Research Article

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Protective effect of green tea against neuro-functional alterations in rats treated with MnO_2 nanoparticles

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Abstract

BACKGROUND: Inhalation of manganese-containing metal fumes at workplaces can cause central nervous damage including a Parkinson-like syndrome. Oxidative stress is likely to be involved in the pathomechanism, due to the presence of nano-sized metal oxide particles with high biological and chemical activity. Oxidative damage of the nervous system could be prevented or ameliorated by properly applied antioxidants, preferably natural ones such as green tea, a popular drink. The aim of this work was to see if orally applied green tea brew could diminish the functional neurotoxicity of manganese dioxide nanoparticles introduced into the airways of rats.

RESULTS: Young adult male Wistar rats were treated intratracheally for 6 weeks with a suspension of synthetic MnO_2 nanoparticles (4 mg/kg body weight), and received green tea brew (1 g leaves 200 mL^{-1} water) as drinking fluid. Reduced body weight gain, indicating general toxicity of the nanoparticles, was not influenced by green tea. However, in rats receiving green tea the nervous system effects – changes in the spontaneous and evoked cortical activity and peripheral nerve action potential – were diminished.

CONCLUSION: The use of green tea as a neuroprotective functional drink seems to be a viable approach.

Keywords: antioxidant; protection; neurotoxicity; oxidative damage; cortical and peripheral electrical activity

INTRODUCTION

Oxidative stress is a phenomenon known or suspected to be involved in the toxic mechanism of numerous environmental and occupational agents, including heavy metals, and it is supposed that antioxidant agents might be applied to avert the health damage caused by exposure to such agents.[1] Optimally, these agents would be available not as pharmaceutical products but as natural substances easily included in the daily diet.

In this aspect tea, especially green tea, would be a strong candidate. Tea is a popular drink worldwide and is a major contributor to total antioxidant intake in many populations. The neuroprotective effect of catechins and other tea flavonoids is based on antioxidant and metal chelating activity;[2] but beyond acting directly as radical scavengers, they also activate transcription factors and antioxidant enzymes.[3] Regarding the prevalence of chronic degenerative central nervous system (CNS) diseases and the popularity of tea, this protective effect has been extensively studied, and the possible beneficial effect of phytochemicals in neuroprotection has been mentioned repeatedly.[4,5]

One of the toxic heavy metals often encountered in industrial settings is manganese (Mn). Significant exposure and health damage have been described first of all in the metal industry, most frequently in welding jobs, but also in the manufacture of zinc–carbon and alkaline dry cells.[6,7] The CNS is an important target for Mn, and the Mn-induced chronic health damage – a Parkinson-like syndrome called manganism[8] – is dominated by CNS symptoms. Mn induces oxidative stress via the oxidation of dopamine and other catecholamines, as Mn is accumulated in

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dopamine-rich brain regions, especially in the basal ganglia,[9] and via mitochondrial damage by inhibition of complexes II and III.[10,11] The activity of two major antioxidant enzymes, Mn superoxide dismutase and glutathione peroxidase, is also decreased if there is a local overdose of Mn.[12]

In welding fumes, Mn is nearly always present for technical reasons. Suspended Mn-containing particles in fumes, both in the micrometre and nanometre range,[13] induced oxidative stress under experimental circumstances both *in vivo* and *in vitro*.[14] The presence of nanoparticles (NPs) may greatly contribute to the pathogenicity of welding fumes as, due to their small size, they have a large specific surface area and typically a high number of particles per volume of air, leading to high biological and chemical activity, including generation of reactive oxygen species.[15] NPs of various composition have been detected in the brain of rats after application through the airways, which points to a direct way of damaging.[16] The role of oxidative stress in the mechanism of neurodegenerative diseases has been described[1] and neuro-functional alterations in rats treated with metal oxide NPs have likewise been demonstrated.[17]

Taken together, the above facts suggest that antioxidant agents might be usable against the CNS damage caused by exposure to welding fumes and similar industrial emissions. Based on that, as well as on our previous experiences with nervous system toxicity in rats upon intratracheal application of metal oxide NPs[17] and the effect of certain antioxidants,[18] the aim of the present work was to test the putative protective effect of green tea brew in a rat model of occupational Mn exposure in which the animals received subacute treatment with MnO_2 NPs.

MATERIALS AND METHODS

Animals, substances and treatment

Young adult (6 weeks old, body weight 210 ± 15 g) male Wistar rats were used, obtained from Toxi-Coop (Budapest, Hungary). The animals were housed with two or three rats in one cage (polypropylene, floor 27 × 39 cm, height 19 cm), under GLP equivalent conditions (22 ± 1 °C, 40–60% relative humidity, 12 h light/dark cycle with light on at 06:00), and had free access to standard rodent pellet and drinking fluid (plain tap water or green tea brew). Altogether 40 rats were used, four groups of rats having 10 animals each. The complete scheme of the experiment is given in Table 1.

