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MINIREVIEW ARTICLE

New perspectives of nanoneuroprotection, nanoneuropharmacology and nanoneurotoxicity: modulatory role of amino acid neurotransmitters, stress, trauma, and co-morbidity factors in nanomedicine

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Abstract Recent advancement in nanomedicine suggests that nanodrug delivery using nanoformulation of drugs or use of nanoparticles for neurodiagnostic and/or neurotherapeutic purposes results in superior effects than the conventional drugs or parent compounds. This indicates a bright future for nanomedicine in treating neurological diseases in clinics. However, the effects of nanoparticles per se in inducing neurotoxicology by altering amino acid neurotransmitters, if any, are 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 understanding nanoneurotoxicology in detail is utmost important. In addition, how co-morbidity factors associated with neurological disease, e.g., stress, trauma, hypertension or diabetes, may influence the neurotherapeutic potentials of nanomedicine are also necessary to explore the details. Recent research in our laboratory demonstrated that engineered nanoparticles from metals or titanium nanowires used for nanodrug delivery in laboratory animals markedly influenced the CNS functions and alter amino acid neurotransmitters in healthy animals. These adverse reactions of nanoparticles within the CNS are further aggravated in animals with different co-morbidity factors viz., stress, diabetes, trauma or hypertension. This effect, however, depends on the composition and dose of the nanomaterials used. On the other hand, nanodrug delivery by TiO_2 nanowires enhanced the neurotherapeutic potential of the parent compounds in CNS injuries in healthy animals and do not alter amino acids balance. However, in animals with any of the above co-morbidity factors, high dose of nanodrug delivery is needed to achieve some neuroprotection. Taken together, it appears that while exploring new nanodrug formulations for neurotherapeutic purposes, co-morbidly factors and composition of nanoparticles require more attention. Furthermore, neurotoxicity caused by nanoparticles per se following nanodrug delivery may be examined in greater detail with special regards to changes in amino acid balance in the CNS.

Keywords Nanoparticles · Neurotoxicity · Nanomedicine · Neural injury · Blood–brain barrier disruption · Co-morbidity factors · Stress · Diabetes · Hypertension neuroprotection · Neuropathology

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Introduction

Central nervous system (CNS) injury is a complex in which several factors and neurochemicals play collective roles

(Sharma 2012; Sharma and Westman 2004). With higher incidences of environment pollution, industrial wastes and contamination of drinking water, food and plants in recent years resulted in an enhanced disease processes affecting mankind including heart attack, diabetes, cancer, neurodegeneration, Alzheimer's, Huntington's and neurovascular disabilities (Sharma 2009a, b; Sharma and Sharma 2012a). Recent data suggests that breathing of microfine particles from the environment could enhance cardiovascular and CNS dysfunctions (Singh 2010; Singh and Nalwa 2007; Zhao and Nalwa 2007; Sharma and Sharma 2007). However, the detail mechanisms and/or functional significance of such observations are still not well supported by the scientific evidences.

Nanoparticles or microfine particles present in the environment when entering into the body fluid compartments through breathing could affect brain functions (Sharma 2009a, b). Engineered nanoparticles from metals, industrial byproducts, motor vehicle exhaust, or from the polluted environment and/or accidental or regular exposure to microfine particles, e.g., silica dust in desert environment, could cause serious health consequences in Humans depending on the magnitude and intensity of the initial exposure (Sharma et al. 2009a, b, c, 2010a, b, c; Sharma and Sharma 2012b). However, studies focusing on the role of nanoparticles in inducing neurotoxicity in the CNS in vivo situations are still lacking.

There are reasons to believe that nanoparticles when entering into the microenvironment of the CNS could affect neurochemical metabolism and induce oxidative stress (Sharma 1998; Sharma and Sharma 2010a, 2012a). A possibility exists that these nanoparticles could also enhance excitotoxicity leading to neuronal death (Lafuente et al. 2011). However, role of nanoparticles on amino acid neurotransmission is still a new subject and require detailed investigations.

On the other hand, recent pharmacological studies explored new ways to enhance drug delivery to the brain using a variety nanoformulations or nanodrug delivery techniques (Sharma et al. 2009c; Tosi et al. 2011; Tian et al. 2012). In addition, nanoparticles are used for neurodiagnostic purposes (Fisher et al. 2012; Uchegbu and Siew 2012). It is believed that nanodrug delivery or nanoformulation of drugs will enhance greater therapeutic success by readily crossing the blood–brain barrier (BBB) or remaining for long periods within the CNS due to slow release and/or degradation because of nanodrug-binding in vivo (Menon et al. 2012; Sharma et al. 2007, 2009c; Sharma and Sharma 2012a, b; Tian et al. 2012). An enhanced binding of nanoparticles to targets by nano-antibody/tools complex, precision neurodiagnosis is also possible within the CNS (Sharma and Sharma 2012d; Sharma 2009a).

However, in developing nanoformulations or for neurodiagnoses or therapy, the effects of nanoparticles per se causing possible adverse effects on the cells and tissues or alterations in the amino acid neurotransmitters within the CNS leading to brain pathology are still being largely ignored (Sharma 2000, 2002, 2007a, b; Sharma et al. 2009a, b, c, d, 2010a, b, c; Sharma and Sharma 2007, 2012a, b; Muresanu et al. 2011a, b; Lafuente et al. 2011, 2012). Thus, additional efforts should be made to attenuate adverse effects of nanoparticles or nanoneurotoxicity in relation to amino acids metabolism while developing new tools for nanomedicine or nanoproducts in healthcare.

Another important issue in developing nanomedicine for routine clinical therapy is to understand the role of nanoparticles in biological system in normal and stressful situations (Sharma and Westman 2004; Sharma 2009a, b; Sharma and Sharma 2010a, b). Stressors of various kinds are known to open the BBB and induce brain pathology (Sharma 1982, 1999, 2004a). Thus, it is quite likely that in situations of stress, nanoparticles could exacerbate their neurotoxic effects in the CNS (Sharma and Sharma 2007, 2012a, b). An increased penetration of nanoparticles within the CNS due to stress-induced disruption of the BBB could paly important detrimental roles (Sharma and Westman 2004).

Furthermore, this is still not known whether infliction of additional stress or trauma in nanoparticles intoxication will exaggerate brain pathologies. Likewise, nanoparticle-induced neurotoxicity may also be affected by different vascular or metabolic diseases (Feng et al. 2010, 2011; Sharma et al. 2009e). Thus, there is an urgent need to understand the nanoparticle-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 (Sharma and Sharma 2012a, b). 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.

However, on one hand, enhanced passage of drugs with or without nanoformulations is the need of the hour to treat brain diseases such as, tumors, bacterial or viral infections, inflammation and/or local or global ischemic-hypoxic damages; the nanodrug induced neurotoxicity on the other hand is an equally important aspect to explore seriously (Sharma 2009a, b; Sharma and Sharma 2012a, b).

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 detail. The salient new trends and emerging concepts on

nanoneurotoxicity in nanomedicine based on our own investigations are discussed briefly in this review.

Nanoparticles affect blood–brain barrier dysfunction

Blood–brain barrier (BBB) strictly regulates the fluid microenvironment of the brain strictly within a narrow limit (Sharma 1999, 2009a, b; Sharma and Westman 2004) (Fig. 1). Peripheral alterations in protein, neurochemicals, peptides, hormones and many other toxins are thus not allowed to entering into the brain fluid compartments by this physiological dynamic barrier (Sharma 2004a, b). The anatomical composition of the barrier lies within the endothelial cells of the cerebral capillaries that are connected with the tight junctions, a feature lacking in peripheral vessels (Sharma 1982, 1999; Sharma and Westman 2004). Moreover, the cerebral capillaries normally do not posses microvesicles for intracellular transport, although this form of transport is quite common in non-cerebral capillaries (Rapoport 1976). Thus, the endothelial cell membrane joined by tight junctions represent an extended plasma membrane barrier that could only allow passage of essential nutrients from blood to brain based on their physicochemical properties and also excretion of waste materials from the brain to the vascular compartments (see Rapoport 1976).

When this barrier is broken down either due to alterations in structural integrity of the cell membrane of the endothelia or widening of the tight junctions, peripheral proteins, toxins, vasoactive material, neurochemicals and other immunologically active substances could gain entry into the CNS (Rapoport 1976; Sharma and Westman 2004; Sharma 1999; Sharma 2009a, b; Sharma and Sharma 2010a). This could lead to adverse cellular reactions or injuries within the brain. Moreover entry of serum proteins could allow passage of water from the vascular comportment to the bran microenvironment causing edema formation and subsequently cell injury or death (Sharma 2009a).

