

Influence of Nanoparticles on Blood–Brain Barrier Permeability and Brain Edema Formation in Rats

Hari Shanker Sharma, Saber Hussain, John Schlager, Syed F. Ali, and Aruna Sharma

Abstract Nanoparticles are small sized (1–100 nm) particles derived from transition metals, silver, copper, aluminum, silicon, carbon and metal oxides that can easily cross the blood–brain barrier (BBB) and/or produce damage to the barrier integrity by altering endothelial cell membrane permeability. However, the influence of nanoparticles on BBB integrity is still not well-known. In this investigation, effect of nanoparticles derived from Ag, Al and Cu (50–60 nm) on BBB permeability in relation to brain edema formation was examined in a rat model. Intravenous (30 mg/kg), intraperitoneal (50 mg/kg) or intracerebral (20 µg in 10 µL) administration of Ag, Cu or Al nanoparticles disrupted the BBB function to Evans blue albumin (EBA) and radioiodine in rats 24 h after administration and induced brain edema formation. The leakage of Evans blue dye was observed largely in the ventral surface of brain and in the proximal frontal cortex. The dorsal surfaces of cerebellum showed mild to moderate EBA staining. These effects were most pronounced in animals that received Ag or Cu nanoparticles compared to Al nanoparticles through intravenous routes. These observations are the first to suggest that nanoparticles can induce brain edema formation by influencing BBB breakdown in vivo.

Keywords Nanoparticles • silver • copper • aluminum • blood–brain barrier • brain edema • Evans blue • radioiodine

H.S. Sharma (✉) and A. Sharma

Laboratory of Cerebrovascular Research, Department of Surgical Science, Anesthesiology and Intensive Care Medicine, University Hospital, Frodingsgatan 12:28, Uppsala, SE-75421, Sweden and Neurochemistry Laboratory, Division of Neurotoxicology, National Centre for Toxicological Research/FDA, Jefferson, AR, USA
e-mail: Sharma@surgsci.uu.se

S. Hussain and J. Schlager

Applied Biotechnology Branch, Human Effectiveness Directorate, Air Force Research Laboratory, Wright-Patterson Air Force Base, OH, USA

S. F. Ali

Neurochemistry Laboratory, Division of Neurotoxicology, National Centre for Toxicological Research/FDA, Jefferson, AR, USA

Introduction

Recently there has been a surge to investigate the effects of nanoparticles on biological systems normally present in the environment (1–3). These small sized (1 to 100 nm) particles have novel properties, which can be highly desirable for applications within the commercial, medical and environmental fields (5,6). Most of these nanoparticles are formed from transition metals, e.g., silver, copper, aluminum, silicon, carbon and metal oxides (1,5). Due to their small sizes, these nanoparticles can either easily cross the blood–brain barrier (BBB) and/or produce damage to the barrier integrity by altering endothelial cell membrane function (5,6). Interestingly, in spite of our increased understanding of BBB function, influence of nanoparticles on BBB is still largely unknown (9,13,15).

It is quite likely that nanoparticles when reaching the CNS compartments may induce profound cellular and molecular stress (8) leading to BBB disruption and brain edema formation through a cascade of secondary cellular and molecular events (8,9,12,13). This investigation is focused on the influence of engineered nanoparticles from metals, e.g., Al, Cu and Ag (50–60 nm) on the BBB permeability to protein tracers, brain edema formation and cell injury in a rat model.

Materials and Methods

Animals

Experiments were carried out on Sprague Dawley rats (body weight 250 to 350 g) housed at controlled ambient temperature ($22 \pm 1^\circ\text{C}$) with 12 h light and 12 h dark schedule. Standard laboratory diet and tap water were supplied ad libitum before and after the experiments. All animal experiments described in this review were conducted according to National Institute of Health (NIH), United States Government guidelines for care, handling and maintenance of animals and approved by the Local Institutional Ethics Committee for Animal Care and Research.

