Nanomedicine Journal

Received: Sep. 19, 2013; Accepted: Nov. 12, 2013
Vol. 1, No. 3, Spring 2014, page 180-190

Online ISSN 2322-5904
http://nmj.mums.ac.ir

Original Research

Comparison of MnO_2 nanoparticles and microparticles distribution in CNS and muscle and effect on acute pain threshold in rats

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Abstract

Objective(s): Recently, applications of MnO_2 nanoparticles and microparticles in industry, pharmacology, and medicine have considerably expanded. Mn distribution and clearance from brain and spinal cord tissue compared with muscle tissue of rats after single subcutaneous injection of nanoparticles and microparticle of MnO_2. Pain sensory threshold of rat was evaluated as neurologic consequence of the particles on CNS activity of rats.

Materials and Methods: Rats divided to control and two experimental groups. Each experimental group received a single subcutaneous injection of MnO_2 nano- and microparticles, respectively. Acute pain thresholds of rats were evaluated by tail immersion method and its weight gain was recorded during these weeks. Samples taken from brain, spinal cord and muscle tissues of rats, once every 2 week for 8 weeks. The tissue Mn level was measured by inductively coupled plasma-mass spectrometry method.

Results: Both particles size passed from blood barriers. Unlike brain tissue, manganese completely cleared from spinal tissue after 8 weeks in both groups. Clearance of Mn from muscle tissue is not complete in both of the groups. Weight gain of rats in both groups was slower than control rats. In microparticle group, rats showed progressive analgesia ($p<0.05$). In nanoparticle groups, rats showed hyperalgesia for first 4 weeks and analgesia during remaining weeks.

Conclusion: Change in MnO_2 particles size affect on Mn distribution and clearance from central nervous system. Effect of particles on whole body metabolism varied with its size too. Finally, comparison of pain response of rats among particle treated groups indicates that neurobiological mechanism affected by particles is varied with their size during times after administration.

Keywords: Biodistribution, Clearance, MnO_2, Nanoparticles, Pain

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Introduction

The use of metal oxide nanoparticles is currently developed in all industries; from computer to perfume manufacturing (1). Currently, microparticles and nanoparticles of manganese dioxide (MnO_2) application in industry, medicine, and pharmacology is increased and therefore human exposure with these particles augmented. The rate of contamination with MnO_2 in environments is higher than other forms of Mn because of MnO_2 use as a substrate for synthesis of other Mn-containing compounds (2). Also, in comparison with other forms of Mn particles, MnO_2 nanoparticles have a higher oxidation power (3). Steel casting and dry battery industries are two major industries in which workers are in contact with MnO2 nanoparticles. Besides, due to the use of MnO_2 nanoparticles in composition of methylcyclopentadienyl manganese tricarbonyl (MMT) as a gasoline additive, all people that live in big cities are at risk of its toxic effects (4). This nanoparticle is also used in MRI contrast dye (5). Most of the previous information about MnO_2 neurotoxicity is restricted to its chronic effects (6, 7). The high oxidative activity of these particles can cause stimulation of pathological mechanisms and induce adverse effects on cell function, like autophagy, induction of cell apoptosis (8, 9). Indeed, it is reported that changes in particle size from micrometer to nanometer can augment the oxidation power of the particles. This enhancement of oxidative properties of particles increased the probability of undesirable acute effects of nanoparticles on living organisms. The syndrome resulting from chronic exposure to manganese particles in industrial workers is known as manganism and is similar to Parkinson's disease (10). This finding indicates that dopaminergic neurons are one of the main targets of MnO_2 particles in CNS. Recent findings show a relationship between intensity of metal nanoparticles toxicity with their concentration and size (11). Therefore, the information about size-dependent biodistribution, clearance, and CNS effects of these compounds has practical importance for further applications of these particles (12). Kim and colleagues reported that the application of a certain dose of MnO_2 nanoparticles in a special size does not have any toxic effects on laboratory animals (13). Contrary, others reported that topical administrations of MnO_2 particles with a diameter less than 2 micrometers to monkeys for one day up to 10 months caused wide distribution of Mn in the nervous system and appearance of some neurological symptoms (14). Also, a linear relation between concentration of MnO_2 nanoparticles and production of reactive oxygen substances (ROS) compounds in Hela cells was demonstrated (15). The oxidative power and easily passing of nanoparticles through blood-brain barrier can induce undesirable acute effects on sensory, motor, and cognitive aspects of CNS functions (16). It has been shown that some metal nanoparticles produced some neural dysfunction even after single exposure (17). In contrary, administration of other nanoparticles has no toxic effects on the function of nervous system in animals (18). Therefore, particles on the base of concentration and size have unique neurotoxic effect and must be studied separately. ICP-MS provides a high quality method for measurement of metal particles concentration in various tissues; especially in brain tissues (19). Tail immersion is a method for assessment of animal pain threshold. The efficacy of this method is shown in assessment of pain threshold changes with respect to dopaminergic system manipulation (20). In this research, the Mn level in rat brain, spinal cord, and muscle tissues were measured once 2 weeks for eight weeks by ICP-MS after single subcutaneous injections (100 µg/kg) of MnO_2 micro and nanoparticles for pharmacokinetic comparison of these two particles. Also, body weight gain of rats