There are reasons to believe that nanoparticles of various sizes and composition could induce a breakdown of the BBB function either through a direct or indirect mechanisms leading to extravasation of serum proteins into the brain and edema formation (Sharma et al. 2009a, b, c, d, 2010a, b, c). Previous studies form our laboratory showed that engineered nanoparticles form metals are able to induce leakage of Evans blue albumin and radioiodine in selective regions the brain causing neuronal, glial and axonal injuries (see below). This increase in the BBB permeability to large molecules could also be modulated by alterations in the amino acid neurotransmitters in the CNS (Lafuente et al. 2011; Muresanu et al. 2011a, b, 2012; Sharma 2004b). This idea is supported by the fact that in a rat model of spinal cord injury (SCI) or hyperthermia, an increase in excitotoxicity, i.e., upregulation of glutamate and aspartate levels coincide the BBB damage and neuronal injuries accompanied with edema formation (Sharma

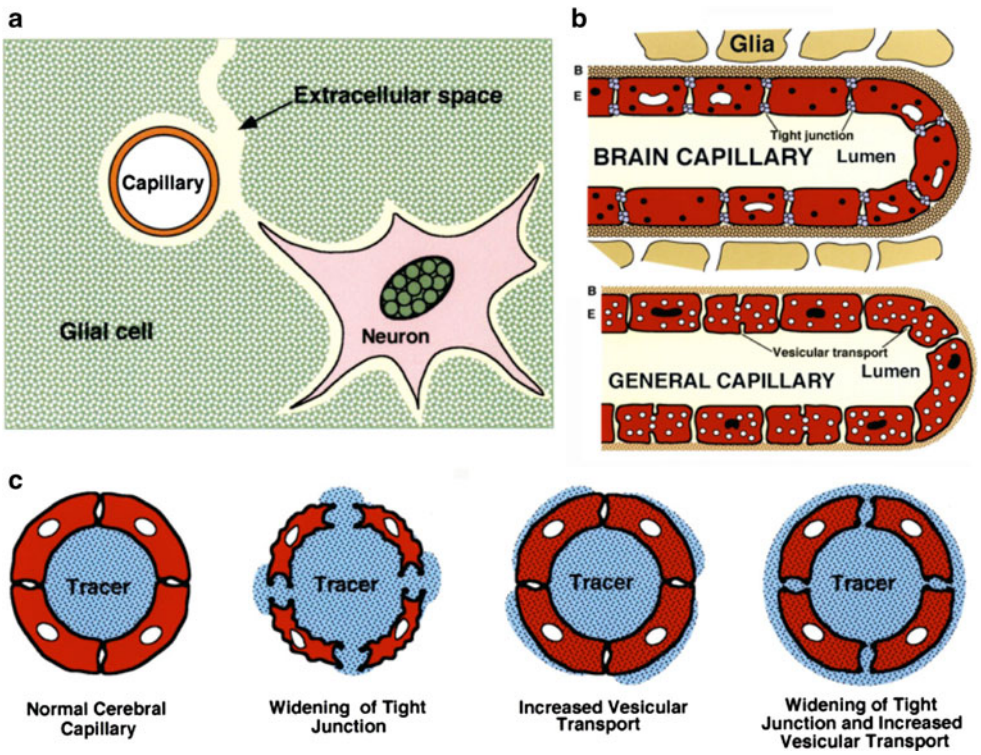

Fig. 1 Blood–brain barrier physiology (**a**), anatomy (**b**) and possible ways for breakdown (**c**). *B* basal lamina, *E* endothelial cell. Data after Sharma (1999) (with permission)

and Sjöquist 2002; Sharma et al. 1998; Sharma 2002). Thus, involvement of nanoparticles in modulation of amino acid neurotransmitters in the CNS is quite likely and requires detailed investigations.

Nanoparticles induce neurotoxicity

Our laboratory data show that engineered nanoparticles from metals, e.g., Cu, Ag, Al or microfine particles like silica dust (SiO_2), MnO_2 in the size range of 50–60 nm, when administered in rats or mice in a dose of 60–80 mg/kg (i.p.), 25–40 mg/kg (i.v.) or 25–75 µg in 20 µl through intracerebroventricular (i.c.v.) route induce neurotoxicity within 4 h (Sharma et al. 2009a, b, c, d, 2010a, b, 2011; Sharma and Sharma 2007, 2012a, b). This is evident with the breakdown of the BBB to Evans blue albumin and neuronal injuries in blue stained brain areas (Sharma 2007a, b, 2009a) (Fig. 2). These changes were further aggravated 24 h after administration of nanoparticles (Sharma et al. 2009a, b, c). This indicates that nanoparticles could influence brain function and induces cellular damage probably by disrupting the BBB function (see Sharma and Sharma 2012a, b).

Our experiments further show that chronic treatment with a mild dose of nanoparticles for 1 week (25–50 mg/kg, i.p. per day for 7 days) resulted in similar breakdown of the BBB and neuronal injuries in normal rats (Sharma et al. 2009c, unpublished observations). This effect was most pronounced by treatment with Cu and Ag nanoparticles followed by SiO_2, MnO2 and Al (Sharma and Sharma 2012a, b; Sharma et al. 2011). This suggests that the composition or inherent properties of nanoparticles 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 were also observed at the tome of the BBB breakdown (Sharma and Sharma 2007). These observations suggest that mild brain injuries and BBB disruption could affect sensory-motor function in healthy rats and mice. However, mice appear to be less sensitive in nanoparticle neurotoxicity as compared to rats indicating a possible species difference in nanoneurotoxicity (Sharma et al. 2009a, b; Sharma and Sharma 2012b).

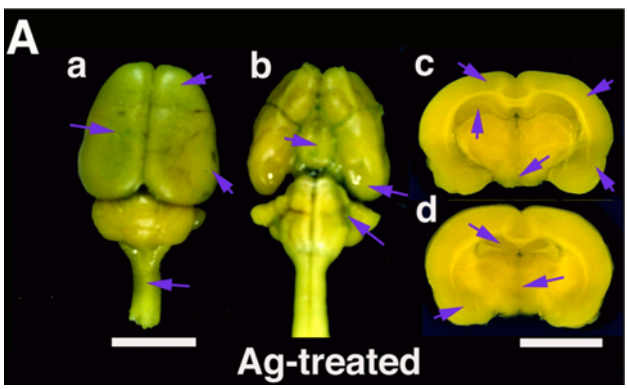

Fig. 2 Ag nanoparticle neurotoxicity. Leakage of Evans blue (*arrows*) could be seen on the dorsal (*a*), ventral (*b*) and coronal (*c*) parts of the brain after Ag nanoparticles intoxication in the rat. Data modified after Sharma et al. (2009a) (with permission). *Bar* 5 mm

Size dependent neurotoxicity of nanoparticles

To further investigate the size effects of nanoparticles, we administer Cu and Ag nanoparticles in the size range of 20–30 nm, 50–60 nm or 80–90 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 nanoparticles and brain damage indicating that smaller sizes of nanoparticles could produce more damages in the brain in vivo situations (Sharma HS unpublished observation). This suggests that size of nanoparticles is also crucial while developing nanomedicine or nanoformulations. However, Ag was more neurotoxic than Cu in all sizes used indicating that both the composition of nanoparticles and size could play important determining roles in neurotoxicity (Sharma 2009a, b). Thus, composition and size of nanoparticles should be carefully evaluated for nanoformulation in therapeutic usage.

Nanoparticles alter amino acid imbalances in the CNS

Previous reports from our laboratory showed that a focal hyperthermia leading to brain pathology alters the balances between excitatory amino acids Glutamate and Aspartate and inhibitory amino acids GABA and glycine (Sharma 2006, 2007a, b). Thus, at the end of 4 h periods of hyperthermia at 38 °C in a biological oxygen demand (BOD) incubator in rats there was a significant increase in glutamate and aspartate in the cerebral cortex, hippocampus, cerebellum, thalamus, hypothalamus and brainstem whereas these brain structures showed a marked decline in the GABA and glycine levels (Sharma 2006) (see Fig. 3). This suggests that an increased excitotoxicity and a reduction in inhibitory amino acid neurotransmitter levels could cause brain pathology. These imbalances in the amino acid neurotransmitter levels were significantly reduced in animals that are treated with various neuroprotective drugs before the heat stress, e.g., naloxone, indomethacin, p-chlorophenylalanine (p-CPA) or brain derived neurotrophic factor (BDNF) (Sharma HS unpublished observations, Sharma 2007a, b; Sharma et al. 1998; Sharma et al. 2000) (Table 1). This suggests that alterations in normal balance

between excitatory and inhibitory neurotransmitters result in brain pathology and corrections in these imbalances will induce neuroprotection.