Administration of Nanoparticles

Under equithesin anesthesia (3 mL/kg, i.p.), engineered nanoparticles from Copper (Cu), Aluminum (Al), or Silver (Ag) in the size range of 50 to 60 nm (obtained from US Air Force Research Laboratory from Dr Saber Hussain; and commercially procured from IoLiTec Ionic Liquids Technologies, 79211 Denzlingen, Germany). The nanoparticles were suspended in 0.05% Tween 80 in 0.7% NaCl solution (cf 10, 11, 13). This solution was administered intravenously (30 mg/kg), intraperitoneally (50 mg/kg) or used as cortical superfusion (c.s., 20 µg/10 µL). The Tween 80 solution alone when injected into the carotid artery does not produce any brain or spinal cord pathology (see 11,15). The animals were allowed to survive 24 h after the administration of nanoparticles.

Blood–Brain Barrier Permeability

The blood–brain barrier (BBB) permeability to Evans blue albumin (2% of a sterile solution, 0.3 mL/100 g body weight) and radioiodine tracer (¹³¹I-iodine, either 100 µCi/Kg, or a minimum of 0.5 million CPM) given intravenously was determined as described earlier (10).

Morphological Investigations

For morphological investigations, the brains were perfused in situ with 4% paraformaldehyde preceded with a brief saline rinse (12–14), taken out and photographed. Then coronal sections passing through hippocampus, cerebellum and brain stem were embedded in paraffin. About 3 µm thick sections were cut and stained for standard histological stains, e.g., Nissl or Hematoxylin and Eosin and Luxol Fast Blue (13).

Brain Edema Formation and Electrolyte Content

Brain water content was measured from the differences in the wet and dry weight of the samples (10–14). Volume swelling from the differences between brain water content was calculated (see 13). Normally, an increase in 1% water content represents marked edema formation. After obtaining the dry weight, the tissue was processed to determine the Na⁺ and K⁺ content according to standard procedures (4).

Physiological Variables

In some group of animals mean arterial blood pressure (MABP) and blood gases including arterial pH were also examined using standard procedures (10).

Statistical Analysis

ANOVA followed by Dunnet's test was used to evaluate statistical significance of the data obtained from one control group. A p-value <0.05 was considered significant.

Results

Effects on Nanoparticles on the BBB Permeability

The Ag, Cu and Al nanoparticles altered the BBB to Evans blue albumin and radioiodine in the rats in a highly selective and specific manner. The leakage of Evans blue was seen on the ventral surface of the brain and in the proximal frontal cortex. The dorsal surfaces of cerebellum and the brain stem showed mild to moderate Evans blue staining (Fig. 1). The effect of Al nanoparticles on the BBB function was much less intense compared to Ag and Cu nanoparticles. Intraperitoneal administration of nanoparticles had least influence on BBB disruption (see Table 1).

Cortical superfusion with nanoparticles resulted in mild to moderate opening of the BBB to protein tracers largely to be seen on the ipsilateral side. However, the cerebellum and dorsal parts of the brain stem showed leakage of Evans blue albumin as well. This effect was most pronounced with Ag and Cu nanoparticles. The Al nanoparticles showed only faint to mild blue staining (Table 1). Intravenous administration of Al, Cu and Ag nanoparticles induced extravasation of Evans blue and radioiodine tracer in different brain areas (Table 1). There was no difference in radiotracer extravasation when the nanoparticles were administered as a suspension in water or mixed with Tween 80 (results not shown). Tween 80 or NaCl given in equimolar concentration did not induce radiotracer extravasation in any brain regions compared to control group.

