high-quality experiments. Current information on animal stroke models is limited and the available data, although encouraging could be enhanced by replication from independent groups, improved blinding and randomization of animals, and ideally replication in different animal species.

Additionally, in the case that the pre-hospital administration is investigated in a clinical trial setting, this should be preceded by demonstration of safety in hemorrhagic animal models: Ischemic and hemorrhagic stroke are essentially clinically indistinguishable without ancillary imaging testing (CT brain scan) which is not readily available in the prehospital setting, with rare, remarkable exceptions of studies that are in a pilot phase and are unlikely to become a widespread standard of care. Therefore if pre-hospital administration is to be considered, the safety in intracerebral hemorrhage should be very robustly demonstrated.

The optimal timing and duration of treatment following acute stroke have not been clarified at the moment. Although there is an acute effect, more prolonged administration has yielded robustly positive results in subjects with cognitive impairment (Craft et al., 2012) and insulin's antiapoptotic and neurotrophic effect makes it biologically plausible that it might be beneficial in the subacute and early chronic phase of ischemic stroke during which neuroplasticity mechanisms are crucial. This raises the question whether intranasal insulin administration should not be limited to the acute phase. Proper dosing is also not clearly defined for stroke yet. Animal studies suggest a dose-dependent beneficial effect (Liu et al., 2001) and human data do not raise safety concerns with higher doses. Both of these critical questions could be assessed with early Phase 1 or 2a duration and dose-response studies in acute stroke subjects that.

In summary, intranasally administered insulin possesses many of the ideal properties for acute stroke neuroprotection, due to its plurifunctional mechanism of action, wide applicability, safety and simplicity of CNS distribution. Well-designed animal and phase I human studies are necessary to improve our understanding of its neuroprotective potential in acute stroke.

Dr VN has received grants from the NIH-National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK) (5R21-DK-084463-02 and 1R01 DK103902-01A1) and National Institute on Aging (NIA) (1R01-AG-0287601-A2) and National Institute of Diabetes and Digestive and Kidney Diseases (1R01DK103902-01A1) related to this study, and VN received salaries from these grants.

The authors wish to thank Katharina Dormanns, Ph.D. candidate Brains Trust Research Group, BlueFern Supercomputing Unit (University of Canterbury, NZ), for her contribution in the design of the figure presented in the paper.

Vasileios-Arsenios Lioutas*, Vera Novak

Department of Neurology, Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, MA, USA

***Correspondence to:** Vasileios-Arsenios Lioutas, M.D., vlioutas@bidmc.harvard.edu.*
***Accepted:** 2015-11-12*
***doi:** 10.4103/1673-5374.179040* ***http**://www.nrronline.org/*
***How to cite this article:** Lioutas VA, Novak V (2016) Intranasal insulin neuroprotection in ischemic stroke. Neural Regen Res 11(3):400-401.*

References

Albers GW, Goldstein LB, Hess DC, Wechsler LR, Furie KL, Gorelick PB, Hurn P, Liebeskind DS, Nogueira RG, Saver JL (2011) Stroke Treatment Academic Industry Roundtable (STAIR) recommendations for maximizing the use of intravenous thrombolytics and expanding treatment options with intra-arterial and neuroprotective therapies. Stroke 42:2645-2650.

Berkhemer OA, Fransen PS, Beumer D, van den Berg LA, Lingsma HF, Yoo AJ, Schonewille WJ, Vos JA, Nederkoorn PJ, Wermer MJ, van Walderveen MA, Staals J, Hofmeijer J, van Oostayen JA, Lycklama à Nijeholt GJ, Boiten J, Brouwer PA, Emmer BJ, de Bruijn SF, van Dijk LC, et al. (2015) A randomized trial of intraarterial treatment for acute ischemic stroke. N Engl J Med 372:11-20.

Craft S, Baker LD, Montine TJ, Minoshima S, Watson GS, Claxton A, Arbuckle M, Callaghan M, Tsai E, Plymate SR, Green PS, Leverenz J, Cross D, Gerton B (2012) Intranasal insulin therapy for Alzheimer disease and amnestic mild cognitive impairment: a pilot clinical trial. Arch Neurol 69:29-38.

Duarte AI, Moreira PI, Oliveira CR (2012) Insulin in central nervous system: more than just a peripheral hormone. J Aging Res 2012:384017.

Garg R, Chaudhuri A, Munschauer F, Dandona P (2006) Hyperglycemia, insulin, and acute ischemic stroke: a mechanistic justification for a trial of insulin infusion therapy. Stroke 37:267-273.

Goyal M, Demchuk AM, Menon BK, Eesa M, Rempel JL, Thornton J, Roy D, Jovin TG, Willinsky RA, Sapkota BL, Dowlatshahi D, Frei DF, Kamal NR, Montanera WJ, Poppe AY, Ryckborst KJ, Silver FL, Shuaib A, Tampieri D, Williams D, et al. (2015) Randomized assessment of rapid endovascular treatment of ischemic stroke. N Engl J Med 372:1019-1030.

Huang SS, Lu YJ, Huang JP, Wu YT, Day YJ, Hung LM (2014) The essential role of endothelial nitric oxide synthase activation in insulin-mediated neuroprotection against ischemic stroke in diabetes. J Vasc Surg 59:483-491.

Lioutas VA, Alfaro-Martinez F, Bedoya F, Chung CC, Pimentel DA, Novak V (2015) Intranasal insulin and insulin-like growth factor 1 as neuroprotectants in acute ischemic stroke. Transl Stroke Res 6:264-275.

Liu XF, Fawcett JR, Thorne RG, Frey WH, 2nd (2001) Non-invasive intranasal insulin-like growth factor-I reduces infarct volume and improves neurologic function in rats following middle cerebral artery occlusion. Neurosci Lett 308:91-94.

Lochhead JJ, Thorne RG (2012) Intranasal delivery of biologics to the central nervous system. Adv Drug Deliv Rev 64:614-628.

Reger MA, Watson GS, Green PS, Baker LD, Cholerton B, Fishel MA, Plymate SR, Cherrier MM, Schellenberg GD, Frey WH, 2nd, Craft S (2008) Intranasal insulin administration dose-dependently modulates verbal memory and plasma amyloid-beta in memory-impaired older adults. J Alzheimers Dis 13:323-331.

Thorne RG, Pronk GJ, Padmanabhan V, Frey WH, 2nd (2004) Delivery of insulin-like growth factor-I to the rat brain and spinal cord along olfactory and trigeminal pathways following intranasal administration. Neuroscience 127:481-496.