Since engineered nanoparticles intoxication in heat stress exacerbates brain pathology (Sharma et al. 2009a, c), our laboratory investigated the role of amino acid neurotransmitters in such situations. Our observations suggest that chronic intoxication of Ag, Cu and Al nanoparticles (50–60 mg/kg, i.p. daily for 7 days) resulted in exacerbation of brain pathology after identical heat stress in rats (Sharma et al. 2009c, 2011b). In these animals, measurement of amino acid neurotransmitters showed about four- to sixfold increase in glutamate and five- to sevenfold elevation of aspartate in the cortex, hippocampus and in cerebellum. Furthermore, about two- to threefold decrease in GAB and four- to sixfold decline in glycine was observed in these brain areas (Sharma HS and Sharma A, unpublished observations). This indicates that nanoparticles aggravate amino acids neurotransmitter imbalances leading to enhanced brain damage.

Interestingly, cerebrolysin, a smart combination of various neurotrophic factors such as BDNF, glial derived neurotrophic factor (GDNF), ciliary neurotrophic factor (CNTF), insulin like growth factor-1 (IGF-1), nerve growth factor (NGF) and other active peptide fragments (Sharma et al. 2012b, c, d) if administered (2.5 or 5 ml/kg, i.v.) 30–60 min after heat stress in normal animals resulted in restoration of amino acid imbalances in the cortex and hippocampus along with marked neuroprotection (Sharma HS unpublished observations). However, in nanoparticles intoxicated animals, nanodrug delivery of cerebrolysin (2.5 ml or 5 ml/kg, i.v.) at identical periods (30 or 60 min) after heat stress is needed to induce neuroprotection and in restoration of amino acids imbalances in the brain (see below). Normal delivery of cerebrolysin in nanoparticles treated animals after heat stress did not induce marked neuroprotection or restoration of amino acids imbalances. These observations suggest that nanoparticles exacerbate amino acid neurotransmitters release or accumulation in the brain causing neuronal injuries. These imbalances in CNS excitatory and inhibitory amino acids are further aggravated after heat stress in nanoparticles treated animals. Taken together our results suggest that nanoparticles influence amino acid neurotransmitters and thus responsible for enhanced brain damage in our model.

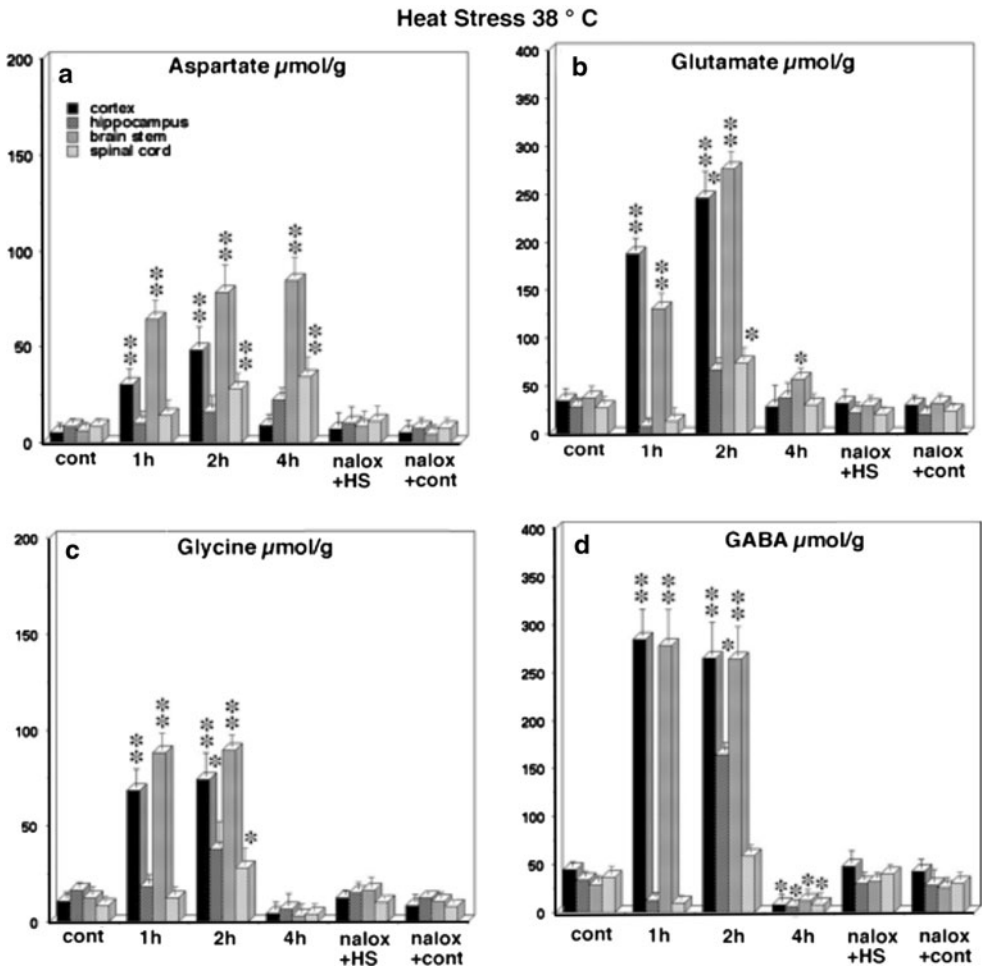

Fig. 3 Changes in amino acid neurotransmitters level in the CNS following heat stress and their modification with naloxone. $^{*}P < 0.05$; $^{**}P < 0.01$ from control value. Student's unpaired t test. Data after Sharma (2006) (with permission)

Table 1 Brain edema and cell injury following heat stress and its modification with naloxone pretreatment

Type of exp.	Regional brain water content (%)				CNS damage/distortion		
	Cortex	Hippocampus	Brain stem	Spinal cord	Nerve cells	Glial cell	Myelin
Control ($n = 5$)	76.86 ± 0.23	78.42 ± 0.21	68.56 ± 0.23	64.35 ± 0.21	Nil	Nil	Nil/?
Naloxone 10 mg/kg, i.p. ($n = 5$)	76.04 ± 0.08	77.82 ± 0.11	67.67 ± 0.37	64.47 ± 0.10	Nil	Nil	Nil/?
1 h heat stress ($n = 6$)	76.38 ± 0.28	78.56 ± 0.23	68.77 ± 0.43	64.85 ± 0.33	Nil/?	Nil/?	Nil/+
2 h heat stress ($n = 6$)	76.78 ± 0.44	78.67 ± 0.42	68.76 ± 0.56	65.78 ± 0.54	Nil/+	Nil/+	Nil/+
4 h heat stress ($n = 6$)	80.54 ± 0.23***	81.34 ± 0.23***	73.24 ± 0.19***	67.34 ± 0.14***	++++	++++	++++
Naloxone 10 mg + 4 h heat stress ($n = 6$)	77.45 ± 0.18**[a]	79.48 ± 0.11**[a]	69.38 ± 0.45**[a]	65.67 ± 0.21**[a]	++	++	++
Naloxone 5 mg +4 h heat stress ($n = 6$)	80.23 ± 0.56***	80.76 ± 0.45***	72 ± 0.33***	66.86 ± 0.34	++++	+++?	+++?
Naloxone 1 mg +4 h heat stress ($n = 6$)	81.76 ± 0.34[b]	82.34 ± 0.22[b]	74.65 ± 0.26[b]	67.67 ± 0.13[b]	++++	++++	++++

Naloxone was given (1 mg, 5 mg or 10 mg/kg, i.p.) 30 min before the onset of heat stress

Regional brain water content was measured in identical sample sizes (80–300 mg) used to determine amino acid neurotransmitters in various experimental groups

Nil, absent; +, occasional; ++, mild; ++++, severe; ?, unclear. (Data after Sharma 2006 with permission)

Values are mean ± SD, ** $P < 0.01$; *** $P < 0.001$, compared from control; a, $P < 0.001$, compared from 4 h heat stress; b, $P < 0.05$, compared from naloxone 10 mg + heat stress; Student's unpaired t test