during this period was compared between MnO_2 microparticles and nanoparticles treated groups. Also, pain thresholds of rats in the two groups were determined by tail immersion methods during these weeks for comparison of acute neurological effects of these particles.

Materials and Methods

Animals

Male albino Wistar rats (Pasteur's institute, Tehran, Iran) weighing 150±5 g were housed in an air-conditioned colony room on a light/dark cycle (21-23°C and a humidity of 30-40%) and supplied with standard diet and tap water ad libitum. Procedures involving animals and their care were conducted in conformity with the NIH guidelines for the care and use of laboratory animals.

Selecting the dose

The dose of MnO_2 nanoparticles and microparticles selected on the basis of You and coworkers report (21). Rats were divided into two groups and treated subcutaneously with solution containing nano- and microparticles of MnO_2 (100 µg/kg) dissolved in saline, respectively. Control rats only received normal saline. MnO_2 microparticles (Figure 1) used in this research were purchased from MERCK Company, Germany.

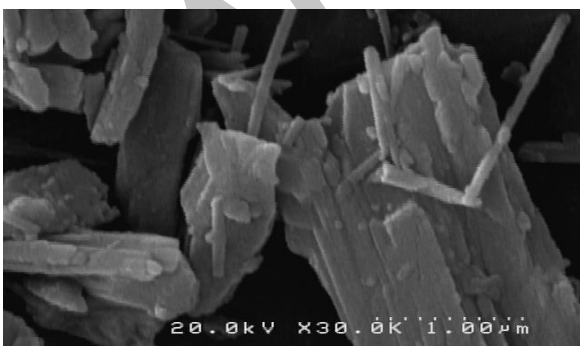

Figure 1. A scanning electromicrograph of MnO_2 micropaticles.

The MnO_2 nanoparticles preparation

MnO_2 nanoparticles were prepared via the hydrothermal procedure proposed by Zhang et al., with some modification (22). In practice, 20 ml of $KMnO_4$ (0.2 mM/lit) were mixed with 16 ml MnO_4 (0.125 mM/lit) for 5 minutes. The resulting mixture was taken directly into a steel autoclave with Teflon covering and kept for 16 hours at 160°C and then was cooled at room temperature. The resulting brown product was collected, washed with distilled water and ethanol 3 times, and dried with hot air current 80°C for 12 hours. The resulting particles were scrutinized by an electron microscope and it was ensured that they were 25 to 85 micrometers in size (Figure 2). Preparing MnO_2 nanoparticles and microparticles injecting solution.

First, the particles were dissolved in normal saline solution. The prepared solutions were sonicated for 30 minutes before injection.

