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Review Article

New Perspectives of Nanoneuroprotection, Nanoneuropharmacology and Nanoneurotoxicity

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Abstract

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. 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. 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 of 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. 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.

INTRODUCTION

Nanoparticles (NPs) or microfine particles present in the environment could enter into the body fluid compartments through inhalation are liable to affect brain functions [1]. Engineered NPs from metals, industrial by products, motor vehicle exhaust, or from the environment e.g., regular exposure of silica dust in desert could affect Humans health depending on the magnitude and intensity of the initial exposure [2,3]. However, detailed studies on NPs induced neurotoxicity in the central nervous system (CNS) in vivo situations are still lacking.

Recent pharmacological studies explored new ways to enhance drug delivery to the brain using a variety nano-formulations or nano-drug delivery techniques [4,5] or their use for neurodiagnostic purposes [2]. Nano drug delivery using nanoformulation enhances greater therapeutic success by readily crossing the blood-brain barrier (BBB) and/or remaining in the CNS for longer periods of time due to their slow release and/or degradation [2,4-6]. Using this principle, NPs when attached to specific-antibodies could precisely be used for better neurodiagnosis for tumor and other neurological diseases [2,4-6].

Thus, while developing nanoformulations or for neurodiagnoses or therapy, the effects of NPs *per se* causing possible adverse effects on the cells and tissues in the biological system should be evaluated in great details. For this purpose, additional efforts should be made to attenuate the adverse effects of NPs or nanoneurotoxicity while developing new tools for nanomedicine or nanoproducts in healthcare.

Another important issue in developing nanomedicine for future clinical therapy is to understand the role of NPs in biological system in normal and in stressful situations. Stressors of various kinds are known to open the BBB and induce brain pathology [7-9]. Thus, it is quite likely that in situations of stress, NPs could exacerbate their neurotoxic effects within the CNS [8,9]. An increased penetration of NPs within the CNS due to stress-induced disruption of the BBB could paly important detrimental roles in health and diseases.

There is still very little indication on the fact that infliction of additional stress or trauma during NPs intoxication could exacerbate brain pathologies. Alternatively, NPs induced neurotoxicity could also be modified by the presence of various vascular or metabolic diseases. Thus, there is an urgent need to understand the NPs-induced alterations in the CNS functions in disease processes and their possible modulation with co-morbidity factors, e.g., hypertension, diabetes, and/or trauma or stress. Without expanding our knowledge in these directions, any attempt to develop nanomedicine for treating neurological disease in patients suffering from various co-morbidity factors would not be successful in clinical practices.

On one hand, enhanced passage of drugs with or without nanoformulations is the need of hour to treat brain diseases such as, tumors, bacterial or viral infections, inflammation and/or local or global ischemic-hypoxic damages; the nano-drug induced neurotoxicity on the other hand is an equally important aspect to explore seriously [see 1-3].

Unfortunately, research on nanoparticle neurotoxicity in vivo situations is still not well recognized. Keeping these views in consideration, our laboratory has focused on the potential adverse effects of nanoparticles on the CNS structure and function in different animal models in great details. The salient new trends and emerging concepts on nanoneurotoxicity in nanomedicine based on our own investigations is discussed briefly in this review.

Nanoparticles induce neurotoxicity

Data from our laboratory show that engineered NPs from metals e.g., Cu, Ag, Al or microfine particles like silica dust (SiO_2) and MnO_2 in the size range of 50 to 60 nm when administered in rats or mice in a dose of 60 to 80 mg/kg (i.p.), 25 to 40 mg/kg (i.v.) or 25 to 75 µg in 20 µl through intracerebroventricular (i.c.v.) route induce neurotoxicity within 4 [10]. Thus, breakdown of the BBB to Evans blue albumin was seen in several areas that are associated with and neuronal injuries [10]. These changes were further enhanced 24 h after administration of these NPs [1,10]. This indicates that NPs could influence brain function and induces cellular damage probably by disrupting the BBB function.