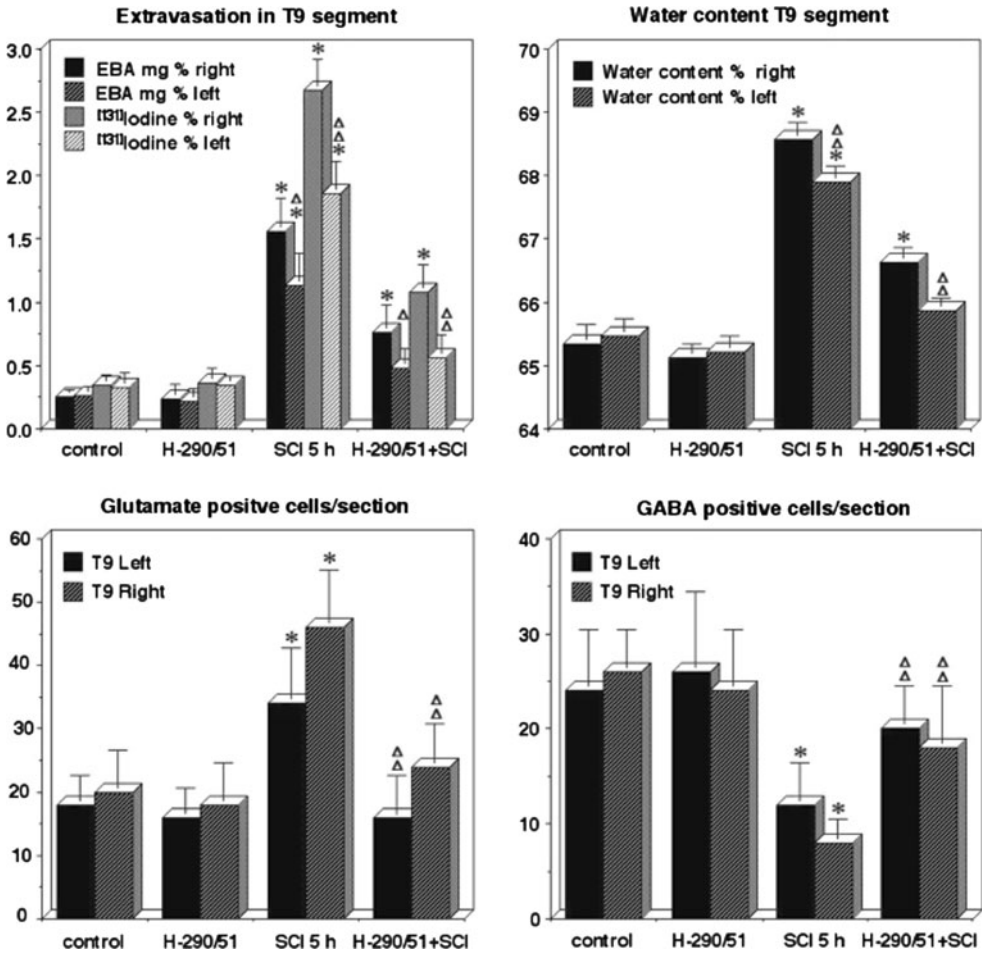

Fig. 4 Blood–spinal cord barrier (BSCB) permeability, edema formation in spinal cord injury (SCI) in relation to Glutamate and GABA positive cells in the T9 segment of the cord and their modification with antioxidant compound H-290/51 in the rat. Data after Sharma and Sjöquist (2002) (with permission). *$P < 0.05$ from control, $D = P < 0.05$ from SCI

Nanoparticles alter amino acid neurotransmitters imbalances in spinal cord trauma

Apart from exacerbation of heat stress induced brain pathology by nanoparticles intoxication, engineered nanoparticles from metals also aggravate spinal cord pathology following trauma (Sharma et al. 2009d, e; Menon et al. 2012). Previous reports from our laboratory showed that a focal SCI induces widespread alterations in amino acid neurotransmitters, e.g., glutamate, GABA, aspartate and glycine in the cord (Sharma and Sjöquist 2002) (Figs. 4, 5). Thus, we examined the influence of engineered nanoparticles form metals on amino acid content of the spinal cord in normal and spinal cord traumatized rats.

Administration of engineered nanoparticles from Cu and Ag (50–60 nm) once daily (50 mg/kg, i.p.) for 1 week resulted in a marked increase in Glutamate and aspartate (+50 to 70 %) content in the T9 and T12 segment of the normal spinal cord whereas GABA and glycine showed a significant decline (−20 to 40 %) (Sharma HS unpublished observations). A focal SCI in these nanoparticles treated rats further enhanced the Glutamate and aspartate content in the cord (+150 to 180 %) whereas, GABA and glycine showed marked decline (−60 to 80 %). These effects on amino acid contents were most marked in Ag treated rats. Interestingly, the neurological dysfunction and cord pathology were also exacerbated in nanoparticles treated animals after SCI (Sharma HS unpublished observations). These observations clearly suggest that nanoparticles induced exacerbation of cord pathology following SCI probably through alterations in imbalances between excitatory and inhibitory amino acids neurotransmission. Thus, further investigations on nanoparticle-induced amino acid neurotransmitter regulation are urgently needed.

Nanoneurotoxicity are exacerbated in stress or trauma

SiO_2 nanoparticle exposure is quite common in human populations in desert environment in association with high environmental temperature (Sharma et al. 2010a, b, c). Thus, normal population, military personal during combat exercise or peace keeping forces in desert environments are frequently exposed to SiO_2 nanoparticles together with high environmental heat conditions (Lafuente et al. 2012; Sharma and Sharma 2012a, b). In such situations, spinal cord or head injuries in military personals during combat operations is quite frequent. Thus, it is interesting to examine whether in these individuals SiO_2 exposure may further aggravate neurotoxicity in combination with

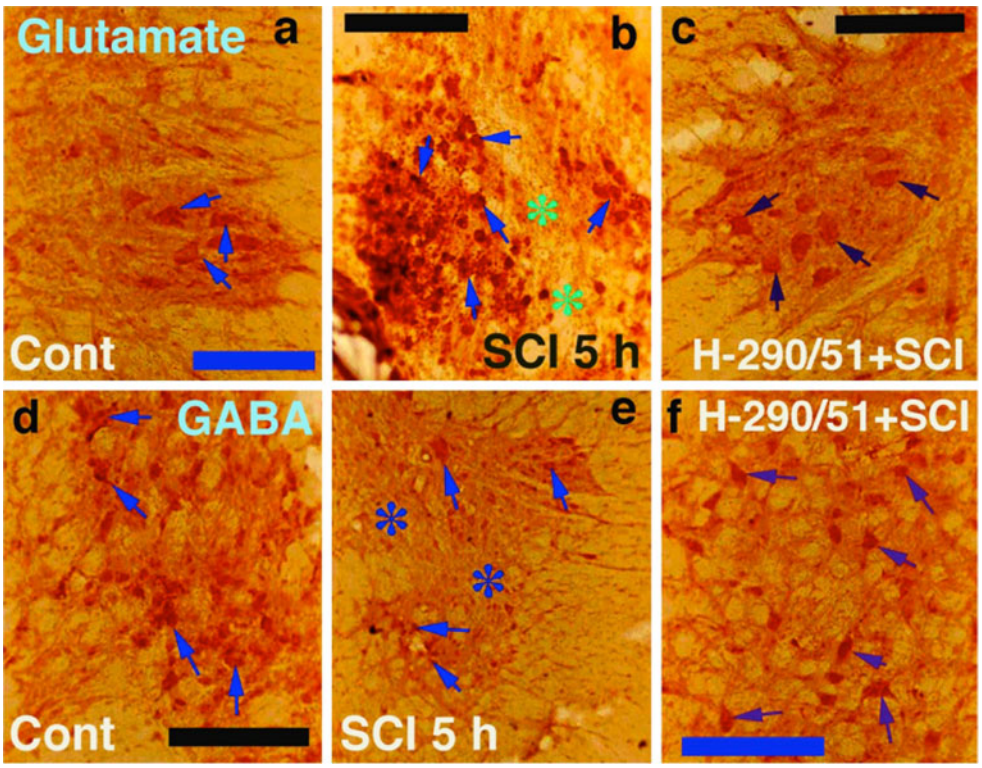

Fig. 5 Representative example of glutamate and GABA immunohistochemistry (*arrows*) in spinal cord of normal, spinal cord injured (SCI) rats and their modification with H-290/51. Edematous swelling (*) is clearly apparent in untreated inured rat. Data from Sharma and Sjöquist (2002) (with permission). *Bar* 30 μm

hyperthermia and/or trauma using model experiments (see Sharma et al. 2010c; Lafuente et al. 2012).

SiO_2 treated rats (50–60 nm, 50 mg/kg, i.p., once daily for 7 days) when subjected to a focal SCI (Lafuente et al. 2012) or closed head injury (CHI, Sharma HS unpublished observations) exhibited 50–180 % more increase in edema formation and neuronal injuries. In these animals the BBB breakdown of Evans blue albumin and radioiodine was exacerbated by 200–350 %. This indicates that nanoparticles treatment exaggerate pathophysiology of CNS injuries (Sharma et al. 2009a, c). In addition, heat exposure alone leads to significant brain damage in several parts of the brain (Figs. 6, 7) (Sharma 2006).

In other experiments, when nanoparticle 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 20–25 cm/s), they exhibited 300–450 % higher brain edema formation and 350–310 % increase in $^{[131]}$Iodine leakage in their brains (Sharma and Sharma 2007, 2012a, b; Sharma et al. 2009c, 2011a, b). The magnitude and intensity of neuronal, glial and myelin damage were 4–6 times higher than rats exposed to identical heat esters treated with saline instead of nanoparticles (Sharma and Sharma 2007, 2012a, b; Sharma et al. 2009a, c, 2010c). This suggests that nanoparticles could exacerbate BBB damage (Tables 2, 3). Although the detailed mechanism underlying exacerbation of nanoparticle-induced brain damage is unclear, it seems likely that enhanced transport of neurodestructive elements to the brain than normal animals as compared to nanoparticles treatment could exacerbate CNS damage. Alternatively increased oxidative stress or amino acid metabolism (Figs. 8, 9) by nanoparticles may also affect greater brain damage than in normal animals (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.

