Manganese Distribution in Brains of Sprague Dawley Rats after 60 Days of Stainless Steel Welding-Fume Exposure

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Welders working in a confined space, like in the shipbuilding industry, are at risk of being exposed to high concentrations of welding fumes and developing pneumoconiosis or other welding-fume exposure related diseases. Among such diseases, manganism resulting from welding-fume exposure remains a controversial issue, as the movement of manganese into specific brain regions has not been clearly established. Accordingly, to investigate the distribution of manganese in the brain after welding-fume exposure, male Sprague Dawley rats were exposed to welding fumes generated from manual metal arc stainless steel (MMA-SS) at concentrations of 63.6 ± 4.1 mg/m^3 (low dose, containing 1.6 mg/m^3 Mn) and 107.1 ± 6.3 mg/m^3 (high dose, containing 3.5 mg/m^3 Mn) total suspended particulates for 2 hrs per day, in an inhalation chamber over a 60-day period. Blood, brain, lungs and liver samples were collected after 2 hr, 15, 30, and 60 days of exposure and the tissues analyzed for their manganese concentrations using an atomic absorption spectrophotometer. Although dose- and time-dependent increases in the manganese concentrations were found in the lungs and livers of the rats exposed for 60 days, only slight manganese increases were observed in the blood during this period. Major statistically significant increases in the brain manganese concentrations were detected in the cerebellum after 15 days of exposure and up until 60 days. Slight increases in the manganese concentrations were also found in the substantia nigra, basal ganglia (caudate nucleus, putamen, and globus pallidus), temporal cortex, and frontal cortex, thereby indicating that the pharmacokinetics and distribution of manganese inhaled from welding fumes would appear to be different from those resulting from manganese-only exposure.

Introduction

Chronic human exposure to high concentrations of metal fumes, as observed among welders, has been shown to induce adverse health effects, including neurological and respiratory problems. Several asymptomatic manganese-exposed workers exhibited increased signal intensities on T1-weighed magnetic resonance images (MRI) (Kim et al., 1999), while another MRI study revealed elevated manganese concentrations in the basal ganglia in a welder with manganism (Nelson et al., 1993). Recent case controls study of 15 career welders with parkinsonism by Racette et al. (2001) indicated that parkinsonism associated with welding was not clinically different from idiopathic parkinson disease with the exception of a younger age at onset, suggesting welding exposure may act as an accelerant to cause parkinson disease. Another study of neurological and neurophysiological examination of workers occupationally exposed to manganese within the range of 0.01-2.67 mg/mg^3 revealed that workers of chronically exposed to manganese showed the increased emotional irritability, dysmnesia, concentration difficulties, sleepiness and limb paresthesia predominated among the disorders of the nervous system functions (Sinczuk-Walczak et al., 2001). Severely affected patients develop a progressive and irreversible loss of dopaminergic neurons in the globus pallidus and nigrostriatal pathway (Calne et al., 1994; Pal et al., 1999). Several pharmacokinetic studies on inhalation exposure to manganese