Nanoparticles and Edema Formation

Intravenous administration of Cu nanoparticle resulted in mild but significant edema formation in different parts of the

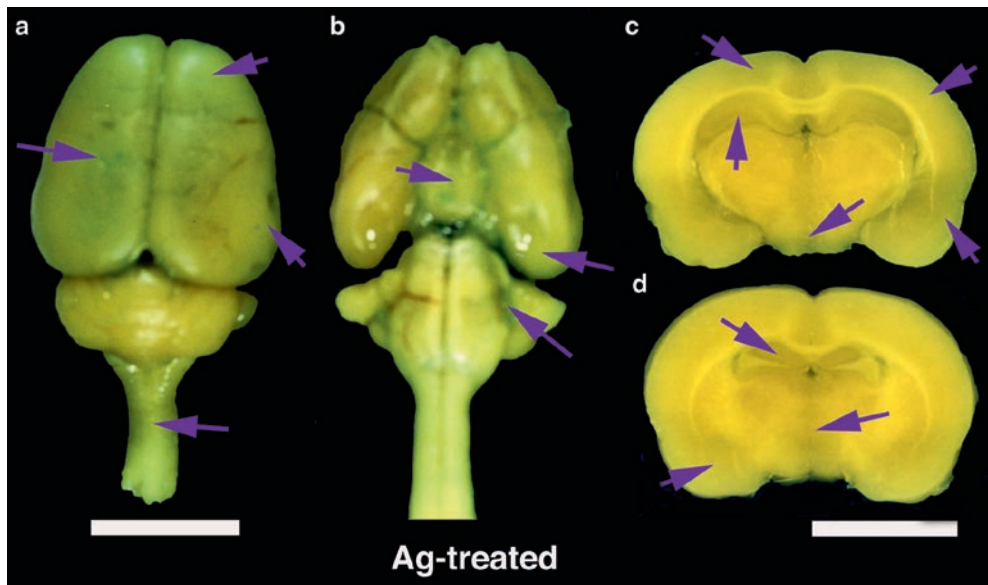


Fig. 1 Shows extravasation of Evans blue on the dorsal (a) and ventral (b) surfaces of rat brain after Ag nanoparticle treatment. The Ag nanoparticle was administered intravenously (35 mg/kg) and the rat is allowed to survive 24 h after injection. Coronal sections of the brain passing through hippocampus (c) and caudate nucleus (d) are also shown. Leakage of Evans blue dye can be seen in various brain

regions (arrows). The deeper parts of the brain, e.g. hippocampus, caudate nucleus, thalamus, hypothalamus, cortical layers including pyriform, cingulate, parietal and temporal cortices, showed moderate blue staining. This indicates widespread leakage of Evans blue albumin within the brain after Ag treatment. Bar = 3 mm (modified after 9 and 15)

cortex compared to the control group. Thus, about 0.5% to 1.2% increase in brain water content was noted in the cingulate, pyriform and temporal cortices. Administration of Al nanoparticle induced mild increase in water content compared to the controls (see Table 1).

Our data further show that Cu treatment increased Na^+ content in the sample with a slight decrease in K^+ content (Table 1). Ag treatment also altered ion content in the brain in a similar way. Al treatment showed minimum changes in Na^+ and K^+ contents in the brain compared to the control groups (Table 1).

Nanoparticles and Physiological Variables

Administration of nanoparticles either into the jugular vein or into the femoral artery slowed the heart rate immediately and the respiratory rate was temporarily increased. This effect lasted for about 10 min. However, none of the animals showed gasping. The effect of Cu and Ag nanoparticles were most pronounced on heart rate and respiration compared to Al nanoparticles (Table 1).

At the onset of nanoparticle (Cu and Ag) administration, the mean arterial blood pressure (MABP) was decreased by 20 to 30 torr for about 5 to 8 min that recovered partially, but remained depressed from the pre-injection value even 90 min after the nanoparticle injection. The decline in MABP following Al nanoparticle was least pronounced (Table 1). Arterial PaO_2 increased slightly after nanoparticle adminis-

tration whereas PaCO_2 was either unchanged or decreased slightly in some animals. The arterial pH was not affected significantly (Table 1).

Nanoparticles and Morphological Changes in the Brain

Nerve cell damage in several brain regions showing Evans blue extravasation (Fig. 2) was seen following administration of Ag and Cu nanoparticles (Fig. 2). Alterations in glial cell and myelin also occurred (Fig. 2). These neuropathological changes were least affected by Al nanoparticles (Table 1). Normal animals that received saline or Tween 80 did not show any cell changes in the brain (see Table 1).

