**Nanodelivery of growth hormone in spinal cord injury and Neuroprotection**

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Trauma to spinal cord results in growth hormone (GH) deficiency that is often coupled with insulin like growth factor-1 (IGF-1) reduction in the plasma. Thus, exogenous supplement of GH in SCI may enhance the IGF-1 levels in the cord and induce neuroprotection. Our previous results showed that repeated GH application over the traumatized spinal cord (SC) starting from 30 min before (but not after 1 h) after injury causes neuroprotection.

Thus, other possibility is to enhance the GH effects in SCI by delivering through nanobiotechnology.

In present investigations we delivered TiO2 nanowired (NW) GH after a focal SCI in our rat model and compared the results with normal GH therapy on IGF-1 and GH contents in the plasma and in SC in relation to blood-spinal cord barrier (BSCB) disruption, edema formation and neuronal injuries.

A focal SCI was inflicted in Equithesin anesthetized rats over the right dorsal horn of the T10-11 segments and the animals were allowed to survive 12 or 24 h after trauma. In separate group of rats TiO2 NW-GH (1µg/kg|min for 10 min) was administered over the injured segment 2 h and 4 h trauma using an intrathecally implanted PE-10 polyethylene tubing before the experiment. Another group of rats received normal GH under identical conditions. The plasma (89±8 ng/ml) and SC T9-12 segments (0.34±0.04 ng/g) showed a significant reduction in IGF-1 content as compared to normal controls (plasma 128±10 ng; SC 2.34±0.10 ng) at 12 h that was further diminished 24 after injury (plasma 60±7 ng; SC 0.21±0.06 ng). At this time the GH levels also declined significantly (12 h SCI plasma 5±1 ng/ml; SC 0.21±0.03 ng/g; 24 h plasma 3±1 ng; SC 0.11±0.05 ng) from control (plasma 12±4 ng/ml; SC 0.98±0.04 ng/g). About 400% increase in BSCB permeability to Evans blue albumin (EBA), 12% increase in edema formation and 350 % increase in neuronal injuries were seen 12 h after SCI that was further increased by 2 to 3- fold in all parameters 24 after injury. NW-GH administration markedly enhanced IGF-1 levels (plasma 134±13 ng/ml; SC 3±1 ng/g 12 h; 148±10 ng/ml; 3.6±0.23 ng/g) and GH content (plasma 10±3 ng/ml; SC 0.96±0.12 ng/g 12 h; 13±7 ng/ml; 1.12±0.10 ng/g 24 h) after SCI. Whereas, normal GH was unable to enhance IGF-1 or GH levels in plasma or cord significantly after 12 or 24 h SCI. Interestingly, NW-GH was also able to reduce BSCB disruption, edema formation and neuronal injuries 12 h and 24 h after trauma to the SC whereas, normal GH was ineffective on these parameters as well at all time points examined.

Taken together our results are the first to demonstrate that NW-GH is quite effective in enhancing IGF-1 and GH levels in the cord and plasma that may be crucial in reducing pathophysiology of SCI.

**Neurodegeneration and neuroregeneration in the central nervous system and nanomedicine**

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Previously a combination of BDNF and GDNF (without NGF) induced neuroprotection when given 90 min after spinal cord injury (SCI). In present investigation, we explored the role of CNTF in combination with BDNF and/or GDNF treatment for enhanced neuroprotection given after 90 to 120 min SCI. Since neurotrophins attenuate nitric oxide (NO) production in SCI, the role of carbon monoxide (CO) production that is similar to NO in inducing cell injury was also explored using immunohistochemistry of the constitutive isoform of CO synthesizing enzyme hemeoxygenase-2 (HO-2). SCI inflicted over right dorsal horn (T10-11 segment, 2 mm deep and 5 mm long) increased the HO-2 immunostaining in the T9 and T12 segments at 5 h in the areas associated with breakdown of the blood-spinal cord barrier (BSCB), edema development and cell injuries. Topical application of CNTF with BDNF and GDNF (but not with BDNF or GDNF alone) in combination (10 ng each) 90 and 120 min after SCI over the injured cord significantly reduced the BSCB breakdown, edema formation, cell injury and overexpression of HO-2 in the cord. These observations are the first to show that an addition of CNTF with BDNF and GDNF is enhances neuroprotection during later phase of SCI probably by downregulation of CO production, not reported earlier.

