PERSPECTIVE

Natural product based novel small molecules with promising neurotrophic, neurogenic and anti-neuroinflammatory actions can be developed as stroke therapeutics

Stroke is the world's second leading cause of death and a major cause of adult disability. Among the different kinds of stroke, ischemic stroke is the most widely prevalent type, causing high morbidity and mortality worldwide (Moskowitz et al., 2010). It is an intricate neurological disorder, characterized by reduced blood flow to the brain, deprivation of nutrients and oxygen and hence fails to maintain sustained ATP levels required to meet brain metabolic demands. This triggers diverse adaptive or maladaptive molecular and biochemical pathways and leads to changes in neural and glial functions. The therapeutic use of recombinant tissue plasminogen activator (rt-PA) at early time points of cerebral ischemic stroke, facilitates reperfusion and mitigates the loss of neuronal cells by intrinsic neural plasticity (Mehta et al., 2007). However, the delay of treatment makes it ineffective causing irreversible loss of cells in neural circuitry affecting the functional integrity due to infarct, eventually leading to neuromotor impairment, post-stroke depression, cognitive impairment, *etc.* Stroke deprives the brain nourishment, triggers diverse biochemical changes like reactive oxygen species (ROS) production, inflammation, calcium influx, glutamate excitotoxicity, *etc*, in the neuro-glial milieu of the brain. Drugs developed against these molecular targets proved effective in impeding the stroke damage, when given in combination with reperfusion agents. However, these therapeutics are ineffective in providing neuroprotection for the stroke affected areas and also fall outside the clinical time window.

Like in most of the neurodegenerative disorders, the impairment of neurological functions in ischemic stroke is the result of compromised intrinsic neuroprotective, neurotrophic and neurogenic equilibrium, leading to irreversible damage to the structural and functional integrity of the brain (Chen et al., 2014). The attenuation in neurotrophin levels has been the hallmark of pathogenesis in various neurological and psychiatric disorders and upon the administration of exogenous neurotrophic factors or stimulating the endogenous supply of these neurotrophins effectively ameliorated the severity of the disorders (Nagahara and Tuszynski, 2011; Longo and Massa, 2013). The most widely studied critical neurotrophin, brain derived neurotrophic factor (BDNF), is known to regulate dendritic branching, synaptic plasticity, spine density, long term potentiation (LTP) in the hippocampal region of rodent brain (Lu et al., 2013). Exogenous supply of BDNF ameliorates the ischemic stroke-induced cortical neuronal loss while the impediment in endogenous BDNF functioning exacerbate the damage following the ischemic stroke (Nagahara and Tuszynski, 2011). Though, the approach of exogenously administering neurotrophins seems to be a potent therapeutic alternative in treating neurological disorders, they have so far failed to cross preclinical or clinical stages as they are limited by their clinical utility owing to its proteinaceous nature, stability in the system (bioavailability), blood-brain barrier permeability and non-selectivity. These suboptimal pharmacological properties of neurotrophins can be overcome by using small molecules which mimic neurotrophins or which can augment the expression of neurotrophins (Moskowitz et al., 2010). Some of such compounds are: Atorvastatin, when administered in middle cerebral artery occlusion (MCAO) focal ischemic stroke model have been shown to boost BDNF and vascular endothelial growth factor (VEGF) expression leading to amelioration in stroke damage by promoting the neural repair and regeneration (Chen et al., 2005); Memantine and Clenbuterol, have been reported to reduce the infarct size in mouse focal cerebral ischemia model by boosting the neurotrophin expression, acting synergistically (Culmsee et al., 2004); Ding XS group demonstrated, fluoxetin, a widely prescribed antidepressant which upregulates BDNF expression in normal and ischemic brain, ameliorates the post-ischemic cognitive deficit by promoting hippocampal neurogenesis; Keqiang Ye et al. observed, 7,8-dihydroxyflavone, the agonist of the BDNF receptor tyrosine receptor kinase B (TrkB), which mimics BDNF and shows neuroprotection.

Hence, there is an urgent need to develop drugs which would stimulate the endogenous repair and regeneration of cells in the affected neural area or circuitry to treat post-stroke conditions (Cai et al., 2014). This is indeed the reason the endogenous neurotrophins (BDNF, GDNF, NGF, NT-3 and NT4/NT5, *etc.*) and their receptors are potential targets in the development of stroke therapeutics which are involved in neural remodelling, regeneration and repair of the nervous system (Cai et al., 2014). Recent research efforts in this direction by our groups (Mehta et al., 2012; Chakravarty et al., 2015) and that from others (Butler, 2005; Xu et al., 2014) led to the discovery of a number of molecules based on the scaffolds from natural products that showed remarkable potential to be developed as stroke therapeutics. Our published findings on the novel compounds based on 2-oxa-spiro[5.5]-undecane derived from the natural product Paecilomycine A (Mehta et al., 2012; Chakravarty et al., 2015), as well as some unpublished recent results suggest that the strategy to develop natural product based small molecules which can boost neurite growth and neural regeneration, in addition to their attenuating effect on the neuroinflammation, would effectively ameliorate the post-stroke pathologies and restore the lost structural and functional properties of the infarct region. These and other small molecules or compounds like gambogic amide, maslinic acid, ginsenoside Rb1 might also induce faster recovery if administered in combinatorial therapy with agents that facilitate reperfusion.