Discussion

The present results are the first to suggest that engineered nanoparticles from metals when administered systemically are able to induce breakdown of the BBB permeability, depending on the route of administration and the type of nanoparticles. Thus, administration of Ag and Cu nanoparticles intravenously or superfused over the cortical surface profoundly induced the breakdown of the BBB to protein tracers compared to Al nanoparticles. On the other hand, intraperitoneal administration of nanoparticles was least effective in BBB disruption. These observations suggest that

Table 1 Effect of nanoparticles on Blood-Brain Barrier (BBB) permeability, brain edema formation and cell changes in rats and mice at 24 h after administration

Type of experiment	BBB permeability			Brain edema			Cell injury variables			Physiological variables		
	Evans blue (mg%)	¹³¹ Iodine %	Water content %	%f	Na ⁺ (mM/Kg dry wt)	K ⁺ (mM/Kg dry wt)	Neuron Nissl	MABP (torr)	Aretrial	PaO ₂ (torr)	PaCO ₂ (torr)	
A. Control Group (saline treatment equimolar concentration)												
i.v.	0.23 ± 0.08	0.34 ± 0.03	76.34 ± 0.28	316 ± 8	218 ± 10	Nil	120 ± 8	7.38 ± 0.04	80.34 ± 0.12	34.56 ± 0.21		
i.p.	0.24 ± 0.08	0.30 ± 0.08	76.12 ± 0.32	310 ± 9	220 ± 12	Nil	123 ± 7	7.38 ± 0.06	80.54 ± 0.23	34.53 ± 0.22		
c.s.	0.28 ± 0.03	0.34 ± 0.06	76.10 ± 0.21	312 ± 12	224 ± 08	Nil	124 ± 9	7.36 ± 0.04	80.61 ± 0.12	34.45 ± 0.12		
B. Nanoparticles intraperitoneal (50 mg/kg)												
Ag	0.23 ± 0.05	0.32 ± 0.07	76.23 ± 0.12	320 ± 11	210 ± 12	+/-	118 ± 8	7.34 ± 0.06	80.56 ± 0.17	34.45 ± 0.23		
Cu	0.24 ± 0.12	0.37 ± 0.17	76.45 ± 0.43	317 ± 10	220 ± 15	+	120 ± 6	7.34 ± 0.04	80.59 ± 0.19	34.57 ± 0.26		
Al	0.28 ± 0.23	0.38 ± 0.21	76.78 ± 0.44	289 ± 12	209 ± 12	+/-	108 ± 12	7.34 ± 0.06	80.38 ± 0.29	34.52 ± 0.31		
C. Nanoparticles intravenous (30 mg/kg)												
Ag	0.67 ± 0.12**	0.89 ± 0.11**	77.34 ± 0.21**	+4	350 ± 18*	160 ± 21**	+++	90 ± 8**	7.34 ± 0.08	81.56 ± 0.67	33.45 ± 1.23	
Cu	0.60 ± 0.19**	0.76 ± 0.08**	77.18 ± 0.08**	+4	328 ± 12**	174 ± 10**	++	89 ± 6**	7.30 ± 0.08	81.89 ± 0.89	32.67 ± 0.56	
Al	0.54 ± 0.12*	0.60 ± 0.12*	76.89 ± 0.12*	+2	330 ± 16*	195 ± 12*	+	96 ± 8**	7.32 ± 0.05	81.34 ± 0.89	33.58 ± 0.41	
D. Nanoparticles Cortical superfusion (20 µg/10 µL)												
Ag	0.78 ± 0.20**	0.98 ± 0.12**	77.68 ± 0.11**	+15	328 ± 14**	144 ± 12**	++++	97 ± 10*	7.30 ± 0.04	82.46 ± 0.94	32.66 ± 0.67	
Cu	0.69 ± 0.11**	0.78 ± 0.14**	76.80 ± 0.14**	+12	308 ± 9**	150 ± 7**	+++	106 ± 4*	7.30 ± 0.08	82.13 ± 0.45	32.36 ± 0.41	
Al	0.46 ± 0.10*	0.56 ± 0.10*	75.21 ± 0.23*	+3	282 ± 6*	181 ± 10	+	98 ± 10*	7.33 ± 0.06	81.48 ± 0.36	33.27 ± 0.35	