Our experiments further show that chronic treatment with a mild dose of NPs for one week (25 to 50 mg/kg, i.p. per day for 7 days) resulted in similar breakdown of the BBB and neuronal injuries in normal rats [10, unpublished observations]. This effect was most pronounced by treatment with Cu and Ag NPs followed by SiO_2, MnO_2 and Al [1,10-12]. This suggests that the composition or inherent properties of NPs are important contributors in nanoneurotoxicity. Furthermore, a mild alteration in sensory and cognitive functions on Rota-rod performances, inclined plane angel test and grid walking sessions was also observed at the time of the BBB breakdown [10]. These observations are in line with the idea that mild brain injuries and BBB disruption could affect sensory-motor function in healthy rats or mice. However, mice appear to be less sensitive in NPs neurotoxicity as compared to rats indicating a possible species difference in nanoneurotoxicity.

Size dependent neurotoxicity of nanoparticles

To further investigate the size related neurotoxicity of NPs, we administer Cu and Ag NPs in the size range of 20-30 nm, 50-60 nm or 80 to 90 nm in rats in a dose of 50 mg/kg, i.p. for 7 days. On the 8th day we evaluated BBB disruption and neuronal injuries. Our results showed an inverse relationship between size of the NPs and brain damage [Sharma HS unpublished observation]. This suggests that size of NPs is also crucial while developing nanomedicine or nanoformulations. However, Ag was more neurotoxic than Cu in all sizes used indicating that the composition of NPs and size both could play important determining roles in neurotoxicity [1,10,11]. Thus, composition and size of NPs should be carefully evaluated for nanoformulation for therapeutic purposes.

Nanoneurotoxicity are exacerbated in stress or trauma

Exposure of SiO2 NPs is quite common in human populations in desert environment in association with high environmental temperature. Thus, civilians or military personnel during combat exercise or peace-keeping forces in desert environments are frequently exposed to SiO_2 NPs at high environmental heat conditions [11,13]. In such situations, spinal cord or head injuries in military personnel during war like situations is quite frequent. Thus, it is interesting to examine whether in these individuals SiO_2 exposure may further aggravate neurotoxicity in combination with hyperthermia and/or trauma using model experiments.

SiO2 treated rats (50-60 nm, 50 mg/kg, i.p., once daily for 7 days) when subjected to a focal spinal cord injury (SCI) (ref. 13) or closed head injury (CHI, Sharma HS unpublished observations) exhibited 50 to 180 % more increase in edema formation and neuronal injuries. In these animals the BBB breakdown to Evans blue albumin and radioiodine was exacerbated by 200 to 350 %. This indicates that NPs treatment exacerbate pathophysiology of CNS injuries [11,13].

In another experiments, when NPs treated rats were exposed to 4 h heart stress in a biological oxygen demand incubator (BOD) maintained at 38° C (relative humidity 45-47 %, wind velocity 20 to 25 cm/sec) they exhibited 300 to 450 % higher brain edema formation and 350 to 310 % increase in $^{[131]}$ Iodine leakage in the brain [1,14]. The magnitude and intensity of neuronal, glial and myelin damage were 4 to 6 times higher than rats exposed to identical heat stress treated with saline [1,13,14]. This suggests

that NPs intoxication exacerbates BBB damage. Although the detailed mechanism underlying exacerbation of NPs induced brain damage is unclear, it seems likely that enhanced transport of neurodestructive elements released after trauma or stress in the periphery to the brain induced by NPs could play key roles. Alternatively, increased oxidative stress by NPs may also account for greater brain damage (see below). Thus, therapeutic aspects of nanomedicine and nanoformulations require additional caution based on the external or internal disturbances in the homeostasis of patients either caused by trauma or hyperthermia.

Co-morbidity factors exacerbate nanoneurotoxicity

In addition to stress or trauma, many neurological diseases, e.g., stroke, dementia are associated often with different co-morbidity factors viz., hypertension and/or diabetes. Under such situations, treatment strategies with neuroprotective agents normally do not work effectively. Thus, use of nanomedicine under such circumstances may also require additional modification. This is because of the reason that NPs toxicity could be further affected by diabetes and /or hypertension in these patients.

Using animal models of hypertension or diabetes we examined neurotoxicity of NPs or nanowires used for drug delivery. Chronic hypertension was produced by 2-kidney one clip (2K1C) procedure [15]. Diabetic rats were made by streptozotocine administration (75 mg/kg, i.p. daily for 3 days) [16]. These animals do not exhibit BBB breakdown, brain edema or neural injuries. However, when these hypertensive or diabetic animals were administered Cu or Ag NPs (50-60 nm) for 1 week (50 mg/kg, i,p,) profound brain edema formation (+140 to 180 %), BBB breakdown to radioiodine (+220 to 260 %) along with neural damages (+80 to 120 %) were seen in different parts of the brain as compared to identically treated healthy animal. This indicates that co-morbidity factors, e.g., hypertension or diabetes could exacerbate NPs-induced neurotoxicity. It appears that brain tissues or cerebral endothelial cells in hypertensive or diabetic animals are more susceptible to NPs-induced toxicity, the details of which are still unclear.

