Mn nanoparticles exacerbates concussive head injury induced blood-brain barrier disruption, brain pathology, and sensory motor dysfunctions. Neuroproetction by Nanowired delivery of 5-HT2 receptor antagonists Ketanserin and Ritanserin

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ABSTRACT

This innovation deals with adverse effects of Mn nanoparticles (NPs) on brain pathology in concussive head injury. Also Mn nanoparticles treatment alters therapeutic modalities. The novel data presented in the abstract show that Mn exposure to our military personal from various industries, welding or deep mine work could make them more vulnerable to any additional head injury. This work also for the first time showed that neuroprotective agents in Mn treated traumatized group require in high concentration to treat effectively the brain pathologies. These data are strategically very important for further development of industrial or military medicine for health benefits. We have shown that nanowired delivery of 5-HT2 receptor antagonists e.g., ketanserin and ritanserin remarkably attenuated exacerbation of brain pathology after CHI in MN NPs treated group, not reported earlier.

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Keywords: Mn NPs, brain pathology, blood-brain barrier, military, ketanserin, ritanserin, neuroprotection

1 INTRODUCTION

Manganese (Mn) is an essential component for human brain function. However, excessive exposure of Mn may induce neurotoxicity [1-3]. Our military personnel are often exposed to Mn nanoparticles (NPs) from industrial sources, welding machine, battery assembly, hazardous wastes and/or deep mines resulting in physical disability and/or mental sickness that correlates well with their blood level of Mn [2,3]. However, whether additional injury or head trauma in these Mn exposed personals will induce exacerbation of brain pathology or alter treatment modalities are still unclear [3,4]. In present investigation effects of Mn NPs on Blood-brain barrier (BBB) breakdown, edema formation and cell injury was examined in a rat model of closed head injury (CHI). In addition, disturbances in sensory motor functions are also evaluated in Mn NPs treated normal and traumatized rats.

2 MATERIALS & METHODS

Experiments were carried out on Male Wistar rats (200-250 g body weight) housed at controlled room temperature (21±1°C) with 12 h light and Dark Schedule. Food and water were supplied ad libitum before experiment. All the experiments were carried out according to the Guidelines & Care for laboratory animals as described by national Institute of Health and approved by local Institutional Ethics Committee.

2.1 Exposure to MnNPs

Mn NPs (30-40 nm size, Product Nr # 45508, Alfa Aesar, Karlsruhe, Germany) were administered in a dose of 10 or 20 mg/kg, i.p. once daily for 7 days. Control rats

received saline instead of MnNPs. On the 8th day, one group of Mn NPs or saline treated treated rats were subjected to CHI.

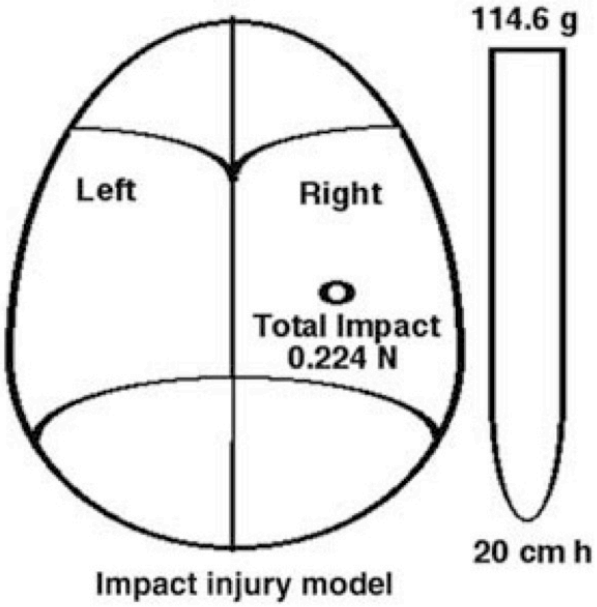

Fig. 1. Rat model of Concussive head injury (CHI). A metal weight is dropped over the right parietal skull inducing an impact force of 0.224 N resulting in more severe damage on the left side due to concussion [For details see Text].

2.2 Concussive head injury

CHI was produced by dropping a weight of 114.6 g from a 20 cm height through a guided tube over the right parietal bone under Equithesin anesthesia (3 ml/kg, i.p.) [Fig. 1]. This combination delivers an impact of 0.224 N on the skull surface resulting in very similar to clinical concussion as evident with severe damage of contralateral side (left half) aftre injury [4,5].

2.3 Nanodelivery of drugs

We used normal (2 or 4 mg/kg, i.p.) or TiO2 nanowired Ketanserin or Ritanserin (1 or 2 mg/kg, i.p.) in normal or brain injured rats following MnNPs exposure. The drugs were given in separate groups of animals once daily starting from 3 days after MnNPs treatment and continued until 7th days [5,6]. In these treated and untreated traumatized rats neuropathological and behavioral parameters were evaluated (see below).