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demonstrated that manganese readily accumulates in the olfactory regions and brain regions. Mn administered intra-nasally to rats was found to be taken in the CNS via the olfactory system (Tjälve *et al.*, 1996, Henriksson, *et al.*, 1999) and produced an initial effect on the astrocytes (Heriksson and Tjälve, 2000). Manganese phosphate-exposed rats showed elevated manganese concentrations in the olfactory bulb, striatum, and lungs following 14 exposure doses of 0.3 mg Mn/m^3 (Vitarella *et al.*, 2000). Another manganese phosphate exposure to rats also indicated that the olfactory bulb and caudate/putamen are the main brain tissue for manganese accumulation after subchronic 90 day inhalation exposure (Normandin *et al.*, 2002), while rats exposed for 13 weeks of manganese dust exhibited significantly higher concentrations of manganese in the lungs, putamen, and cerebellum compared to a control group (St-Pierre *et al.*, 2001). Manganese accumulation studies using primate indicated that manganese showed selective affinity to globus pallidus and pituitary (Newland *et al.*, 1989). The important difference between rodents and primates is that the over signs of basal ganglia disorders are easy to detect in primates, and are unlikely to appear in measures of locomotor activity (Newland *et al.*, 1992 & 1999). The kinetics of manganese distribution in primates indicated that inhalation could prolong exposure to other tissues by releasing manganese slowly. The elimination of manganese from the brain after inhalation was about 4 times slower than after subcutaneous administration, suggesting the manganese deposited in the lung conti-nued to supply the brain long after exposure terminated (Newland *et al.*, 1987 & 1989). Manganese was detected in the lungs for 500 days with a half-time of 94 to 187 days after inhalation exposure (Newland, 1999). In contrast, dietary manganese intake does not affect brain manganese concentrations in rats following manganese tetroxide or manganese sulfate inhalation in rats maintained on either a manganese-deficient or high manganese diet (Dorman *et al.*, 2001 & 2002). The competition between Mn and iron for intestinal ab-sorption is well known in human and rats (Davis *et al.*, 1992). The subjects with iron deficiency or chronic liver disease like cirrhosis absorb several folds higher Mn than normal subjects (Mena *et al.*, 1969; Malecki *et al.*, 1999). A transport of for Fe^{+2} and Mn^{+2}, DMT-1 (divalent metal transporter) was regulated by the iron status in a human erythrocyte culture model (Han *et al.*, 1999). DMT-1 expression has also been observed in

neurons (Gunshin *et al.*, 1997). It is also possible that Mn many compete with iron for transferrin/ transferrin-mediated uptake in the brains (Suárez and Erikson, 1993). Although several previous injection and inhalation studies have shown elevated manganese levels in certain brain regions when experimental animals have been exposed to manganese alone, the movement of manganese in the CNS when experimental animals are exposed to welding fumes has not been clearly established. Welding fumes contain nickel, chrome, and several fold higher concentrations of iron besides manganese. As such, since the accumulation of Mn in the CNS would appear to depend on iron homeostasis, the oxidation state of Mn may represent a determinant in the differential distribution, accumulation, and secretion profiles of Mn (Aschner *et al.*, 1999). In addition, there is no confirmed case of welding fume associated manganism, although increased signal intensities in the globus pallidus and subcortical frontal white matter on the T1 weighted magnetic resonance from asymtomatic manganese-exposed welders has been reported (Kim *et al.*, 1999). Despite welding fume exposure could act as a risk factor to cause parkinson disease, the disease is indistinguishable from idiopathic Parkinson disease except for age at onset (Racette *et al.*, 2001). In this study they could not prove manganese was the toxic agent for the idiopathic parkisonism. Therefore, the pharmacokinetics of manganese inhaled from welding fumes could be different from those related to manganese-only exposure. Accordingly, in the current study, Sprague Dawley rats were exposed to manual metal arc stainless steel (MMA-SS) welding fumes for 60 days to investigate changes in the manganese levels in the brain region.

Materials and Methods

Generation of MMA-SS Welding Fumes

The welding fumes were generated as described in previous reports (Yu *et al.*, 2000, 2001); that is, the fumes were generated using a rotating stainless steel disc plate (SUS 304, 50 cm diameter, 1 cm thick) as the base metal, and a welding rod (KST 308, 2.6 × 300 mm, Korea Welding Electrode Co. LTD, Seoul) was restrained in a welding-rod holder support. When the welding rod was moved by a pulley and approached the rotating disc, an arc was produced and the rod consumed, thereby generating welding fumes. The

fumes were then moved into an exposure chamber (whole body type, 1.3 m^3, Dusturbo, Seoul). The exposure chamber was a rectangular shape made of metal with a plexi-glass window and with conical shape bottom to collect excretions. The chamber was cleaned everyday after exposure.