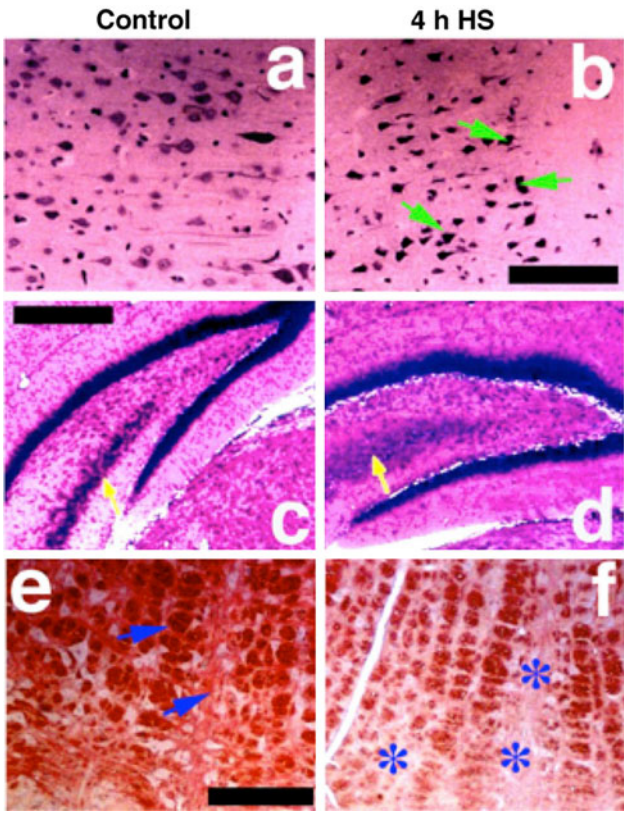

Fig. 6 Morphological changes in the cerebral cortex (**a**, **b**), hippocampus (**c**, **d**) and brain stem (**e**, **f**) in control (**a**, **c**, **e**) and 4 h heat stressed (**b**, **d**, **f**) rats. Neuronal damages in cortex (**b**) and hippocampus (**d**) are clearly seen (*arrows*). Myelin damage as evident with myelin basic protein (MBP) immunohistochemistry is apparent in heat stressed rat (*). Loss of MBP immunostaining (**f**) is clearly visible than control (**e** *arrows*). *Bars* 30 µm. Data from Sharma (2006) (with permission)

Co-morbidity factors exacerbate nanoneurotoxicity

In addition to stress or trauma, many neurological diseases, e.g., stroke or dementia, is often associated with different co-morbidity factors viz., hypertension and/or diabetes. Under such situations, treatment strategies with neuroprotective agents normally do not work effectively. Thus, the use of nanomedicine under such circumstances may also require additional modification of the drug dosage. It is also quite likely that nanoparticle toxicity may be further affected by diabetes and/or hypertension in clinical situations. Thus, using animal models of hypertension or diabetes we examined neurotoxicity of nanoparticles or nanowires used for drug delivery (Muresanu and Sharma 2007; Muresanu et al. 2012; Sharma 2007a, b; Sharma and Sharma 2007, 2012a, b). Chronic hypertension was produced by 2-kidney one clip (2K1C) procedure (Muresanu and Sharma 2007). Diabetic rats were made by streptozotocin administration (75 mg/kg, i.p. daily for 3 days) in rats (Sharma et al. 2010a, b). These animals normally do not exhibit BBB breakdown, brain edema or neural injuries. However, when these hypertensive or diabetic animals were administered Cu or Ag nanoparticles (50–60 nm) as well as TiO_2 nanowires for 1 week (50 mg/kg, i.p.) profound brain edema formation (+140 to 180 %), BBB breakdown to radioiodine (+220 to 260 %) and neural damages (+80 to 120 %) were seen in different parts of the brain as compared to nanoparticles treated healthy controls (Sharma and Sharma 2012a, b; Sharma et al. 2009a, d). This indicates that co-morbidity factors, e.g., hypertension or diabetes, could exacerbate nanoparticle-induced neurotoxicity. It appears that brain tissues or cerebral endothelial cells in hypertensive or diabetic animals are

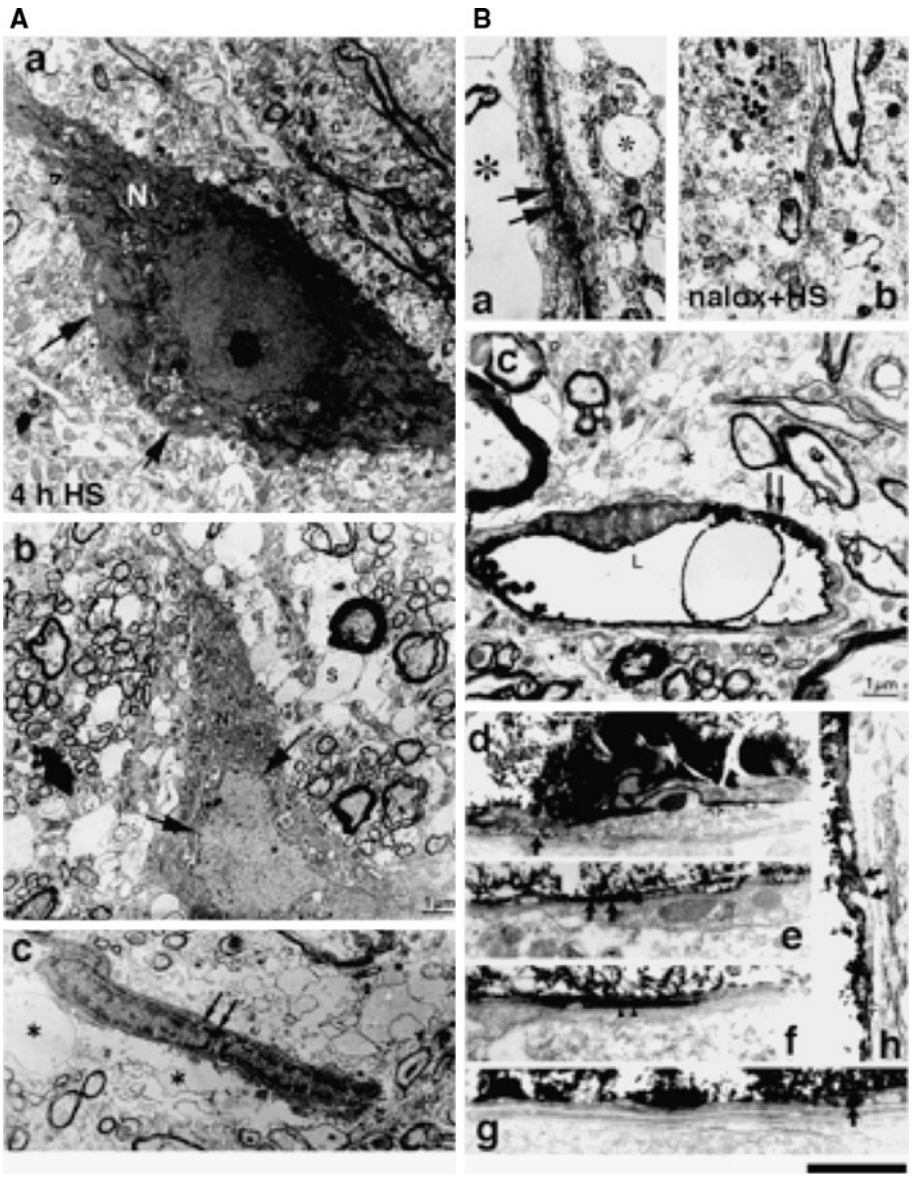

Fig. 7 Ultrastructural changes in the neuron (**A***a*), cortical microvessels (**A**, *c*; **B**, *a*, *c*, *d–h*) in heat stressed rat and its modification with naloxone (**A**, *b*; **B**, *b*). Naloxone treatment reduces amino acid neurotransmitter changes and attenuated neuronal damage (**A**, *b*) and edema formation in the neuropil (**B**, *b*). Leakage of lanthanum across the microvessels (**A**, *c*; **B**, *c*; **B**, *d–f*) is clearly seen across the endothelial cell membrane without widening of the tight junctions. Data from Sharma (2006) (with permission). *Bar* 1 μm

more susceptible to nanoparticle-induced toxicity, a possibility that requires further investigation.