Values are Mean ± SD of 5 to 8 animals. * P < 0.05; ** P < 0.01, ANOVA followed by Dunnet's test for multiple group comparison from one control group. c.s. = cortical superfusion, Nil = absent, Na = sodium, K = potassium; %f = volume swelling calculated from changes in water content (for details see text). + = faint, ++ = moderate, +++ = extensive, ++++ = unclear, ? = boarder line.

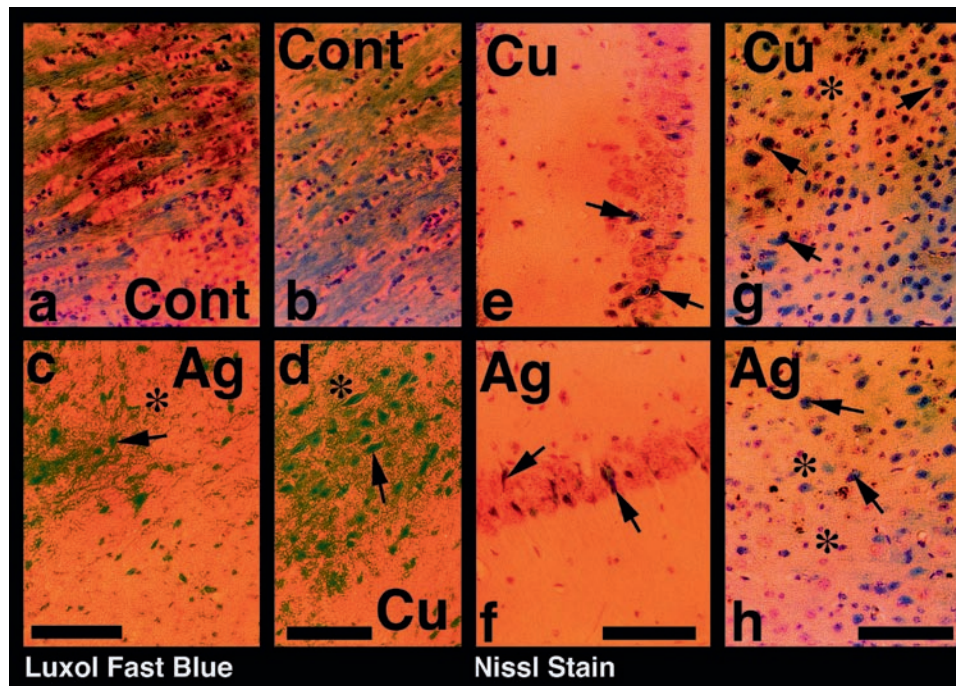


Fig. 2 Shows loss of myelin and nerve cell damage in rats treated with Ag (c, f, h) or Cu (e, g, d) nanoparticles. Luxol fast blue staining was done to check myelin loss in nanoparticle treated rats compared to controls (Cont a, b). Significant loss of myelin was seen in loss of blue-green staining following Ag[®] and Cu (d) treatment (arrows). The areas devoid of luxol fast blue (*) are clearly seen (c, d). Nissl staining

(e, f, g, h) shows dark and distorted neurons in the hippocampus following Cu (e) or Ag (f) treatment. In the cerebral cortex (g, h) Nissl stain showed many dark and distorted neurons following Cu (g) or Ag (h) treatment. Dark neurons (arrows), and loss of nerve cells (*) are clearly seen in the neuropil. Bar = 60 μ m (after Sharma et al., 2009, Ref. 15)

the amount of nanoparticles reaching the cerebral circulation is largely determining their effects on BBB permeability. Obviously, intravenous administration or cortical superfusion exposes the brain microvessels to these nanoparticles more effectively compared to their administration through intraperitoneal route.