The chemicals used were from Sigma-Aldrich Hungary, except rutin standard (Gonmisol Fine Ingredients, Barcelona, Spain) and fluorescein (Fluka Analytical, Japan).

The MnO_2 NPs given to the rats were synthesized at the Department of Applied Chemistry, University of Szeged Faculty of Science and Informatics. Aqueous $KMnO_4$ solution was mixed with ethylene glycol and sonicated with a Hielscher UIP1000 (Hielscher, Teltow, Germany) ultrasound device. The resulting dark suspension was heated at 200 °C for 16 h in a Teflon-lined autoclave and then allowed to cool to room temperature naturally. The brownish precipitate formed was filtered and washed with 80 °C preheated distilled water, and dried at 100 °C for 1 h. Chemical purity of the nanoparticles was checked by X-ray diffraction, and particle size (27.4 ± 6.5 nm) by X-ray diffraction and transmission electron microscopy.

The rats were exposed to nanoparticles of manganese dioxide (MnO_2 NPs) by intratracheal instillation, performed in brief ether anaesthesia. Treatment was done once daily, 5 days per week, for 6 weeks. For administration, the MnO_2 NPs were suspended at 4 mg mL^{-1} concentration in 1% hydroxyethyl cellulose (HEC) dissolved in phosphate-buffered saline (PBS; pH 7.4). This vehicle slowed the aggregation of the NPs. The suspension was intensively sonicated as it was made, and again before each administration. The dose given to the rats (4 mg MnO_2 NPs kg^{-1} body weight (b.w.), administration volume 1 mL kg^{-1} b. w.) was found to cause clear general and nervous system effects in a previous study.[18]

Table 1. Treatment groups and corresponding treatment doses

Group	Code	Treatment[a]
Control	*Con*	Vehicle for suspension of nanoparticles (NPs), intratracheally instilled (1 mL kg^{-1} b.w.)
Green tea treated	*Tea*	Green tea brew as drinking fluid (2.5 g tea leaves 500 mL^{-1} boiled tap water)
Nano-Mn treated	*Mn*	Suspension of MnO_2 NPs, intratracheally instilled (4 mg MnO_2 kg^{-1} b.w.)
Combination treated	*MnT*	Green tea brew as drinking fluid + MnO_2 NPs, intratracheally instilled

[a] Treatment was done once daily, 5 days per week, for 6 weeks.

The green tea used was a commercially available kind (Chunmee China Green Tea 9366, non-fermented, Fujian Tea Import & Export Co. Ltd, China). Green tea infusion was prepared by brewing 2.5 g tea leaves in 500 mL boiled tap water. After 10 min, the infusion was filtered and cooled to room temperature. This brew was given as drinking fluid to groups *Tea* and *MnT*, for the whole 6-week treatment period, with a fresh brew given each Monday, Wednesday and Friday.

General toxicology

The rats' body weight was measured, and their general health state observed, daily before treatment. Body weight data were used to determine the daily doses and to graphically demonstrate the effects on weight gain. Drinking fluid consumption was measured at each change to calculate antioxidant intake. Eventual signs of toxicity (e.g. rough fur, hunched back, unusual aggressive behaviour) were noted.

After electrophysiological recording (see below) had been completed, the rats were sacrificed by an overdose of urethane (twice the dose used for anaesthesia) and dissected. Organs were removed and weighed, and the relative organ weight (as an indicator of toxicity) of the liver, lungs, heart, kidneys, spleen, thymus and adrenal glands was calculated, related to brain weight. During dissection, macroscopic abnormalities were recorded. From the groups *Tea*, *Mn* and *MnT*, 3 rats were randomly chosen for metal level determination and biochemical measurements. These were transcardially perfused with 500 mL PBS to remove blood from the organs. Whole brain, liver and 2–3 mL red blood cells (RBCs, separated from the freshly drawn heparinised blood by centrifugation) were shock-frozen in liquid nitrogen and stored at −20 °C.