Nanodrug delivery induces neuroprotection

The possibility that drugs delivered with nano-formulations may have enhanced neuroprotective effects was examined in a rat model of SCI. We labeled three different types of drugs to TiO_2 nanowires (50-60 nm) using standard procedures [6,17]. Our observation shows that nanowired drug delivery enhanced neuroprotection in SCI at 5 h as compared to the parent compound alone. However, among the three compounds chosen, the best effects was always observed in SCI with the drug that was most superior among them in reducing spinal cord pathology given without nanowired delivery [17]. This indicates that nanowired delivery did not change the property of the drug but only enhances its effects within the CNS.

Enhanced neuroprotective effects of the nanowired drugs may either be due to their ability to penetrate faster into the CNS and/or a reduction in drug catabolism due to their binding with nanomaterials. Obviously, nanowired drugs could enhance the half-life of the compound. However, our observations indicate that TiO_2 nanowires itself when administered induced some minor pathological changes in the cord in normal animals [Sharma HS unpublished observations]. Thus, long-term effects of nanowired drugs should be examined in great details.

Nano-drug delivery requires dose adjustment with co-morbidity factors

TiO_2 Nanowired attached to neuroprotective drugs was also able to reduce brain damage in hyperthermia caused by heat stress more effectively than the parent compound [14]. Thus, an antioxidant compound H-290/51 was nanowired and administered 30 min after 4 h heat stress at 38°C in saline treated group markedly reduced the brain pathology. On the other hand, when NPs treated rats ware subjected to identical heat stress, the nanowired treatment failed to attenuate brain damage [14]. This indicates that nanowired drugs could not reduce nanoneurotoxicity following a combination of NPs and heat stress.

Likewise, nanowired H-290/51 treatment given in diabetic rats after identical heat stress did not affect brain pathology. However, when the dose of nanowired drug was increased by 100 %, moderate neuroprotection could be seen in NPs treated or diabetic animals after heat exposure [18]. This suggests that the dose of nanowired drugs require considerable adjustment to achieve neuroprotection in animals associated with co-morbidity factors.

Nanoparticles induce oxidative stress in the CNS

Available evidences suggest that NPs induce oxidative stress in the CNS causing nanoneurotoxicity [19]. Interestingly, many drug carriers used for nano delivery, e.g., nanowires, liposomes or carbon nanotubes may also induce mild to moderate oxidative stress [20]. Studies carried our in our laboratory showed that engineered NPs e.g., Cu, Ag, Al, microfine particles SiO_2, MnO_2, or synthetic nanowires TiO_2 when administered systemically are capable to cause oxidative stress in different brain regions [19; Sharma HS unpublished observations]. In general, a significant decline in glutathione levels and marked increase in malondialdehyde, myeloperoxidase and luciferases are seen in cerebral cortex, hippocampus, thalamus, hypothalamus, cerebellums, brain stem and spinal cord after nanoparticle treatment [19]. The magnitude and intensities of oxidative stress caused by these nanoparticles was further exacerbated in diabetic or hypertensive rats as compared to normal healthy animals. The changes in oxidative stress parameters correlate well with neuronal damage and BBB breakdown to radioiodine in several brain areas.

Obviously, future development of nanomedicine requires great caution to avoid neurotoxicity caused by NPs in neurological diseases. Furthermore, the nanoneurotoxicity could be further enhanced in patients suffering simultaneously with other vascular or metabolic diseases.

CONCLUSION AND FUTURE PERSPECTIVES

In conclusion, further research on NPs is needed to understand whether nanomedicine or nanodrug delivery could cause any potential neurotoxicity in healthy individuals. In addition, efforts should be made that co-morbidity factors e.g., diabetes,

hypertension, trauma or hyperthermia that are often associated with neurological diseases may not exacerbate nanoneurotoxicity following nano-drug delivery. The dose of nanomedicine may be adjusted or modified according to external or internal environmental factors as they could influence the outcome with regards to nanoneuroprotection and/or nanoneurotoxicity.

DISCLOSURE

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