Although, the mechanisms by which nanoparticles influence the BBB function are still unclear, it appears that nanoparticles depending on their characteristics may induce cellular or oxidative stress within the brain microvessels (1, 8, 15). Cellular or oxidative stress is known to induce release of various neurochemicals, cytokines and other neurodestructive factors, e.g., lipid peroxidation, generation of free radicals and nitric oxide (see (9–14)). These neurodestructive elements may then act on the cerebral microvessels either from lumen (when the nanoparticles are administered intravenously) or on the abluminal side (in case of cortical superfusion with nanoparticles) to disrupt the endothelial cell membrane permeability allowing intravascular tracers to leak within the brain microfluid environment (13, 15). Our investigation thus further suggested that nanoparticles are able to disrupt both the blood–brain and brain–blood barriers, not reported earlier.

This breakdown of BBB caused by nanoparticles is unrelated to the possible hyperosmotic effects of Al, Ag or

Cu in saline or Tween 80 solution (7, 9, 10). This is apparent from the fact that Tween 80 alone or NaCl solution of equimolar concentration did not affect the BBB function. Moreover, selective effects of nanoparticles with least BBB breakdown by Al nanoparticles compared to Ag and Cu also rule out hyperosmotic effects of solutions per se on BBB dysfunction (7).

Breakdown of the BBB to protein tracers, e.g., Evans blue and radioiodine, leads to vasogenic edema formation and subsequent brain damage (12–14). Significant increase in brain water and volume swelling in the areas showing Evans blue leakage induced by nanoparticles is in line with this idea. Alterations in Na⁺ and K⁺ content following nanoparticle treatment further support the development of vasogenic edema formation (4). When the brain fluid microenvironment is altered, then various biochemicals, immunological agents, and neurodestructive factors can easily be transported from blood to brain. Entry of these restricted elements into the fluid microenvironment of the brain will thus initiate serious immunological, biochemical, and cellular or molecular stress leading to nerve cell, glial cell and myelin injury (Sharma HS unpublished observation). Obviously, leakage of BBB and exposure of neurons, glial cells and myelin to exogenous serum factors will induce cell reaction in the brain.

Conclusion

In conclusion, our observations suggest that engineered nanoparticles when administered systemically are capable to induce BBB disruption, brain edema formation and lead to abnormal cell reactions. These effects of nanoparticles on brain function are most pronounced with Cu and Ag nanoparticles, compared to Al. It remains to be seen whether nanoparticle-induced brain dysfunction is related to dose and size of the nanoparticles. Furthermore, whether this acute exposure of nanoparticles will further enhance early neurodegenerative changes leading to various brain diseases is unclear and currently being investigated in our laboratory.

Conflict of interest statement We have no conflict of interest with any organizations mentioned below.

Acknowledgements This investigation is partially supported by the Air Force Office of Scientific Research (London), Air Force Material Command, USAF, under grant number FA8655-05-1-3065. The U.S. Government is authorized to reproduce and distribute reprints for Government purpose notwithstanding any copyright notation thereon. The views and conclusions contained herein are those of the authors and should not be interpreted as necessarily representing the official policies or endorsements, either expressed or implied, of the Air Force Office of Scientific Research or the U.S. Government. We express sincere gratitude to several laboratories where a part of the work is done or some data is recorded and evaluated. Financial support from Acure Pharma (Sweden); Astra-Zeneca, Mölndal, Sweden, Alexander von Humboldt Foundation (Germany); The University Grants Commission, New Delhi, India, Department of Science and Technology, Govt. of India, New Delhi is gratefully acknowledged. The authors have no conflict of interest with any financial agencies mentioned above. Technical assistance of Inga Hörte, Kerstin Flink, Madeleine Jarild, Mari-Anne Carlsson, Margareta Butler of Uppsala University are highly appreciated.