Chemical and biochemical measurements

To determine Mn concentration, RBC samples were divided and, from the frozen brain, the cortex was cut off and divided. These, and ca. 1 g of liver samples, were dried at 80 °C to constant weight, and were digested in 65 % HNO_3 at 90 °C for 90 min (4 mL acid

Table 2. Chemical characterization of green tea brew

Chemical parameter	Tea brew, fresh	Tea brew, 48 h old
Total phenolics content ($\mu g\ mL^{-1}$)	239 ± 0.00	210 ± 0.50
Total flavonoid content ($\mu g\ mL^{-1}$)	62.61 ± 0.27	*
Epigallocatechin-3-gallate (EGCG) content ($\mu g\ mL^{-1}$)	28.37 ± 1.55	Not measured
Radical scavenging capacity (μg Trolox equivalents mL^{-1})	13.84 ± 2.62	5.22 ± 0.80
Antioxidant capacity (EC_{50}, mg ascorbic acid equivalents mL^{-1})	1.34 ± 0.14	1.98 ± 0.08

*Total flavonoid could not be determined in 48 h old tea because of the strong spontaneous darkening.

g^{-1} wet tissue). The digested matter was diluted as needed with distilled water, and measurement was done by inductively coupled plasma mass spectrometry (Agilent 7700X) at the Department of Inorganic and Analytical Chemistry, University of Szeged Faculty of Science and Informatics.

Lipid peroxidation as an oxidative stress indicator was measured from the other half of the divided RBC and cortex samples. Protein content (for calculation basis) was measured – after haemolysing RBCs, homogenizing the brain samples and diluting them as appropriate – by a standard method.[19] Lipid peroxidation (as a metric of oxidative damage) was characterized by measuring the level of thiobarbituric acid-reactive substances (TBARS).[20] These measurements were performed at the Department of Biochemistry and Molecular Biology, University of Szeged Faculty of Science and Informatics.

The tea brew was chemically characterized in fresh state and after using it in the watering bottles for 48 h. Total phenolics[21] and total flavonoid content were measured (against a calibration series of rutin, based on complexation with $AlCl_3$).[22] Additionally, epigallocatechin-3-gallate (ECGC) content of the fresh sample was also measured. For that, 1.000 g green tea leaf was extracted with 10.00 mL HPLC-grade methanol for 10 min in a tempered (25 °C) ultrasonic bath, and the extract was filtered with a 0.45 µm syringe filter. This extract, and a series of ECGC standard solutions (0.001034 – 2.180 mg mL^{-1}) were measured by HPLC in a Lichrospher RP-18e (5 µm, 4.6 × 200 mm) column with gradient elution (from 90% H_2O + 10% acetonitrile with 0.1% formic acid to 100% acetonitrile with 0.1% formic acid in 15 min; 23 °C; detection at 280 nm).

The radical scavenging capacity of the tea brew was measured by oxygen radical absorbance capacity (ORAC) assay performed on a 96-well microplate. Each well contained 20 μL of a member of Trolox ((±)-6hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid) calibration series or 20 μL tea brew in triplicate, 60 μL AAPH free radical solution (40 mmol L^{-1}) and 120 μL fluorescein solution