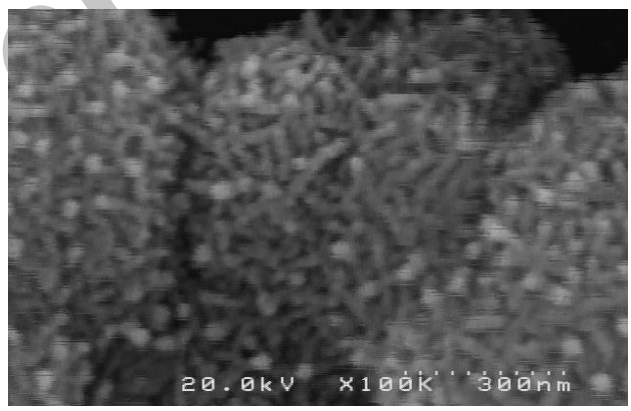

Figure 2. Scanning electromicrograph of MnO_2 nanopaticles**.**

Tissue Samples Preparation for ICP/MS measurement

Male Wistar rats (140 ±10 g) were used in this experiment. Rats were divided into three groups: control, microparticle, and nanoparticle groups. The experimental groups injected subcutaneously with solution containing MnO_2 (100 µg/kg) microparticles or nanoparticles, respectively.

Control rats received only normal saline. Five rats were chose from each group every two weeks and were deeply anesthetized with diethyl-ether (Merck).

Then, rats scarified and their brains transformed into ice-cold ACSF solution (4°C). The spinal cords of rats were pushed from vertebral canal with perfusion of high-pressure of cold ACSF solution into the vertebral canal. Muscle tissue sample was taken from hind paw of rats (squadriceps femoris).

All samples were frozen with liquid nitrogen after weighting and kept in a -20°C until ICP-MS measurement.

The tissue Mn was extracted by the following procedure: 1/2 gram of each tissue sample was selected and one ml of HNO_3 %63 (Merck, Germany) was added to it (23). Then, the samples were kept for 24 hours at 4°C for complete digestion of the tissues. The digested samples were centrifuged at 4500 rpm for 5 minutes. The upper clear solution was diluted with extra-pure water and the MnO_2 level was measured by ICP-MS (24).

The ICP-MS measurement of tissue Mn level

A standard Agilent 7500 ICP-MS, fitted with a crossflow nebulizer and a quartz spray chamber was used throughout (25). Operational parameters are given in Table 1. A solution containing 100 PPM of Mn (Merck) was used as the standard solution.

Table 1. Operational parameters of ICP/MS for measurement of Mn level in liver tissue extract.

Parameter	Value	Units
Argon Gas Flow Rates		
Plasma	15.0	L/min
Auxiliary	1.0	L/min
Carrier	1.2	L/min
RF Power	1350	W
Sampling Depth	6.7	mm
Nebulizer	Cross-flow	
Spray Chamber	Double-pass, quartz	
Spray Chamber Temp.	2	°C
Sample Uptake	0.4	ml/min

Tail immersion test

The rat pain threshold was assessed using tail immersion test. After the adaptation, rat tail was immersed in warm water (49°C) and the tail flick response latency (withdrawal response of tail) was observed as the end-point response.

Each experiment was repeated 4 times for each animal with an interval of 2 min and its average was reported. Cut-off time of 25 sec was considered (26, 27).

Results

Tissue Mn level

The basal level of Mn in brain, spinal cord, and muscle tissues of control rats is 1 (µg/g tissue wet weight). The maximum content of Mn in brain tissue of microparticles group is higher than nanoparticles group.

In MnO_2 microparticles group, Mn level in the brain tissues reached to its maximum level 4 weeks after injection (Figure 3).

Then, the Mn level in this tissue returned to 77.5% its maximal value after 8 weeks. In MnO_2 nanoparticles group, Mn level in brain tissue was reached to its maximum level 2 weeks after injection and its level reduced to 42% its maximum valve after 8 weeks.Variation of Mn level in spinal cord tissues during 8 weeks after the injection of particles is shown in Figure 4.

Mn concentration in spinal cord tissues following the injection of MnO_2 microparticles attained its maximum level after 4 weeks, but this time for the nanoparticles receiving group was 2 weeks.

The variation of Mn level in muscle tissues during 8 weeks after the injection of MnO_2 microparticles and nanoparticles is shown in figure 5. In the MnO_2 microparticle groups, Mn level in muscle tissue increase very slowly and reach to a plateau after 2th week.

But in MnO_2 nanoparticle group, the tissue level of Mn increased very fast and reached to high level in second weeks after this particle injection.