2.3.1 Blood-brain barrier

The BBB leakage was measured using Evans blue albumin (EBA, 2 % 3 ml/kg i.v.) and radioiodine ([131]-Iodine] 100 µCi/kg, i.v.) extravasation in the brain given 10 min before termination of the experiment [5]. After intravascular washout of the tracers with 0.9 % saline in situ, the brains were dissected out and examined for blue staining and the radioactivity determined in a Gamma Counter (Packard, USA). Leakage of these tracers in brain was expressed as percentage increase over blood concentration [3,4,6].

2.3.2 Brain Edema formation

The brain edema formation was measured using water content in the brain by obtaining wet and dry weights of samples. According to standard protocol [5,6].

2.3.3 Neuronal injury

For this purpose, animals were perfused in situ with 4 % buffered paraformaldehyde preceded with a brief saline rinse though cardiac puncture and coronals sections of the brain were cut and embedded in paraffin. Using Nissl or Haematoxylin & Eosin (HE) staining on 3-µm thick paraffin sections obtained from brains of different groups neuronal damages were analyzed. [5,6].

2.3.4 Rota Rod performance

In all these animals, behavioral dysfunctionswas examined on a Rota Rod apparatus (Harvard Apparatus) manually using a speed of 16 rpm as described earlier [6].

2.3.5. Inclined plane angle test

Animals from different groups wer placed on an inclined plane angle apparatus (at 60°) and exmimed their stay on the plane for 5 sec. Those animals that could not stand on this steep plane the angle of the plane was lowered down to record their saty and noted. [6]

2.3.5. Walking on a mesh grid

Animal were allowed to walk on a steel mesh grid inclined at 45° and faulty placement of forepaw was recorded over a period of 120 sec. Normal animals could walk easily without any problems [5,6].

2.4 Statistical analyses

ANOVA followed by Dunnett's test for multiple group comparison with one control group was used to analyze statistical significance of the data obtained. A p-value less than 0.05 was considered significant.

Table 1. Neuroprotective effects of TiO2-nanodelivered 5-HT2 receptor antagonists Ketanserin and Ritanserin on Mn NPs induced brain pathology in CHI.

Expt. Type	Control	CHI 5 h LH	CHI+MnNPs LH	CHI+MnNPs +KTN LH	TiO2-KTN+ +CHI+MnNPs LH
		A. Neuropathological Parameters			
EBA mg%	0.24±0.08	2.34±0.10**	3.67±0.14**#	1.04±0.09*a	0.54±0.16*#b
$^{[131]}$Iodine %	0.34±0.08	2.83±0.16**	3.98±0.12**#	1.68±0.11*a	0.64±0.13*#b
Brain Water %	75.36±0.12	81.54±0.24**	83.28±0.34**#	77.34±0.26*a	75.88±0.22*#b
Neuron Injury Nr	1±2	284±65**	485±72**#	122±43*a	24±12*#b
		B. Behavioral Parameters			
Rota-Rod Performances Sec	120±3	74±6**	56±8**#	94±8*a	112±8*#b
Inclined Plane Angle test °	60±2	34±3**	23±3**#	48±5*a	54±3*#b
Placement Error Nr	1±1	15±2**	24±4**#	8±32*a	3±2#b

*Values are Mean±SD of 5 to 6 rat; .LH = Left Half, non –traumatized; KTN = Ketanserin, * P <0.05, ** P <0.01 from control, # P <0.05 from CHI =Concussive head injury, a P <0.05 from CHI+MnNPs, b P <0.05 from CHI+MnNPs+TiO2-KTN = nanodelivered Ketanserin. For details see text.*

3 RESULTS

3.1 MnNPs induce neurotoxicity

Normal animals treated with MnNPs exhibited cognitive and motor dysfunction on the 8th day [Table 1]. At this time, BBB disruption, brain pathology and brain edema formation were most marked in the sensory-motor cortex (+134 %), hippocampus (+180 %), caudate putamen (+87%), cerebellum (+167%) and thalamus (+98%), hypothalamus, (+56%) pons (+52%), medulla (+48%) and spinal cord (+156%). Neuronal injuries were present in all these brain regions [Table 1].

3.2 MnNPs exacerbate CHI pathology

MnNPs intoxicates rats aftre CHI exhibited about 2- to 4-fold exacerbation of BBB breakdown, edema formation and neuronal injuries. In these animals, the cognitive and sensory motor functions were further worsened than CHI in normal animals [see Table 1].

These adverse effects of MnNPs in CHI are dose dependent [results not shown].

3.3 Effect of Drug Treatment

Treatment with potent neuroprotective compounds ketanserin or ritanserin (selective 5-HT2 receptor antagonists) induced marked neuroporetction in normal animals exposed to MnNPs. This protective effects of 5-HT2 receptro antagonists was dose dependent [Fig. 2]. However, when these drugs were administered in MnNPs intoxicated rats aftre CHI, high doses of the drugs (4 mg) is needed to induce appreciable neuroproetction. High dose of the drugs (4 mg instead of 2 mg) was also needed to improve behavioral responses after CHI in MnNPs exposed rats [Table 1].