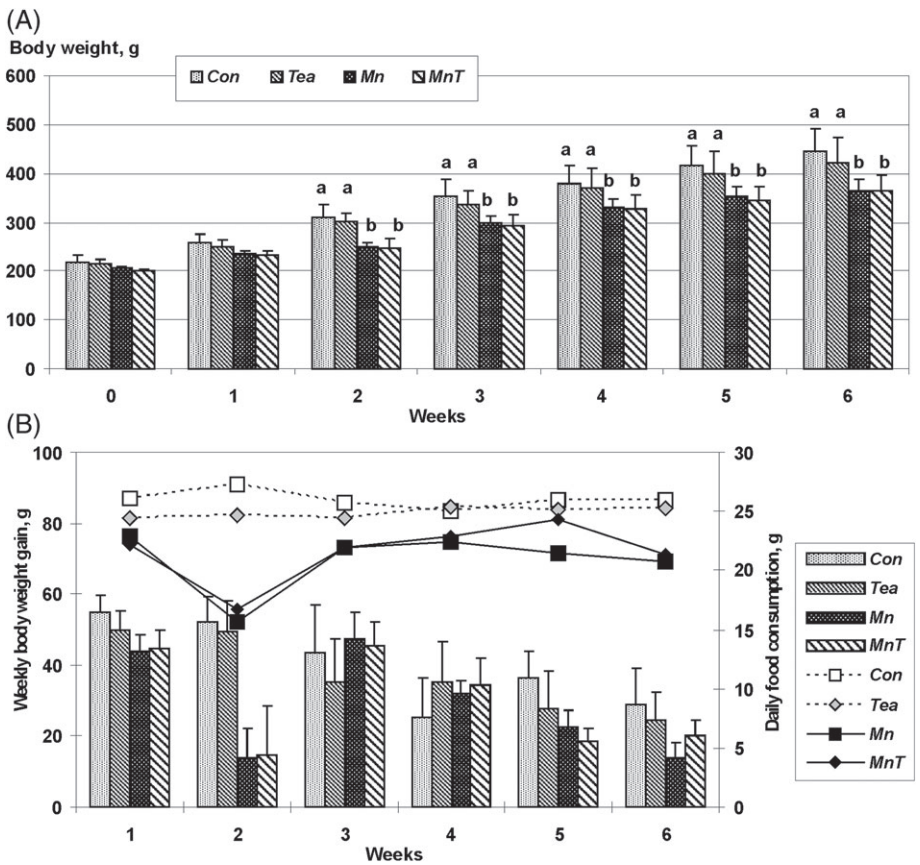

Figure 1. (A) Time course of weekly body weights, measured on Friday each week (week 0: before treatment commenced). Mean values of whole rat groups ($n = 10$); error bars represent SD. Weight values of one week, marked with non-identical letters, are significantly different at $P < 0.05$. (B) Weekly body weight gains (Friday to Friday, left ordinate, bars) and food consumption (right ordinate, lines). Mean values of whole rat groups ($n = 10$); error bars represent SD (for food consumption data, SD is omitted for clarity).

Table 3. Relative organ weights (ratio of organ weight to brain weight) of liver and adrenals in control and treated rats

	Liver	Adrenals
Con	6.53 ± 1.05a	0.026 ± 0.003ab
Tea	6.92 ± 1.48a	0.027 ± 0.004ab
Mn	5.25 ± 0.76b	0.034 ± 0.007c
MnT	5.26 ± 0.54a	0.032 ± 0.004ab

Mean ± SD for whole rat groups ($n = 10$).
Values of one organ, marked with non-identical letters, are significantly different at $P < 0.05$.

(1.40 μmol L^{-1}). Fluorescence was measured for 3 h with a BMG Labtech FluoStar Optima plate reader.[23]

Antioxidant capacity by the DPPH (2,2′-diphenyl-1-picrylhydrazyl) assay was measured in a 96-well microplate. A dilution series of the green tea stock extract, starting from 50 μL stock extract per well, was made in three parallels. To each well, 150 μL DPPH solution (100 μmol L^{-1}) was added. Absorbance was measured after 30 min at 550 nm.[24] On another 96-well microplate, the same measurement was performed on a dilution series of ascorbic acid used as standard. From the absorbance values, the EC_{50} value of green tea extract was calculated in ascorbic acid equivalents. The EC_{50} value of the fresh green tea was 265.67 mg g^{-1} dry tea leaves in ascorbic acid equivalents, which changed to 393.58 mg g^{-1} after 48 h (increasing EC_{50} meant that the antioxidant effect of the tea brew decreased during the 48 h). Chemical characterization of the tea brew was performed at the Department of Pharmacognosy, University of Szeged Faculty of Pharmacy.

Electrophysiology

One day after the last intratracheal NP application treatment, the rats were anaesthetized by intraperitoneal injection of 1000 mg kg^{-1} b.w. urethane and the left hemisphere was exposed (for details of preparation, see Oszlánczi *et al.*[17]). To record cortical activity (electrocorticogram, ECoG; and evoked potentials, EPs), ball-tipped silver recording electrodes (tip diameter ∼1 mm) were placed on the dura over the primary somatosensory (SS) projection area of the whiskery pad (barrel field), and over the primary visual (VIS) and auditory (AUD) foci. A stainless steel clamp, attached to the cut skin edge, was used as indifferent electrode.

The electrophysiological session started with recording ECoG simultaneously from the three areas for 6 min. From the records, the band power spectrum was calculated using the standard human EEG bands.[25] EPs were then recorded from the same cortical locations. Square electric pulses (3–4 V; 0.05 ms) delivered via a pair of needles inserted into the contralateral whiskery skin, were used as SS stimuli. VIS stimulation was performed by flashes of a high-luminescence white LED placed directly against the contralateral eye of the rat. For AUD stimulation, clicks (∼40 dB) were applied into the contralateral ear of the rat from a mini earphone through the hollow ear bar of the stereotaxic frame. VIS and AUD stimuli were given with 1 Hz frequency (one train of 50 stimuli) and SS stimuli with 1, 2 and 10 Hz (one train of 50 stimuli of each frequency – it was supposed that the frequency-dependent lengthening of EP latency could be another indicator of the neurotoxic effect). The 50 EPs were averaged, and onset latency was measured between time zero (stimulus artefact in the case of SS stimulation) and the start of the main wave of the EP.