But the tissue Mn level quickly decreased to a basal level at 4th week and remained stable till the end of experiment.

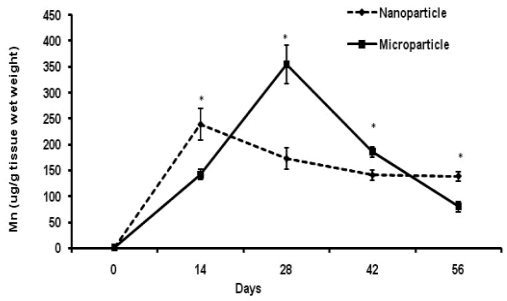

Figure 3. Change in Mn level in brain tissues of rat during 8 weeks after single treatment with MnO_2 (100µg/kg) microparticles and nanoparticles. Groups compared by ANOVA and tukey post-test, *p<0.05 (n=5).

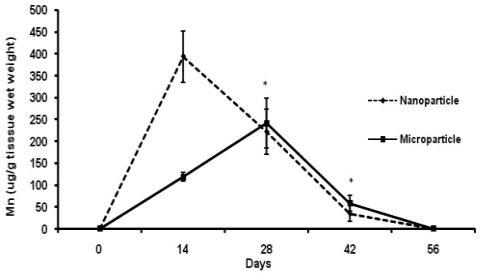

Figure 4. Comparison of Mn level in spinal cord tissues of rat (n=5) in MnO_2 microparticles or nanoparticles (100 µg/kg) treated groups during 8 weeks. ANOVA and tukey post-test showed a significant (p<0.05) difference in Mn tissues level in between groups.

Weight gain changes

The rate of weight gain in rats in nanoparticle group in comparison with control rats significantly (p<0.05) reduced during 2th to 4th week after injection (Figure 6).

Weight gain of rats receiving the same dose of microparticles only at the 4th weeks was significantly (p<0.05) lower than control rats (Figure 6).

Tail immersion measurement of acute pain

The results of tail withdrawal latency of rats in groups during 8 weeks after injections are shown in Figure 7.

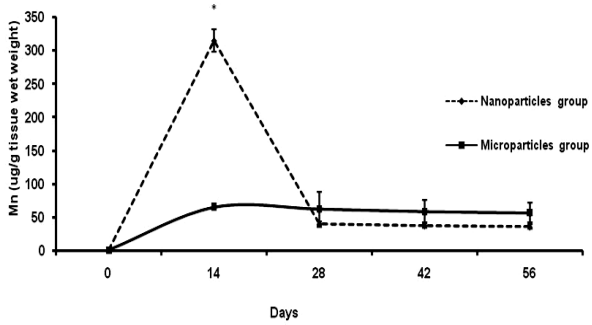

Figure 5. Change in Mn level in muscle tissues of rats (n=5) in MnO_2 microparticles or nanoparticles (100µg/kg) groups during 8 weeks after injection. The ANOVA and Tukey post-test show a significant difference (p<0.05) between two groups.

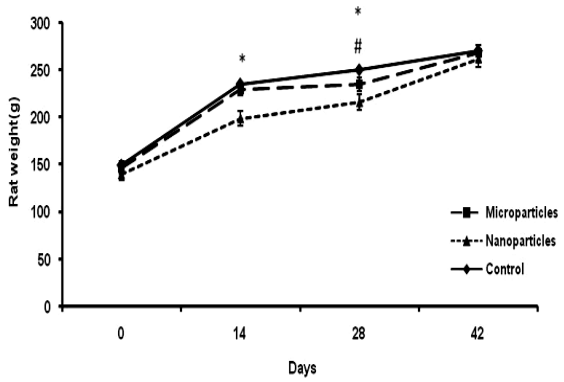

Figure 6. Rat weight gain changes during 8 weeks after single subcutaneous injections of MnO_2 microparticles or nanoparticles (100 µg/kg) in comparison with control rats (n=5). *control vs. nanoparticle groups, # control vs. microparticle groups (p<0.05).

The mean tail withdrawal latency of rats before the particles injections was 10 seconds.

The cut-off time was chosen 25-second.

The microparticles injection caused a significant (p<0.05) progressive increase in tail flick latency of rats during 8 weeks.