Analysis of Welding Fumes

The welding fumes in the chamber were sampled using a personal sampler (MSA 484107, Pittsburgh) with a flow rate of 2 liter/min. The samples were obtained from the center of chamber and half distance away from the center. The welding fume particulates captured on the membrane filters (pore size 0.8 μm, 37 mm diameter, Millipore AAWP 03700, Bedford) were analyzed for its metal composition with an atomic absorption spectro-photometer (SpectAA-800, Varian, Palo Alto) using the NIOSH 7300 method (1999). Meanwhile NIOSH method 7604 (1999) was used to evaluate the Cr^{VI} in the welding fumes. The samples used for measuring the total Cr were stored in vials containing a base solution (2% NaOH, 2% Na$_2$CO$_3$), whereas the samples used for measuring the Cr^{VI} were kept in vials containing deionzed water. The total Cr concentration was measured with an atomic absorption spectro-photometer after a pretreatment of ashing using a microwave oven. The Cr^{VI} concentration was measured using an ion chromatography (DX-500, Dionex, Sunnyvale) after extracting the base and deionized water.

The gaseous fumes, O$_3$, NO$_2$, and nitrous fumes, were measured using Drager tubes (Cät No. 6733181, CH 31001, CH 30001, respectively). The gaseous fumes were sampled by stroking a gas detector pump (6400000, Dräger, Lubeck), according to the manufacturer's direction, 1 hour after beginning the welding fume exposure.

Study of Inhalation Toxicity

Six-week-old male, specific pathogen-free (SPF) Sprague Dawley rats, purchased from Biogenomics (Korea), were acclimated to a 12-h light, 12-h dark cycle with light from 08:00 to 20:00 h. The rats were fed LabDiet$^®$ 5002 (Purina Mills Inc, USA) and tap water *ad libitum* for 1 week before the initiation of the experiment. Rats weighing 218±10 g were randomly assigned to six groups and exposed to welding fumes for 2 hr per day in the exposure chamber. One group was

sacrificed immediately after the first 2-hour exposure, while the other groups were sacrificed after 15, 30, or 60 days of exposure. The food and water were not provided during two hours exposure. The rats were taken out of the chamber at the end of each two hours of exposure. Each group consisted of 8 unexposed, 8 low dose exposed, and 8 high dose exposed rats. Maximum forty rats (8 rats/dose × 5 time points) in a chamber were exposed during two hours of exposure period. From each group, four rats were processed to determine the organ distribution of the manganese from the welding fumes, while other four rats were used for lung fibrosis recovery study. The rats were fed LabDiet$^®$ 5002 (Purina Mills Inc, USA) and tap water *ad libitum* for 1 week before the initiation of the experiment. Manganese and iron concentrations of in this diet were 75 ppm and 210 ppm, respectively. The right lungs were used for a histopathological examination and the left lungs were processed to determine the organ distribution of the metal components from the welding fumes. The TWA concentrations in the exposure doses were 63.6±4.1 mg/m^3 (low dose) and 107.1±6.3 mg/m^3 (high dose) total suspended particulates per 2hr. More than 90% of the fume particles had aerodynamic diameters of less than 1 μm. Fifty percent of the diameters were between 0.65 and 0.43 μm (Yu *et al.*, 2001).

Brain section

After exposure, the rats were anesthetized with diethyl ether, and blood was collected from the abdominal aorta. The lungs, trachea, and liver were removed and fixed in a 4% paraformaldehyde solution containing neutral phosphate buffered saline. The brains were removed from the skull and fixed in the same 4% paraformaldehyde for 48 hrs. Thereafter, representative coronal tissue sections were cut into 1 mm slices using a vibratome, starting from the bregma according to guidance (Paxinos & Watson, 1997). The topographical target sites were defined as follows: Caudate putamen: 1.60~0.4 mm from the bregma; frontal associated cortex: 5.20~3.20 mm from the bregma; temporal associated cortex: -6.04~-7.64 mm from the bregma; substantia nigra: -4.5~-6.05 mm from the bregma; and cerebellum: -11.30~-13.60 mm from the bregma.