Finally, the compound action potential was recorded from the rat's tail nerve. Two stimulating needles (delivering 4–5 V, 0.05 ms pulses at 1 Hz) were inserted into the tail base and another two, for recording, 50 mm distally. Conduction velocity was calculated from this distance and the onset latency of the action potential. Relative refractory period was determined, using double stimuli with 1–10 ms inter-stimulus interval, from the extra delay of the second action potential. The complete electrophysiological recording and evaluation were performed using the software NEUROSYS 1.11 (Experimetria, Budapest, Hungary).

Data analysis

The data were checked for normality by the Kolmogorov–Smirnov test. Significant differences were determined using one-way analysis of variance (ANOVA) with post hoc LSD test (IBM SPSS Statistics version 22). Linear correlation between datasets was sought for by the 'linear fit' function of MS Excel. The level of significance was set to $P < 0.05$.

Table 4. Results of chemical and biochemical measurements on rat samples

		Group		
		Tea	*Mn*	*MnT*
Summed external Mn dose (mg per rat)		–	36.12 ± 6.61a	35.51 ± 6.76a
Tissue Mn (mg kg^{-1} dry weight)	RBCs	0.80 ± 0.11a	1.37 ± 0.21a	1.53 ± 0.40a
	Cortex	20.94 ± 6.96 a	46.40 ± 2.39b	54.04 ± 2.57c
	Liver	7.93 ± 0.51a	10.27 ± 0.51b	10.67 ± 1.88b
Summed dose of active substances[a] (mg Trolox equivalents per rat)		27.95 ± 5.83a	–	20.21 ± 5.81a
TBARS (nmol mg^{-1} protein)	RBCs	0.26 ± 0.04a	0.32 ± 0.05a	0.28 ± 0.02b
	Cortex	0.24 ± 0.04a	0.30 ± 0.03b	0.28 ± 0.03c

Mean ± SD for three randomly chosen rats per group ($n = 3$; data from the same rats were also used in the correlation diagrams in Fig. 5).
Abbreviations: RBCs, red blood cells; TBARS, thiobarbituric acid-reactive substances.
Values in one row (one sample), marked with non-identical letters, are significantly different at $P < 0.05$.
[a] Calculated from the mean of the radical scavenging capacity of green tea brew in fresh and 2-day-old state; see Table 2.

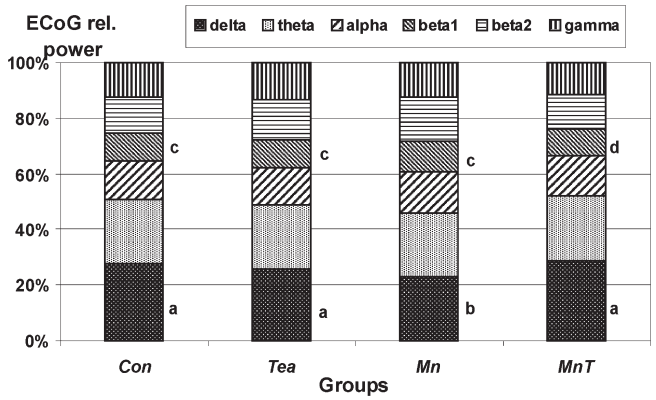

Figure 2. Band power spectrum of ECoG recorded from the somatosensory area of control and treated rats. Mean values of whole rat groups ($n = 10$). For the bands delta and beta1, values marked with non-identical letters are significantly different at $P < 0.05$.