The compounds we reported recently as potent neurotrophic, neurogenic and neuroprotective molecules (Chakravarty et al., 2015) are based on natural product inspired scaffolds of Paecilomycine A, synthesised by Diverted Organic Synthesis (DOS). Paecilomycine A promises possible neuroactive properties, as it exhibits potent *in vitro* neurotrophic activity (Xu et al., 2014); however, the complexity of Paecilomycin A structure limits its ease of synthesis. It stimulates us considering Paecilomycin A based scaffolds comp#1 (±)-(1R,2S,4a′S)-2-hydroxy-4-methyl-4a′,5′-dihydro-3′H-spiro[cyclohexane-1,4'-cyclopenta[c]pyran]-3-en-6'(1'H)-one and comp#2 (±)-(1R,2S,4a′S,6′R)-4-methyl-1′,4a′,5′,6′-tetrahydro-3′H-spiro[-cyclohexane-1,4′-cyclopenta[c]pyran]-3-ene-2,6′-diol with different functional groups to check for *in vitro* and *in vivo* neuroactive properties. Comp#1 and comp#2 exhibited potent *in vitro* (N2A) neurotrophic activity when we first evaluated (Mehta et al., 2012). Further evaluation led us to uncover that comp#2 alone and not comp#1 had promising *ex vivo* neurogenic activity, as shown by utilizing neonatal mouse hippocampal neurosphere culture (Chakravarty et al., 2015). Similarly, other groups reported an Iridoid inspired scaffolds demonstrating the neuritogenic activity in primary rat hippocampal neurons and ESC-derived motor neurons (Xu et al., 2014). Jiadifenolide, Jiadifenin and (1R,10S)-2-oxo-3,4-dehydroxyneomajucin (4, ODNM), 03 highly neurotrophic Illicium natural products and its natural product inspired analogues exhibited potent *in vitro* (PC12 cells) neurotrophic activity (Trzoss

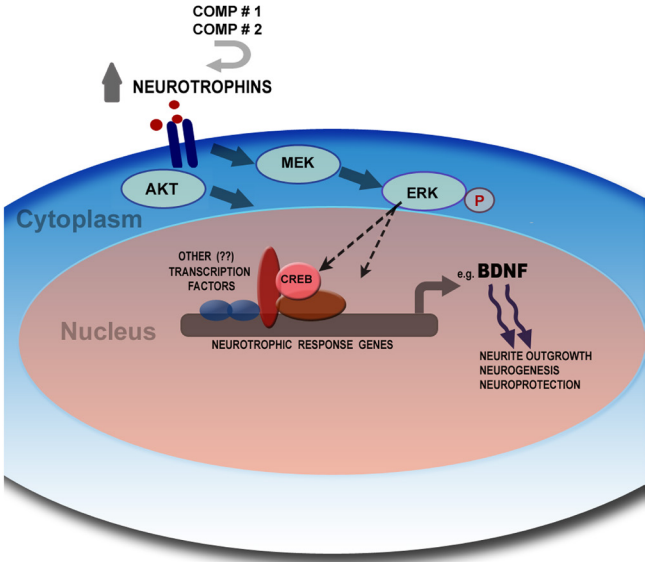

Figure 1 Schematic representation of the possible signalling pathway for the compounds (comp#1 and comp#2).
AKT: Protein kinase B; BDNF: brain derived neurotrophic factor; CREB: cAMP response element binding protein; ERK: extracellular signal-regulated kinase; MEK: mitogen-activated potein kinase; P: phosphorylated.

et al., 2013). These and other findings led us to assess further, whether comp#2 crosses the blood-brain barrier (BBB) and induces neurogenesis, and for that we used zebra fish larvae 3 days post fertilization (dpf) when the BBB just gets established. The result was striking as comp#2 treated larvae showed striking T-junction, analogous to the highly neurogenic niche in mammals, the sub ventricular zone (SVZ). Finally, we tested whether this compound also has proneurogenic action in mouse brain and indeed it had, as shown by the significant level of increase in nestin-GFP positive cells in the subgranular zone (SGZ) of the dentate gyrus, the highly neurogenic niche in nestin GFP transgenic mouse hippocampus. The same conclusion we also reached using the BrdU method after counting the BrdU positive highly proliferating neural progenitor cells in SGZ of C57bl/6 mice (Chakravarty et al., 2015).