This progressive increase in tail filck latency of rats continued to end of experiment.

The rats treated with MnO_2 nanoparticles showed a significant (p<0.05) hyperalgesia in the first four weeks after injection and analgesia (p<0.05) during the remaining weeks (Figure 7).

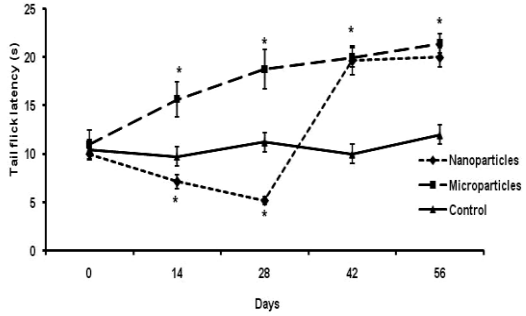

Figure 7. Tail flick latency of rats (n=5) following single subcutaneous injections of MnO_2 microparticles or nanoparticles (100 µg/kg) in comparison with the control group. *Control vs. experimental groups ($p<0.05$).

Discussion

Our results showed that basal amount of Mn in normal rat brain is very low. Previous research also has shown presence of very small amount of Mn in brain tissue of human and other animals (28, 29). The Mn level in brain tissue of control rats in this study was proportional with its level in previous studies (30). Measurement of Mn level in brain and spinal cord of rats following administrations of MnO_2 micro and nanoparticles indicate that both types of particles can pass through blood barrier and entered to spinal cord. Previous research has shown Mn can enter and distributed in brain through olfactory bulb (31). It is shown that water soluble and insoluble forms of manganese particles can easily pass through the BBB (32). In this study, Mn clearance rate from brain and spinal cord is not the same for MnO_2 nano- and microparticles receiving groups. The spinal cord tissues were completely cleared from Mn after 8 weeks in both treated groups but brain tissues were not completely cleared from Mn during this time. Previously, it was demonstrated that bioclearance of nanoparticles varies among different tissues; even in tissues have not blood barriers (33).

The Mn clearance rate from brain tissues in microparticle group is faster than nanoparticles group, but Mn clearance rate from spinal cord tissues was the same for nanoparticles and microparticles treated groups. Sharma and colleagues evaluated permeability range of blood and spinal cord barrier in rats after hyperthermia stimulation with the use of nanoparticles. Their findings suggest that blood-spinal cord barrier permeability variation is more than blood-brain barrier (34).

This report confirms our observation about difference of nanoparticles clearance rates from brain and spinal cord. Previously, it is reported that Mn distributed to all areas of rat brain after intracerebroventricular administration of MnO_2 nanoparticles within three weeks and then concentration of Mn in brain tissue decreased gradually (35, 36).

The time course of this report is equal to time that obtained in our study for reaching of Mn level in brain tissue to maximum value after MnO_2 microparticle administration. Size-dependent differences in tissue bioclearance of particles were shown previously, for example, comparison of fluorescent polystyrene bioclearance microparticles (2 micrometers) with its nanoparticles (200 nanometers) after subconjunctival injections in rats showed that most of nanoparticles disappeared from the periocular tissue during 15 days and only %8 of their initial amount remained in tissues up to 7 days after the injection, but micro particles stayed in the injected tissue for more than two months (37). In nanoparticle group, the Mn slowly accumulated in muscle tissues and reached to plateau at fourth week. In micro particles group, the concentration of Mn in muscle tissue show an initial rise in the second week and then returned to a lower level and stayed at this level till the end of study.A similar situation was demonstrated for clearance of gold nanoparticles from liver and spleen tissues of rats (38).

Thus, the accumulation and clearance pattern of Mn in each tissue special pattern and also changed with particles size. It should be noted that weight gain changes was measured as indicator of general

health status of animals.

The reduction in weight gain of microparticle receiving rats continued for two weeks after the injection, while this effect in nanoparticle-receiving group was longer and continued for four weeks after the injection. Our results show a relation between tissues level of Mn and weight changes of rats. Little information was available about the acute effect of metal oxide nanoparticles on weight changes of animals and most of the researchers have studied the effects of chronic administration of metal oxide particles on the weight changes of animals. Besides, the effect of nanoparticles on weight gain changes of animals varies from one metal oxide nanoparticle to another. For example, intraperitoneal injections of TiO_2 nanoparticles for 3-month to rats have not any significant effects on its weight gain (39). On the contrary, inhalation of Fe_2O_3 nanoparticles significantly affects on rat weight gain (40).