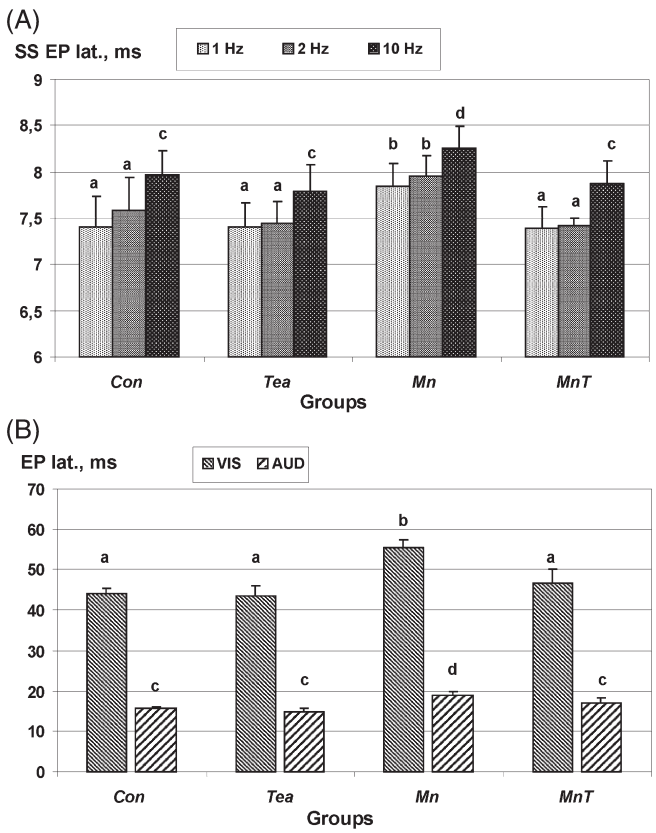

Figure 3. Latency of the sensory evoked potentials. (A) Somatosensory EP recorded with 1, 2 and 10 Hz stimulation. Mean values of whole rat groups ($n = 10$); error bars represent SD. Values marked with non-identical letters are significantly different at $P < 0.05$. (B) Visual and auditory EPs recorded with 1 Hz stimulation. Mean values of whole rat groups ($n = 10$); error bars represent SD. Values marked with non-identical letters (separately for VIS and AUD EPs) are significantly different at $P < 0.05$.

The study was approved by the Ethical Committee for the Protection of Animals in Research of the university. The experimental methods used were licensed by the authority competent in animal welfare issues under No. XXI./151/2013.

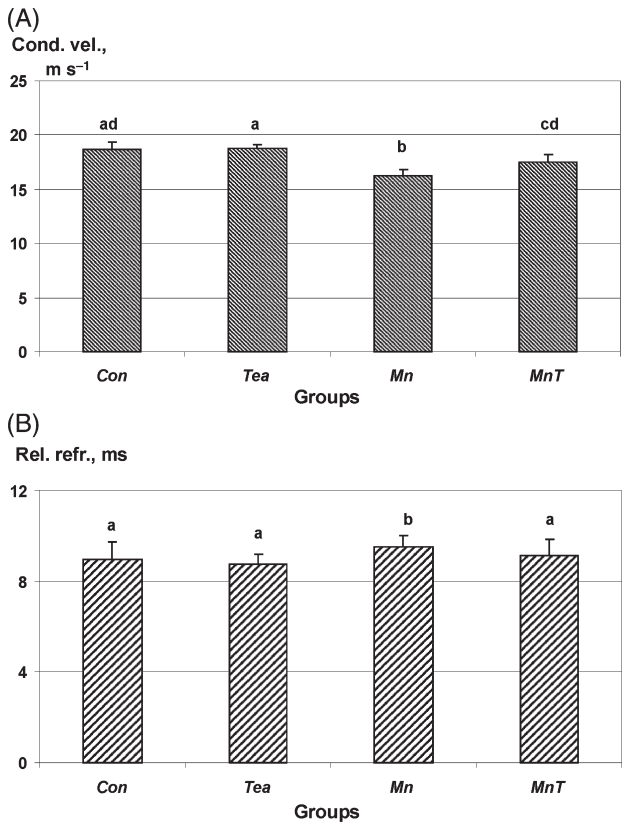

Figure 4. Conduction velocity (A) and relative refractory period (B) of the tail nerve. Mean values of whole rat groups ($n = 10$); error bars represent SD. Values marked with non-identical letters are significantly different at $P < 0.05$.

RESULTS

Properties of the tea brew

The brew made from the green tea proved to be a rich extract of bioactive substances. As shown in Table 2, radical scavenging capacity and antioxidant power of the tea decreased during the 48 h but was not lost. (The summed dose of antioxidants taken up by the rats, calculated from the ORAC data in Table 2 and drinking fluid consumption, is given in Table 4).

Effect of the MnO_2 NPs on body and organ weights. Chemical and biochemical effects

The body weight effect of intratracheal application of MnO_2 NPs was clearly visible from the second treatment week on (Fig. 1A). A parallel drop in food intake from the first to second week (in groups Mn and MnT; see line graph in Fig. 1B) was also observed. Later, food intake was partly normalized (but remained clearly below that of groups *Con* and *Tea*); the body weight deficit, however, did not vanish and was minimally influenced by co-administration of green tea (slight differences in *MnT vs. Mn* only in weekly weight gain, 4th and 6th weeks). In the groups receiving nano-Mn instillation, fluid consumption was also slightly reduced, which had an effect on antioxidant uptake (see below). Abnormal behaviour or other signs of gross general toxicity were not seen. Of all measured organs, only the relative weight of the liver and the adrenals showed any clear effect (Table 3). Green tea had no influence on the significant weight decrease of the liver, and only a slight effect on the weight increase of the adrenals.