Nanodrug delivery induces neuroprotection

The possibility that drugs delivered with nanoformulations may have enhanced neuroprotective effects due to their targeted delivery, long-term effects, slow release of compounds like biological minipumps and less degradation over time (Singh 2010; Sharma et al. 2009d; Tosi et al. 2011; Sharma 2011; Tian et al. 2012). Thus, we examined nanodrug delivery of key compounds in a rat model of SCI. For this purpose, we labeled three different types of drugs to TiO_2 nanowires (50–60 nm) using standard procedures (Sharma 2007a, 2009c) (Figs. 10, 11). Our observation shows that nanowired drug delivery enhanced neuroprotection in SCI at 5 h as compared to the parent compounds (Fig. 12). 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 if given without nanowired delivery (Sharma 2007a, b; Tian et al. 2012). This indicates that nanowired delivery of drugs do not change the property of the compounds but only enhances their efficacy as compared to the parent drug (Muresanu et al. 2012; Sharma 2007a, b, 2009a, b).

This is quite likely (as mentioned above) that this 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 of the compounds due to nano-binding (Sharma 2007a, 2009c; Tian et al. 2012). Obviously, nanowired drugs could enhance the half-life of the compound as compared to parent drug. However, our observations indicate that TiO_2 nanowires

Table 2 Effect of nanoparticles on BBB permeability, cerebral blood flow and brain edema in normal rats and animals subjected to 4 h whole body hyperthermia (WBH) at 38 °C

Expt. type	*n*	BBB permeability		BSCB permeability		Blood flow	Spinal cord	Edema formation		Volume swelling	
		Evans blue mg %	$^{[131]}$Iodine %	Evans blue mg %	$^{[131]}$Iodine %	ml/g/min Brain		Brain water %	Spinal cord water %	% *f* Brain	% *f* Spinal cord
Normal animals											
Saline	6	0.28 ± 0.08	0.36 ± 0.06	0.22 ± 0.04	0.30 ± 0.05	1.16 ± 0.06	0.94 ± 0.04	74.23 ± 0.86	65.25 ± 0.21	Nil	Nil
Cu	8	0.56 ± 0.13**	0.68 ± 0.11**	0.67 ± 0.08**	0.76 ± 0.10**	0.98 ± 0.08*	0.84 ± 0.09*	75.14 ± 0.13*	66.16 ± 0.08*	3.53	2.61
Ag	8	0.64 ± 0.12**	0.74 ± 0.11**	0.64 ± 0.08**	0.75 ± 0.05**	0.96 + 0.10*	0.88 ± 0.06*	75.04 ± 0.08*	66.08 ± 0.11*	3.14	2.38
Al	8	0.46 ± 0.08*	0.58 ± 0.06*	0.58 ± 0.06*	0.64 ± 0.08*	1.04 ± 0.06*	0.88 ± 0.08*	74.84 ± 0.13*	65.75 ± 0.31*	2.36	1.43
Heat stressed animals (38 °C for 4 h)											
Saline	5	2.06 ± 0.25aa	2.86 ± 0.24aa	1.21 ± 0.21aa	1.46 ± 0.22aa	0.76 ± 0.08aa	0.70 ± 0.06a	81.34 ± 0.62aa	68.23 ± 0.21aa	27.59	8.57
Cu	6	2.68 ± 0.33b	3.17 ± 0.26b	2.10 ± 0.8b	2.83 ± 0.08b	0.70 ± 0.06b	0.65 ± 0.07b*	83.12 ± 0.34b*	68.76 ± 0.43*	34.49	10.01
Ag	8	2.64 ± 0.33b	3.14 ± 0.12bb	1.87 ± 0.6b	2.08 ± 0.07bb*	0.74 + 0.08b	0.68 ± 0.06*	82.32 ± 0.26b*	68.56 ± 0.28*	31.39	9.52
Al	8	2.13 ± 0.36*	3.08 ± 0.21b*	1.46 ± 0.32b*	1.89 ± 0.42b*	0.72 ± 0.06*	0.68 ± 0.10b*	82.16 ± 0.24*	68.34 ± 0.21b*	30.77	8.89

For details see text

The nanoparticles were suspended in Tween 80 and administered separately intraperitoneally in rats daily once in a dose of 50 mg/kg (weight/volume)

Values are mean ± SD from 5 to 9 animals at each data point

* $P < 0.05$; ** $P < 0.01$ compared from saline in normal animals, a, $P < 0.05$; aa, $P < 0.01$, compared from 4 h saline treatment in normal vs. heat stressed. b, $P < 0.05$; bb, $P < 0.01$ compared from saline treated heat stressed animals, ANOVA followed by Dunnett's test for multiple group comparison from one control group (Data from Sharma and Sharma 2007, with permission)

Table 3 Effect of nanoparticles on regional BBB permeability and regional brain edema in normal rats and animals subjected to 4 h whole body hyperthermia (WBH) at 38 °C

Expt. type	BBB permeability $^{[131]}$Iodine %					Regional brain edema (water content %)				
	Cerebral cortex	Hippocampus + caudate N	Cerebellum	Thalamus + hypothalamus	Brain stem	Cerebral cortex	Hippocampus + caudate N	Cerebellum	Thalamus + hypothalamus	Brain Stem
Normal animals ($n = 6$–8)										
Saline	0.34 ± 0.06	0.26 ± 0.04	0.12 ± 0.04	0.56 ± 0.07	0.18 ± 0.04	72.34 ± 0.21	80.38 ± 0.21	78.32 ± 0.12	76.33 ± 0.43	66.35 ± 0.21
Cu	0.62 ± 0.11** (+82 %)	0.48 ± 0.08** (+85 %)	0.38 ± 0.05** (+217 %)	0.71 ± 0.10** (+27 %)	0.38 ± 0.06* (+111 %)	74.38 ± 0.19* (+2.82 %)	81.34 ± 0.23* (+1.19 %)	80.33 ± 0.14* (+2.56 %)	78.18 ± 0.23* (+2.42 %)	67.34 ± 0.12* (+1.49 %)
Ag	0.68 ± 0.14** (+100 %)	0.54 ± 0.06** (+108 %)	0.40 ± 0.06** (+233 %)	0.76 ± 0.08** (+36 %)	0.36 + ±0.07* (+100 %)	74.21 ± 0.16* (+2.58)	81.65 ± 0.27* (+1.57 %)	80.67 ± 0.34* (+3.00 %)	78.23 ± 0.13** (+2.48 %)	67.67 ± 0.21* (+1.98 %)
Al	0.60 ± 0.09* (+76 %)	0.50 ± 0.08** (+92 %)	0.41 ± 0.03** (+242 %)	0.70 ± 0.06** (+25 %)	0.32 ± 0.08** (+78 %)	73.67 ± 0.23* (+1.83 %)	81.75 ± 0.43* (+1.70 %)	80.56 ± 0.28* (+2.86 %)	77.65 ± 0.21* (+1.72 %)	67.04 ± 0.14* (+1.04 %)
Heat stressed animals (38 °C for 4 h, $n = 6$–8)										
Saline	1.86 ± 0.15aa (+447 %)	2.06 ± 0.28aa (+692 %)	2.01 ± 0.11aa (+1,575 %)	2.56 ± 0.24aa (+357 %)	0.76 ± 0.08aa (+322 %)	78.34 ± 0.12** (+8.29 %)	82.34 ± 0.12aa (+2.43 %)	81.43 ± 0.08aa (+3.97 %)	78.78 ± 0.21** (+3.21 %)	70.48 ± 0.08** (+6.22 %)
Cu	2.48 ± 0.13b (+612 %)	2.87 ± 0.16b (+1,004 %)	2.20 ± 0.18b (+1,733 %)	2.87 ± 0.18b (+412 %)	1.45 ± 0.06b (+706 %)	79.54 ± 0.18** (+9.95 %)	83.87 ± 0.18b* (+4.34 %)	82.04 ± 0.13* (+4.74 %)	79.56 ± 0.14** (+4.23 %)	71.56 ± 0.22** (+7.85 %)
Ag	2.34 ± 0.14b (+588 %)	3.01 ± 0.21bb (+1,058 %)	2.37 ± 0.16b (+1,875 %)	2.76 ± 0.17bb* (+393 %)	1.70 + ±0.06b (+844 %)	79.16 ± 0.23* (+9.43 %)	83.02 ± 0.16b* (+3.28 %)	81.76 ± 0.12* (+4.39 %)	79.65 ± 0.08* (+4.34 %)	71.48 ± 0.06** (+7.73 %)
Al	2.13 ± 0.12* (+526 %)	2.78 ± 0.14b* (+969 %)	2.16 ± 0.13b* (+1,700 %)	2.89 ± 0.12b* (+416 %)	0.96 ± 0.08* (+433 %)	78.97 ± 0.21* (+9.165 %)	82.96 ± 0.13* (+3.20 %)	81.84 ± 0.17b* (+4.49 %)	79.23 ± 0.11 (+3.79 %)	71.52 ± 0.21* (+7.79 %)

For details see text

The nanoparticles were suspended in Tween 80 and administered separately intraperitoneally in rats daily once in a dose of 50 mg/kg (weight/volume)