**Key words**: Hemeoxygenase 2, spinal cord injury, BDNF, GDNF, CNTF, blood-spinal cord barrier, spinal cord edema,

**TiO2 nanowired cerebrolysin enhances neuroprotective effects of mesenchymal stem cells**

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Neprilysin (NPL) is an endogenous enzyme that functions as rate-limiting step in amyloid-beta peptide (AbP) degradation. It is widely believed that an imbalance between production and clearance of AbP results in its accumulation leading to development of Alzheimer s Disease (AD). In several cases of AD the metalloprotease NPL brain concentration is decreased. Also NPL knocked out mice exhibited AD like brain pathology and behavioural dysfunctions. This suggests that enhancing the NPL concentartions by therapeutic means may reduce brain pathology in AD.

Few studies indicated that mesenchymal stem cells (MSCs) administration into the brain fluid compartment resulted in enhanced NPL concentration and a reduced AbP accumulation that correlates well with the behavioural and pathological deficit in a mouse model of AD.

Our previous studies have shown that TiO2-nanowired cerebrolysin (CBL), a balanced composition of several neurotrophic factors and active peptide fragments is able to thwart AD pathology following AbP infusion model in rats. However, role of CBL on NPL concentration are not well known. Thus, in present investigation we explored the combined effects of of nanowired MSCs and CBL on NPL and AbP levels in our rat model of AD.

Our observations showed that co-administration of TiO2 nanowired MSCs (106 cells) with 2.5 ml/kg CBL (i.v.) once daily for 1 week starting from 2 weeks after AbP infusion resulted in a significant increase of NPL in hippocampus (400 pg/g) from AbP group (120 pg/g; Control 420±8 pg/g brain). Interestingly these changes were 30 to 45 % less by MSCs or CBL treated alone. The brain pathology in terms of neuronal damage, gliosis and myelin vesiculation was also protected in combined treatment with TiO2 MSCs and CBL in our AD model. These observations show that a combination of CBL and MSCs has superior neuroprotective effects in AD.

**Traumatic spinal cord injury & neuroprotection**

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Recent advancement in nanomedicine suggests that nano drug delivery using nanoformulation enhances neurotherapeutic values of drugs or neurodiagnostic tools for superior effects than the conventional drugs or the parent compounds [1,2]. This indicates a bright future for nanomedicine in treating neurological diseases in clinics. However, effects of nanoparticles *per se* in inducing neurotoxicology, if any is still being largely ignored [3]. The main aim of nanomedicine is to enhance the drug availability within the central nervous system (CNS) for greater therapeutic successes. However, once the drug together with nanoparticles enters into the CNS compartments, the fate of nanomaterial within the brain microenvironment is largely remained unknown. Thus, to achieve greater success in nanomedicine our knowledge in expanding our understanding of nanoneurotoxicology in details is the need of the hour.

In addition, neurological diseases are often associated with several co-morbidity factors, e.g., stress, trauma, hypertension or diabetes. These co-morbidity factors tremendously influence the neurotherapeutic potentials of conventional drugs. Thus, this is utmost necessary to develop nanomedicine keeping these factors in mind. Recent research in our laboratory demonstrated that engineered nanoparticles from metals used for nanodrug delivery significantly affected the CNS functions in healthy animals. These adverse reactions of nanoparticles are further potentiated in animals associated with heat stress, diabetes, trauma or hypertension. These effects nanomaterials were dependent on their composition and the doses used. Thus, drugs delivered using TiO2 nanowired enhanced the neurotherapeutic potential of the parent compounds following CNS injuries in healthy animals. However, almost double doses of nanodrug delivery are needed to achieve comparable neuroprotection in animals associated with anyone of the above co-morbidity factors. Thus, cerebrolysin delivered either though TiO2-nased nanowires or PLGA-nanoparticles effectively reduced brain pathology in several diverse neurological diseases often complicated with various co-morbidity factors. Taken together, it appears that while exploring new nanodrug formulations for neurotherapeutic purposes, co-morbidly factors and composition of nanoparticles require great attention. Furthermore, neurotoxicity caused by nanoparticles *per se* should be examined in greater details before using them for nanodrug delivery in patients.

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