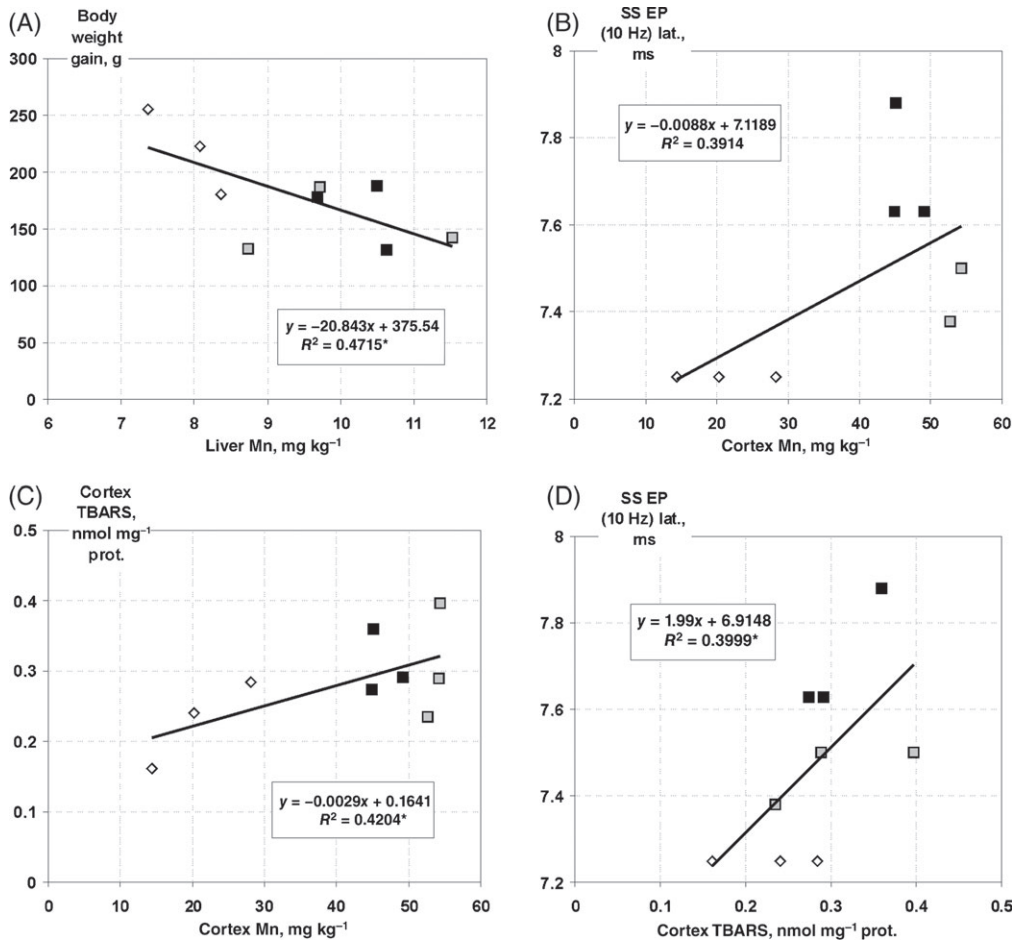

Figure 5. Correlation diagrams of body weight gain and liver Mn level (A), somatosensory EP latency and cortical Mn level (B), cortical lipid peroxidation and Mn level (C), somatosensory EP latency and cortical lipid peroxidation (D). Data of three animals each, chosen randomly from group *Con* (light diamonds), *Mn* (black squares) and *MnT* (grey squares). *Significant correlation ($P < 0.05$, *F*-test).

Tissue Mn level measurements (Table 4) showed massive deposition of Mn in the cortex and moderate but significant deposition in the liver, and this was not influenced by green tea. The level of lipid peroxidation (TBARS reaction) showed both the effect of MnO_2 NPs and of green tea as antioxidant in a more or less clear way, even if the total uptake of active substances of green tea was less in group *MnT* than that in group *Tea* due to the lower fluid consumption in the former.

Electrophysiological effects

MnO_2 NPs caused a moderate shift in the ECoG to higher frequencies in all three cortical areas (Fig. 2 shows this for the SS area; the changes in the VIS and AUD areas were highly similar). The decrease of slow activity in group *Mn* was abolished almost completely by green tea brew in group *MnT*.

Evoked cortical and peripheral responses were slowed by the exposure to MnO_2 NPs. The sensory EPs in all examined modalities (SS, VIS, AUD) showed significant latency increase (Fig. 3) in group *Mn*, which was no more seen in *MnT*. Green tea alone (group *Tea*) had no effect on this parameter. In the frequency dependence of the SS EP latency (latency values at 2 and 10 Hz *vs.* 1 Hz; Fig. 3A) neither MnO_2 NPs nor green tea caused any noteworthy change.