Values are Mean ± SD from 5 to 9 animals at each data point

* $P < 0.05$; ** $P < 0.01$ compared from saline in normal animals, a, $P < 0.05$; aa, $P < 0.01$, compared from 4 h saline treatment in normal vs. heat stressed. b, $P < 0.05$, bb, $P < 0.01$ compared from saline treated heat stressed animals, ANOVA followed by Dunnett's test for multiple group comparison from one control group (Data from Sharma and Sharma 2007 with permission)

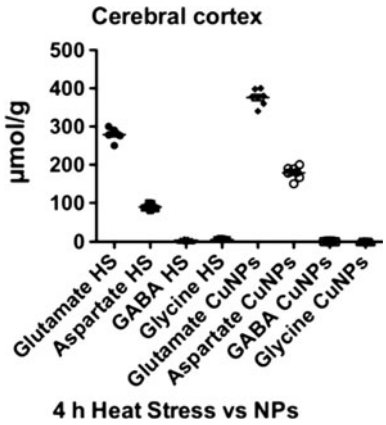

Fig. 8 Changes in amino acid neurotransmitters in the cortex of normal rats after nanoparticles (NPS) treatment

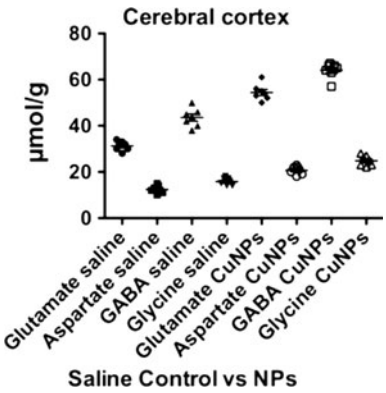

Fig. 9 Changes in amino acid neurotransmitters in the cortex of heat stressed rats after nanoparticles (NPS) treatment

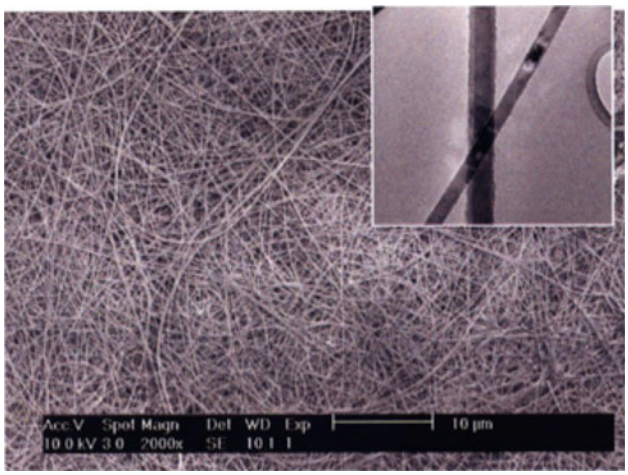

Fig. 10 TiO_2 nanowired mesh for nano drug delivery. Data from Sharma (2007a, b) (with permission)

itself when administered induced some minor but significant pathological changes in the cord in normal animals (Sharma HS unpublished observations). Thus, long-term

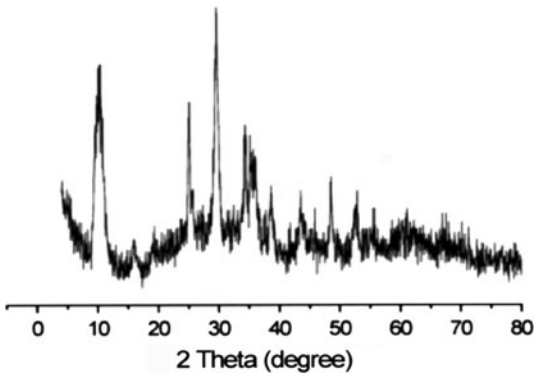

Fig. 11 X-ray diffraction studies of TiO_2 nanowired drug labeling showing smooth and reliable preparation. Data from Sharma (2007a, b) (with permission)

effects of nanowired drugs should be examined in vivo in great detail for the safety of nanomedicine in future.

Nanowired cerebrolysin enhanced neuroprotection in hyperthermia

As mentioned above, hyperthermia induces marked increase in excitatory amino acid neurotransmitters, e.g., glutamate and aspartate in the cerebral cortex, hippocampus, thalamus, and hypothalamus and in spinal cord at the time of neuronal damages and BBB dysfunction (Sharma 2006, 2007a, b) (see Figs. 13, 14). At this time, inhibitory amino acids such as GABA or glycine showed marked decrease in the identical CNS regions (see Sharma 2006, 2007a, b). Since cerebrolysin is a mixture of various neurotrophic factors (Sharma et al. 2007d) and induces marked neuroprotection in hyperthermia (Sharma et al. 2011a), effects of cerebrolysin with or without TiO_2 nanowiring was examined on the glutamate, aspartate, GABA and glycine levels in the CNS following hyperthermia in relation to brain damage.

Rats were treated with cerebrolysin (2.5 ml/kg, i.v.) with or without nanowiring after 30, 60 and 90 min of HS and amino acid neurotransmitters and brain damage were examined. We found that rats receiving cerebrolysin after 30 min markedly thwarted the increase in glutamate and aspartate and reduced the GABA and glycine levels in the CNS resulting in neuroprotection (Sharma HS unpublished observations). However, 60 or 90 min after cerebrolysin administration did not affect the amino acid levels and/or brain damage. On the other hand nanowired cerebrolysin if given at 60 or 90 min after heat stress, thwarted this amino acid imbalance and induced marked neuroprotection (Figs. 15, 16). These observations suggests that nanowiring of cerebrolysin enhances its neurotherapeutic efficacy in hyperthermia induced neuroregeneration probably through modulating amino acid neurotransmission in the CNS.

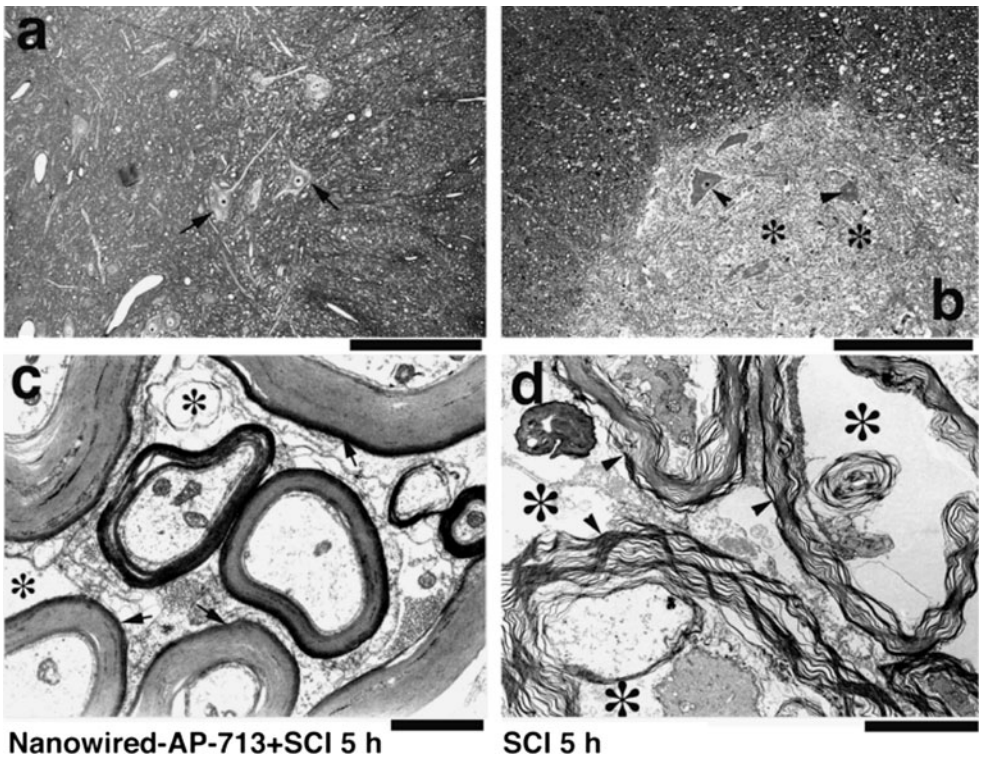

Fig. 12 Nanodrug delivery of AP-713 compound in spinal cord injury (SCI). Morphological damage at light (**b**) and electron microscopy (**d**) in untreated SCI is markedly reduced by nanodrug delivery of the compound in identical regions (**a**, **c**), respectively. *Bar* **a**, **b** = 35 μm, **b**, **d** = 800 nm. Data after Sharma (2007a, b) (with permission)

Nanodrug delivery requires dose adjustment with co-morbidity factors

As mentioned above, TiO_2 nanowire attached to neuroprotective drugs was also able to reduce brain damage in hyperthermia caused by heat stress more effectively than the parent compound (Sharma et al. 2009a, b, c, d, e). Accordingly, when nanowired antioxidant compound H-290/51 (50 mg/kg, p.o. once) was administered 30 min after 4 h heat stress at 38 °C in saline treated group resulted in marked reduction in brain pathology. On the other hand, when nanoparticles treated rats ware subjected to identical heat stress, the nanowired treatment failed to attenuate brain damage (Sharma et al. 2009d). This indicates that nanowired drugs could not reduce nanoneurotoxicity following a combination of nanoparticles and heat stress.