References

- Crüts B, van Etten L, Törnqvist H, Blomberg A, Sandström T, Mills NL, Borm PJ (2008) Exposure to diesel exhaust induces changes in EEG in human volunteers. *Part Fibre Toxicol* 11; 5:4
- Elder A, Gelein R, Silva V, Feikert T, Opanashuk L, Carter J, Potter R, Maynard A, Ito Y, Finkelstein J, Oberdörster G (2006) Translocation of inhaled ultrafine manganese oxide particles to the central nervous system. *Environ Health Perspect* 114:1172–1178
- Kim JS, Yoon TJ, Yu KN, Kim BG, Park SJ, Kim HW, Lee KH, Park SB, Lee JK, Cho MH (2006) Toxicity and tissue distribution of magnetic nanoparticles in mice. *Toxicol Sci Jan*; 89(1):338–347. Epub 2005 Oct 19. Erratum in: *Toxicol Sci. Mar* 2006; 90(1):267
- Kiyatkin EA, Brown PL, Sharma HS (2007) Brain edema and breakdown of the blood–brain barrier during methamphetamine intoxication: critical role of brain hyperthermia. *Eur J Neurosci Sep* 26(5):1242–1253
- Kreuter J (2007) Nanoparticles – a historical perspective. *Int J Pharm Feb* 22; 331(1):1–10
- Oberdörster G, Sharp Z, Atudorei V, Elder A, Gelein R, Kreyling W, Cox C (2004) Translocation of inhaled ultrafine particles to the brain. *Inhal Toxicol* 16:437–445
- Rapoport SI, Hori M, Klatzo I (1971) Reversible osmotic opening of the blood–brain barrier. *Science Sep* 10; 173(4001):1026–1028
- Rogers EJ, Bello D, Hsieh S (2008) Oxidative stress as a screening metric of potential toxicity by nanoparticles and airborne particulate matter. *Inhal Toxicol Jul*; 20(9):895
- Sharma HS (2007) Nanoneuroscience: emerging concepts on nanoneurotoxicity and nanoneuroprotection. *Nanomed Dec*; 2(6): 753–758. Editorial
- Sharma HS (1987) Effect of captopril (a converting enzyme inhibitor) on blood–brain barrier permeability and cerebral blood flow in normotensive rats. *Neuropharmacology* 6(1):85–92
- Sharma HS, Dey PK (1986) Influence of long-term immobilization stress on regional blood–brain barrier permeability, cerebral blood flow and 5-HT level in conscious normotensive young rats. *J Neurol Sci* 72(1):61–76
- Sharma HS, Sjöquist PO (2002) A new antioxidant compound H-290/51 modulates glutamate and GABA immunoreactivity in the rat spinal cord following trauma. *Amino Acids* 23(1–3):261–272
- Sharma HS, Sharma A (2007) Nanoparticles aggravate heat stress induced cognitive deficits, blood–brain barrier disruption, edema formation and brain pathology. *Prog Brain Res* 162:245–273. Review
- Sharma HS, Westman J, Cervós-Navarro J, Nyberg F (1997) Role of neurochemicals in brain edema and cell changes following hyperthermic brain injury in the rat. *Acta Neurochir Suppl* 70:269–274
- Sharma S, Ali SF, Hussain SM, Schlager JJ, Sharma A (2009) Influence of Engineered Nanoparticles from Metals on the Blood-Brain Barrier Permeability, Cerebral Blood Flow, Brain Edema and Neurotoxicity. An Experimental Study in the Rat and Mice Using Biochemical and Morphological Approaches. *J Nanosci Nanotechnol* 9:5055–5072
- Weiss CK, Kohnle MV, Landfester K, Hauk T, Fischer D, Schmitz-Wienke J, Mailänder V (2008) The first step into the brain: uptake of NIO-PBCA nanoparticles by endothelial cells in vitro and in vivo, and direct evidence for their blood–brain barrier permeation. *Chem Med Chem* 9:1395–1403