Mounting evidence proves that small molecules mimic neurotrophins or modulate neurotrophin expression, exhibiting promising neurotrophic and neurogenic activity, and thus we embarked on to evaluate the modulatory effect of comp#1 and comp#2 on the expression of neurotrophins such as brain derived neurotrophic factor (BDNF), glial cell line- derived neurotrophic factor (GDNF), neurotrophin 3 (NT3), neurotrophin 4 (NT4; also known as NT5). Comp#1 elevated endogenous BDNF, GDNF, NT3 and NGF, while comp#2 induced BDNF, GDNF and NT3 gene expression in Zebra fish larvae, with the possible mediation of the neurotrophic activity taking place via MAPK-ERK signalling (**Figure 1**). Further, comp#1 and comp#2 were investigated for their therapeutic potential in a global ischemic mouse model [bilateral common carotid artery occlusion (BCCAO)], as the reduced neurotrophic environment has been reported in stroke condition. Acute dose of comp#1, which is more neurotrophic, countered the ischemic stroke-induced neural damage and reduction in spine density in the striatal region of mouse brain; while comp#2, which is more proneurogenic but less neurotrophic, failed to provide neuroprotection at acute dosing (Chakravarty et al., 2015). However, majority of stroke affected human subjects reach clinics much after the onset of stroke symptoms associated with severe damage, while most of the stroke therapeutics tested in preclinical studies are administered before or at the time of the onset of stroke, limiting their transformation to clinics. Hence, further studies are required to assess the reproducibility of comp#1 induced neuroprotection, administered at much later time point post-ischemic stroke induction. In addition comp#2 having potent neurogenic activity, if given chronically for longer period post-stroke might have therapeutic utility. So, more studies are warranted with these novel compounds for the development of potential therapeutics to treat stroke.

This work was initiated under the Indo-French "Joint Laboratory for Sustainable Chemistry at Interfaces" and supported by the Council of Scientific and Industrial Research (CSIR) network projects [BSC0103UNDO]. We would like to acknowledge Ms. Swati Maitra for technical assistance for figure preparation and Dr. Srihari Pabbaraja for correct naming of the compounds.

Priya Jhelum, R. Gajendra Reddy, Arvind Kumar, Sumana Chakravarty*

Chemical Biology, CSIR-Indian Institute of Chemical Technology, Hyderabad, India (Jhelum P, Reddy RG, Chakravarty S)
CSIR- Centre for Cellular and Molecular Biology, Hyderabad, India (Kumar A)

***Correspondence to:** Sumana Chakravarty, Ph.D., sumanachak@iict.res.in or sumana98@gmail.com.*
***Accepted:** 2016-04-12*
***orcid:** 0000-0001-8223-6508 (Sumana Chakravarty)*
***doi:** 10.4103/1673-5374.184486*
***How to cite this article:** Jhelum P, Reddy RG, Kumar A, Chakravarty S (2016) Natural product based novel small molecules with promising neurotrophic, neurogenic and anti-neuroinflammatory actions can be developed as stroke therapeutics. Neural Regen Res 11(6):916-917.*

References

Cai J, Hua F, Yuan L, Tang W, Lu J, Yu S, Wang X, Hu Y (2014) Potential therapeutic effects of neurotrophins for acute and chronic neurological diseases. Biomed Res Int 2014.
Chakravarty S, Maitra S, Reddy RG, Das T, Jhelum P, Kootar S, Rajan WD, Samanta A, Samineni R, Pabbaraja S, Kernie SG, Mehta G, Kumar A (2015) A novel natural product inspired scaffold with robust neurotrophic, neurogenic and neuroprotective action. Sci Rep 5:14134.
Chen J, Venkat P, Zacharek A, Chopp M (2014) Neurorestorative therapy for stroke. Front Hum Neurosci 8:382.
Chen J, Zhang C, Jiang H, Li Y, Zhang L, Robin A, Katakowski M, Lu M, Chopp M (2005) Atorvastatin induction of VEGF and BDNF promotes brain plasticity after stroke in mice. J Cereb Blood Flow Metab 25:281-290.
Culmsee C, Junker V, Kremers W, Thal S, Plesnila N, Krieglstein J (2004) Combination therapy in ischemic stroke: synergistic neuroprotective effects of memantine and clenbuterol. Stroke 35:1197-1202.
Lu B, Nagappan G, Guan X, Nathan PJ, Wren P (2013) BDNF-based synaptic repair as a disease-modifying strategy for neurodegenerative diseases. Nat Rev Neurosci 14:401-416.
Mehta G, Samineni R, Srihari P, Reddy RG, Chakravarty S (2012) Diverted organic synthesis (DOS): accessing a new, natural product inspired, neurotrophically active scaffold through an intramolecular Pauson–Khand reaction. Org Biomol Chem 10:6830-6833.
Mehta SL, Manhas N, Raghubir R (2007) Molecular targets in cerebral ischemia for developing novel therapeutics. Brain Res Rev 54:34-66.
Moskowitz MA, Lo EH, Iadecola C (2010) The science of stroke: mechanisms in search of treatments. Neuron 67:181-198.
Nagahara AH, Tuszynski MH (2011) Potential therapeutic uses of BDNF in neurological and psychiatric disorders. Nat Rev Drug Discov 10:209-219.
Trzoss L, Xu J, Lacoske MH, Mobley WC, Theodorakis EA (2013) Illicium sesquiterpenes: divergent synthetic strategy and neurotrophic activity studies. Chemistry 19:6398-6408.
Xu J, Lacoske MH, Theodorakis EA (2014) Neurotrophic natural products: chemistry and biology. Angew Chem Int Ed Engl 53:956-987.