Scientists related these differences in nanoparticles effect on body weight to their oxidative power (41). In support of this statement, it is shown that manganese oxides can cause impaired mitochondrial redox activity (18). MnO_2 oxidative power is higher than other form of Mn oxides, such as Mn_3O_4 (42).

Therefore, our results indicate MnO_2 particles in both size can influence on the whole body metabolism in rats, moreover the effect of the nanoparticle is slightly higher than the microparticles.

Tail flick latency of rats was evaluated in order to compare effects of the particles on nervous system activity (43).

Scientists previously are used tail immersion latency test for evaluation of analgesia duration after subcutaneously injected nanoparticles (44).

Tail immersion test considered as a model of acute thermal pain (45). Acute pain results from disease, inflammation, or injury to tissue.

Inflammatory processes contribute to nerve cell toxicity (46). Our results indicate that both size of MnO_2 particles influenced on pain thresholds of rats.

The administration of microparticles led to a gradual, irreversible increase in the pain thresholds of rats during eight weeks. However, nanoparticles produced biphasic response in rat, initial hyperalgesia for four weeks and late analgesic during last four weeks. On the other hand it has been reported that inhalation of MnO_2 particles caused changes in the neurochemistry of brain dopaminergic system (47) and severely affect on expression of genes like MAOA, Park2, and SNCA (48). Dopamine also plays a clear role in pain control at the spinal cord level (49, 50) and dysfunctions in this system in spinal cord level can produce deficits in pain sensation (51). Patients with Parkinson's disease showed a progressive hyperalgesia similar to rats treated the MnO_2 micro particles in our study (52, 53).

As a conclusion we can say that MnO_2 particles effects on acute pain threshold probably mediated via dopaminergic systems and progressive nature of their effect is similar to Parkinson's disease. As explanation for biphasic effect of MnO_2 nanoparticles on rat pain responses and according to Wang’s finding, a direct relation exists between the level of Mn nanoparticles accumulation in central nervous system and its neurological symptoms (54).

Tissue Mn concentration increased considerably after nanoparticle injection during the first four weeks and then decreased and remained constant during the remaining ones.

Thus, it seems that the neural mechanisms that modulated by MnO_2 nanoparticles in the first four weeks after injection are different from late-phase mechanisms.

It is shown that manganese ions can interrupt the transmission of stimulatory signals in the nervous system through changes in the activity of calcium channels at pre-synaptic terminals (26).

Also, neuronal evoked potentials of rats showed a significant reduction after long-

term inhalation of MnO_2 nanoparticles (55).

The reduction in activity of descending pain modulation tract can induce hyperalgesia (56).

Thus, it seems that during first four weeks after injection, nanoparticles activate central descending mechanisms that induced hyperalgesia in rats.

Also these mechanisms can mask the MnO_2 nanoparticle analgesic effects in this phase.

But, at the end of first phase and termination of these fast effects, the long term analgesic effect of nanoparticles via effect on dopaminergic system is appeared.

In support of our hypothesis that central dopaminergic system that mediate analgesic effect of MnO_2 nanoparticles, it is reported that depletion of dopaminergic neurons in periaqueductal grey areas of rat brain can induce analgesia (20).

Conclusions

Administrations of microparticles or nanoparticles of MnO_2 caused Mn accumulation in brain, spinal cord and muscle tissues of rats after single injection for long time. Both sizes of MnO_2 have adverse effects on general metabolism of rats and reduce normal rate of rats weight gain.

The MnO_2 nanoparticle or microparticle has different effect on pain sensation. Probably, change in Mn level in CNS with effect on dopaminergic system mediates its effects on pain modulating systems of rats.

Acknowledgment

This work was supported by Islamic Azad University, Tehran, Iran and contribution of Shahed University of Iran.

We also thanks from Dr Hassani in ICP/MS lab of Tarbiat Modares University for his helps.

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