On the other hand, when TiO2-nanowired ketanserin or ritanserin were adminsitered marked neuroportection was seen with lower doses of the drugs (1 or 2 mg/kg, i.p.) in CHI rats aftre MnNPs intoxication. These animals also showed remarkable improvement in behavioral functions [Table 1]. It appears that ritanserin has more superior effects than ketanserin at idenitical doses in CHI induced barin pathology in normal or MNNPs intoxicated rats [results not shown].

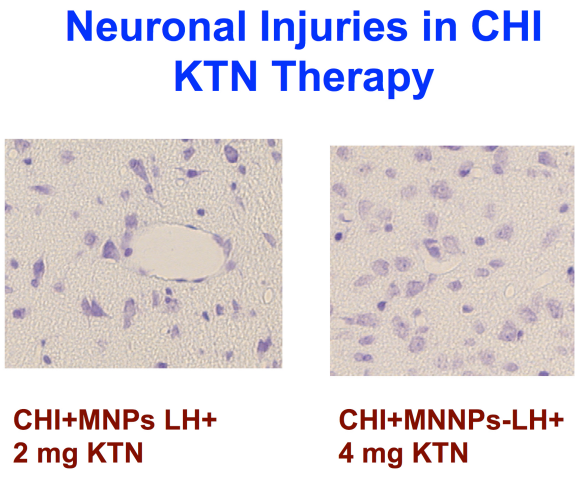

Fig. 2. Shows Higher dose of Ketanserin (4 mg) is able to reudce neuronal injuries in the contralateral half (Left half, LH) aftre concussive head injuruy (CHI). Nissle staining on Paraffin section, x 40.

4 DISCUSSION

Our novel findings clearly show that MnNPs not only induce neurotoxicity in normal animals but also exacerbate brain pathology and behavioral functions after brain injury. This suggests that exposure to MnNPs to healthy populations or military personnel during work related activities could be more dangerous in terms of their mental health following any kinds of head injury or insults to the central nervous system (CNS). Our results are this first to point out that the pathological processes following CHI are exacerbated by prior MnNPs exposure.

The probable mechanisms underlying MnNPs exposure and exacerbation of CHI induced brain pathology are still unclear. However, available evidences show that exposure of MnNPs induces oxidative stress in the brain and alters glutamine (Gln)/glutamate (Glu)-γ-aminobutyric acid (GABA) cycle (GGC) between astrocytes and neurons leading to neurotoxicity [3,4]. Furthermore, an increased production of nitric oxide synthase (NOS) occurs following MnNPs exposure hat may also contribute to damage of neurovascular units [2-4]. A breakdown of the BBB to serum proteins in this investigation is in line with this idea. Consequently brain edema develops and cell injury occurs due to exposure of neurons, astrocytes to unwanted materials from the blood [5,6].

Our observations further show that two potent serotonin 5-HT2 receptor antagonists ketanserin and ritanserin when are able to thwart MnNPs induced neurotoxicity in normal animals. This suggests that serotonin is somehow involved in Mn neurotoxicity. Blockade of 5-HT2 receptors are known to reduce BBB breakdown, edema formation and cell injury [5,6]. This suggests that apart from oxidative stress and altered amino acid metabolism, serotonin could play key roles. A massive increase in plasma and brain serotonin in CHI is associated with brain pathology and behavioral dysfunctions further support this hypothesis [5].

However, when CHI was induced in MnNPs exposed rats, these 5-HT2 receptor blockers were not enough to contain brain pathology. In this group TiO2 nanowired delivery of ketanserin or ritanserin is needed to induce desired neuroprotection. This suggests that nanodelivery of compounds may have superior neuroprotective effects than the parent compounds [see 6].

An improved behavioral function in MnNPs treated traumatized rats by TiO2-nanowoired ketanserin or ritanserin could be due to neuroprotection offered by these agents. Obviously, damage to sensory motor pathways due to edema formation and BBB breakdown resulting in neural injuries contributes to behavioral dysfunction.

5 CONCLUSION

In conclusion, our investigations for the first time point out a role of 5-HT2 receptor blockers in MnNPs neurotoxicity and further show that exacerbation of brain pathology following CHI in MnNPs exposed subjects may beconta8ned by TiO2 nanowired delivery of these drugs achieve better neuroprotection that the parent compounds. The findings have immense clinical significance in military medicine as well as in those populations who are exposed to MnNPs in their work-related environments.

6 ACKNOWLEDGEMENTS

This investigation is partially supported by grants from the Air Force Office of Scientific Research (EOARD, London, UK), and Air Force Material Command, USAF, under grant number FA8655-05-1-3065; Swedish Medical Research Council (Nr 2710-HSS), IT 794/13 (JVL), Govt. of Basque Country and UFI 11/32 (JVL) University of Basque Country, Spain. Technical assistance of Mari-Anne Carlsson and Ingmarie Olsson of Uppsala University is highly appreciated. The U.S. Government is authorized to reproduce and distribute reprints for Government purpose notwithstanding any copyright notation thereon. The views and conclusions are exclusively those of the authors and should not reflect the official policies or endorsements of the Air Force Office of Scientific Research or the U.S. Government or any of the granting organizations or collaborating entities mentioned above.

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