In the tail nerve, the conduction velocity was not influenced by green tea alone but was massively decreased by the MnO_2 NPs (Fig. 4A); this effect was partially, but significantly, reduced in group *MnT*. The effects on the relative refractory period (Fig. 4B) were similar although less marked.

Correlations of the effects obtained

The relationship of the measured toxicological parameters was tested on the basis of linear correlation. The correlations found (Fig. 5) were not very robust but, when regarded together with the data presented above, supported the existence of a causal link between body weight gain during the exposure period and Mn load of the liver, for general toxicity (Fig. 5A); and latency of SS EP and Mn load of the cortex, for neurotoxicity (Fig. 5B). There was also a fair correlation between Mn load and TBARS level in the cortex (Fig. 5C) and SS EP latency and cortical TBARS (Fig. 5D). The correlation plots also suggested, however (mainly those in Fig. 5C, D), that green tea diminished lipid peroxidation without acting on local Mn load, but counteracted the effect of MnO_2 NPs on SS EP latency partly independently of lipid peroxidation.

DISCUSSION

The relationship between Mn exposure and neurologic–neuropsychiatric dysfunctions has been established both in massive workplace exposures and in chronic low-level exposure among workers and in the general public.[26,27] Electrophysiological phenomena related to functional damage, such as myoclonus in

welders [28] or EEG and visual EP alterations in shipyard workers,[29] have been also described.

The causal relationship between the capacity of Mn to cause oxidative stress and Mn-induced functional damages in human or animal CNS – including functional alterations like those presented in this work – is supported by several lines of evidence. Reactive oxygen species have been regarded as a 'final common pathway' in the action of several neurotoxicants.[30] The mechanism may include oxidative damage to membrane lipids of axons and neuronal cell bodies, vulnerable to oxidative damage due to the abundance of unsaturated fatty acids,[31] leading to changes of membrane fluidity and thus to alterations in functions bound to an intact membrane,[32] first in synaptic transmission and regenerative nerve pulse conduction. Products of lipid peroxidation themselves may be responsible for damage to synaptic structure and function.[33]

The generation of cortical EPs depends on synaptic transmission and may directly indicate changes in the latter. In the present work, correlation was found between an electrophysiological functional indicator – EP latency – and an oxidative stress indicator – TBARS level (see Fig. 5D). A similar relationship of TBARS and visual EPs was reported in a disease model based on sulphite-exposed rats, together with the protective effect of lipoic acid, an endogenous antioxidant.[34] Beyond synaptic transmission, regenerative nerve pulse conduction also depends on normal membrane functioning, which explains why the changes in EP latency and tail nerve conduction velocity in the rat groups exposed to nano-Mn and/or green tea were similar (cf. Figs 3 and 4).

There have been attempts to demonstrate a beneficial effect of antioxidants in Mn neurotoxicity. In rats receiving Mn exposure via the drinking water, silymarin (a flavonoid antioxidant of the milk thistle) significantly reduced the Mn-induced oxidative biochemical and brain histological alterations.[35] In a mouse model of Mn-induced Parkinson-like disease, α-tocopherol greatly reduced the increase of oxidative markers (such as prostaglandin E2) and the degeneration of striatal medium spiny neurons.[36] However, neither oral, as in Chtourou *et al.*,[35] nor subcutaneous, as in Milatovic *et al.*,[36] application of Mn was a truly realistic model of human exposure, which takes place mostly by metal fume inhalation. Likewise, application of the antioxidant was, even though efficient, more or less artificial in both mentioned works (intraperitoneal and oral, respectively). Compared to these, our method of administering both Mn (oxide NPs applied in the airways) and the antioxidant (green tea brew as continuously available drinking fluid) seems to be a better approximation of what affected workers may experience. Moreover, our results directly showed the ameliorative effect of tea on both an oxidative stress and a neuro-functional parameter. This may follow from the complex action of flavonoids, the main active substances of green tea brew. These act, on the one hand, as radical scavengers, and on the other as metal chelators.[2,37] This way, they can counteract several nervous system damaging effects of Mn, both related (mitochondrial damage)[5,6] and unrelated (interference with glutamate turnover)[38] to oxidative stress.

CONCLUSION

Green tea is a popular drink with numerous bioactive substances. If its potential neuroprotective action can be verified by results like those presented above, green tea can gain a role in practical health protection, e.g. as a constituent of protective (or functional) drinks.[4]

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest to disclose.

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