Likewise, nanowired H-290/51 treatment given in diabetic rats after identical heat stress was unable to reduce brain pathology. However, when the dose of nanowired drug was increased by 100 %, moderate neuroprotection could be seen in nanoparticle treated or diabetic animals after identical heat exposure (Sharma et al. 2010b). This suggests that the dose of nanowired drugs require considerable adjustment to achieve neuroprotection in animals with co-morbidity factors.

Nanoparticles induce oxidative stress in the CNS

Available evidences suggest that nanoparticles induce oxidative stress in the CNS that could play important roles in causing nanoneurotoxicity (Muresanu et al. 2011a, b). Interestingly, many drug carriers used for nanodelivery, e.g., nanowires, liposomes or carbon nanotubes, may also induce mild to moderate oxidative stress (Feng et al. 2011). Studies carried our in our laboratory showed that engineered nanoparticles, 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 (Muresanu et al. 2011a, b; 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 (Feng et al. 2011; Sharma HS Unpublished observations). The magnitude and intensity of oxidative stress caused by these nanoparticles were further exacerbated in diabetic or hypertensive rats. These changes in oxidative stress parameters correlate well with neuronal damage and the BBB breakdown to radioiodine (Sharma HS unpublished observations).

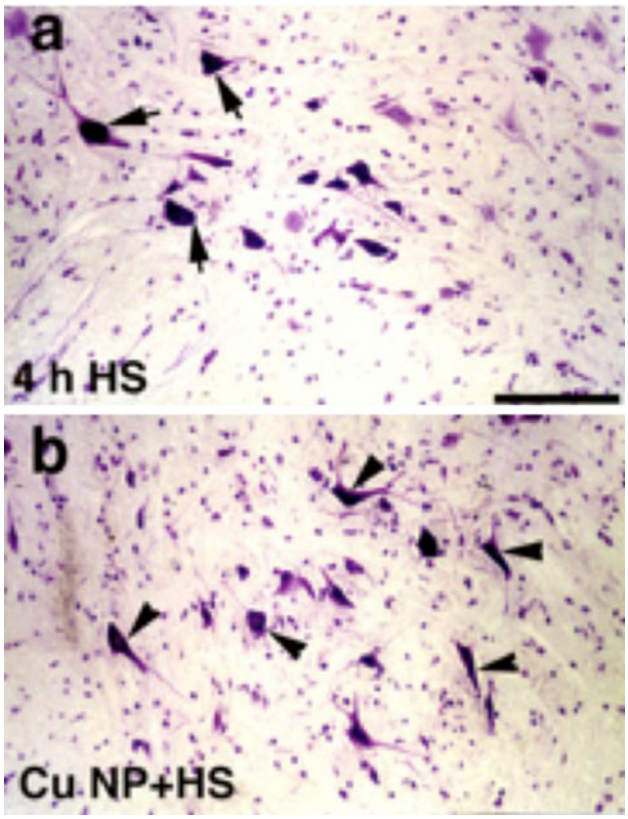

Fig. 13 Exacerbation of neuronal damages in the brain after heat stress in Cu NPs treated group (**b**) as compared to saline treatment (**a**). *Bar* 30 μm. Data after Sharma and Sharma (2007) (with permission)

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

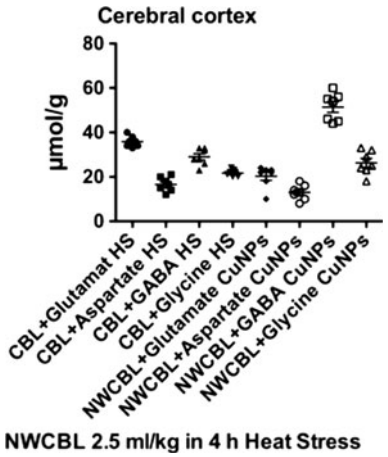

Fig. 15 Changes in the amino acid levels in the cortex following heat stress and their modification by nanowired cerebrolysin treatment

Conclusion and future perspectives

In conclusion, our studies clearly show that nanoparticle-induced exacerbation of brain pathology is modulated by additional stress, trauma or endocrine alterations, e.g., diabetes. It appears that changes in amino acid neurotransmitters and oxidative stress could play important roles in this enhancement of brain pathologies by nanoparticles. Thus, to contain the disease progression and induce neuroprotection in such circumstances, nanodrug delivery could be of great help provided the nanomaterial used to deliver drugs by itself do not cause brain pathology or adverse cellular reactions. Thus, further research is needed to understand whether nanomedicine or nanodrug delivery could cause any potential neurotoxicity in normal animals in relation to alterations in oxidative stress, amino acid neurotransmitters and breakdown of the BBB function. In addition, co-morbidity factors viz., such as diabetes, hypertension, trauma or hyperthermia often associated with

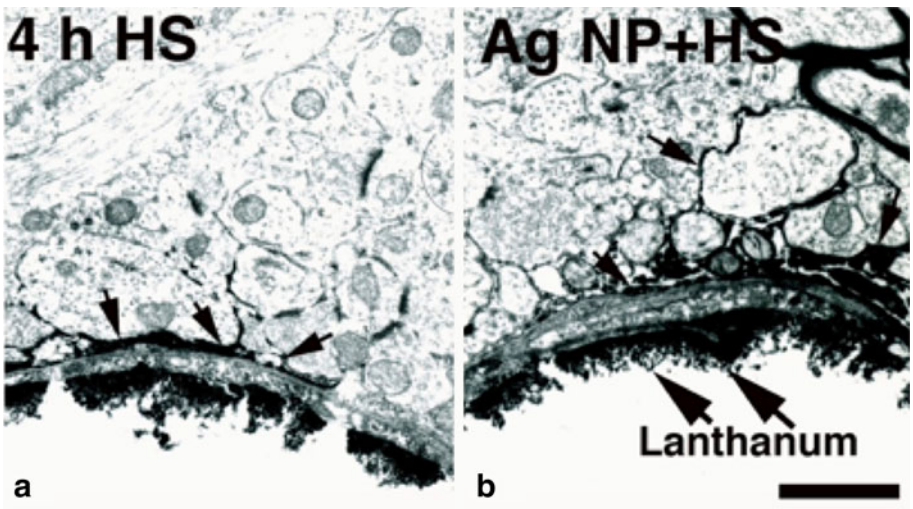

Fig. 14 Exacerbation of lanthanum leakage in the neuropil after heat stress in Cu NPs treated group (**b**) as compared to saline treatment (**a**). *Bar* 1 μm. Data after Sharma and Sharma (2007) (with permission)

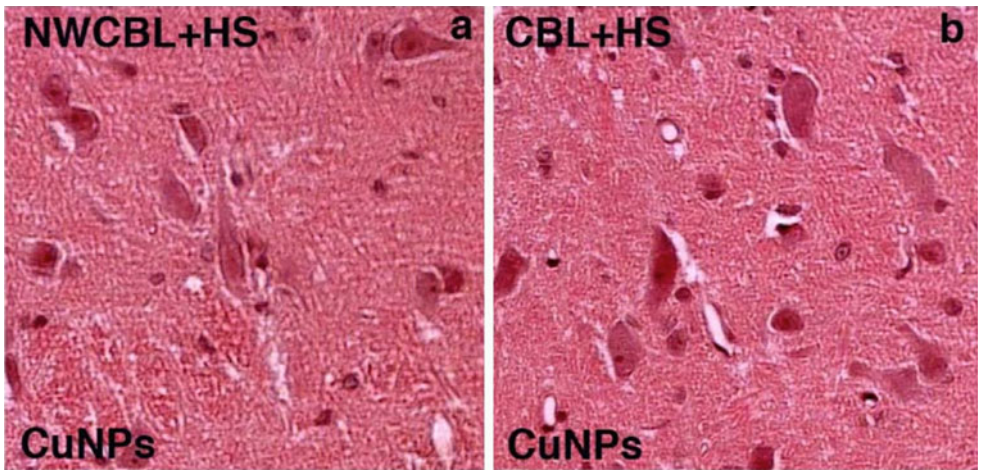

Fig. 16 Neuroprotection by nanowired cerebrolysin (NWCBL) in heat stress treated with Cu NPs as compared to normal CBL treatment. NWCBL is very effective in achieving neuroprotection (×30)

neurological diseases could exacerbate nanoneurotoxicity and in such conditions the dose or delivery schedule require ample modification including nanodrug delivery. Keeping these factors in mind, the drug delivery using nanomedicine may be adjusted or modified to achieve better clinical efficacy and enhanced patient care.

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