Mn analysis

The Mn and Fe concentrations in the whole blood

was analyzed using a Zeeman-corrected flameless (Perkin Elmer 5100ZL Zeeman furnace module, CT) atomic absorption spectrophotometry (Perkin-Elmer 5100PC, CT) after simple dilution with 0.5% triton-X 100. The Mn and Fe concentrations in the lung, liver and brain tissues, including the basal ganglia (caudate, putamen, and globus pallidus), frontal cortex, temporal cortex, substantia nigra, and cerebellum were determined according to a flame or flameless method after wet digestion using a Microwave Digestion System (MDS-2000, CEM, NC).

Statistical analysis Data were expressed as mean ±one standard error. Multiple variance of analysis and Duncan's *post hoc* multiple range tests were used to compare the body weights, and the concentrations of metals in lungs, blood, liver and brain obtained from the two dose groups with those obtained from the unexposed control rats.

Results

Concentrations of MMA-SS welding fume components

The MMA-SS welding fume consisted of mainly Fe, Mn, Cr, and Ni. The metal concentrations and gaseous fractions of the welding fumes are shown in Table 1.

Body weight development and animal observation

The body weight gain was significantly less in the welding fume exposed rats than control during the 60-day exposure period (Figure 1). No systemic behavioral measures were undertaken.

Concentrations of Mn in lungs, blood, and liver.

Table 1. Concentrations of MMA-SS welding fume components.

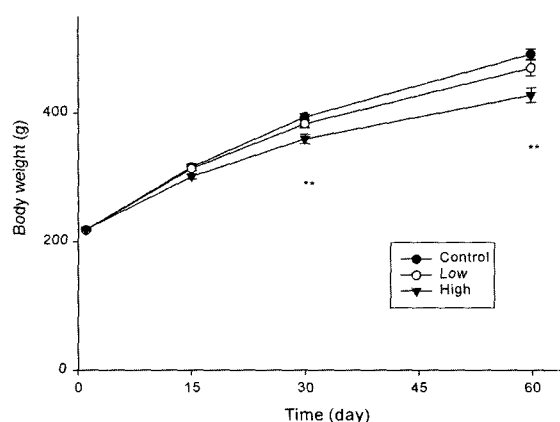|	Low dose	High Dose
Metal	mg/m^3	mg/m^3
Fe	4.6 ± 0.2	10.1 ± 1.0
Cr	2.0 ± 0.6	5.4 ± 0.1
Cr ^{VI}	1.1 ± 0.2	1.5 ± 0.3
Mn	1.6 ± 0.3	3.5 ± 0.2
Ni	0.6	1.2
Gas	ppm	ppm
O$_3$	0.05	0.1 - 0.15
NO$_2$	0.5	0.5
Nitrous fumes	10	20
SO$_2$	2.1	3.0

Fig. 1. Body weight changes during 60 days of MMA-SS welding-fume exposure.

The manganese concentrations in the lung tissue of the group exposed to 60 days of welding fumes revealed statistically significant (P < 0.01) dose-dependent increases (Figure 2A). The blood manganese concentration increased slightly after 30 days of exposure, yet statistically significant (P < 0.05) increases were observed after 60 days of exposure (Figure 2B). In contrast, clear increases (P < 0.05) were observed in the livers of the groups exposed for 15 days and up to 60 days (Figure 2C). Although the results showed some variability in Mn concentration in the control group, there were no significant statistical differences in manganese concentrations in lungs, blood, and liver among control groups in different time points.

Mn concentrations in Brain

Slight increases in the manganese concentrations were found in the substantia nigra, basal ganglia (caudate nucleus, putamen, and globus pallidus), temporal cortex, and frontal cortex after 60 days of welding-fume exposure, yet the increases were not statistically significant. Only statistically significant increase was found in the basal ganglia of the 30 day low dose exposed rats. However, statistically significant increases (62% for low dose and 55% for high dose) in the manganese concentrations were observed in the cere-bellum after 60 days of welding fume exposure (Figure 3). Increases were also observed in the both low and high dose groups after 15 days until the end of the exposure period. There were no significant statistical differences in manganese concent-rations in different brain regions among control groups in different time points.
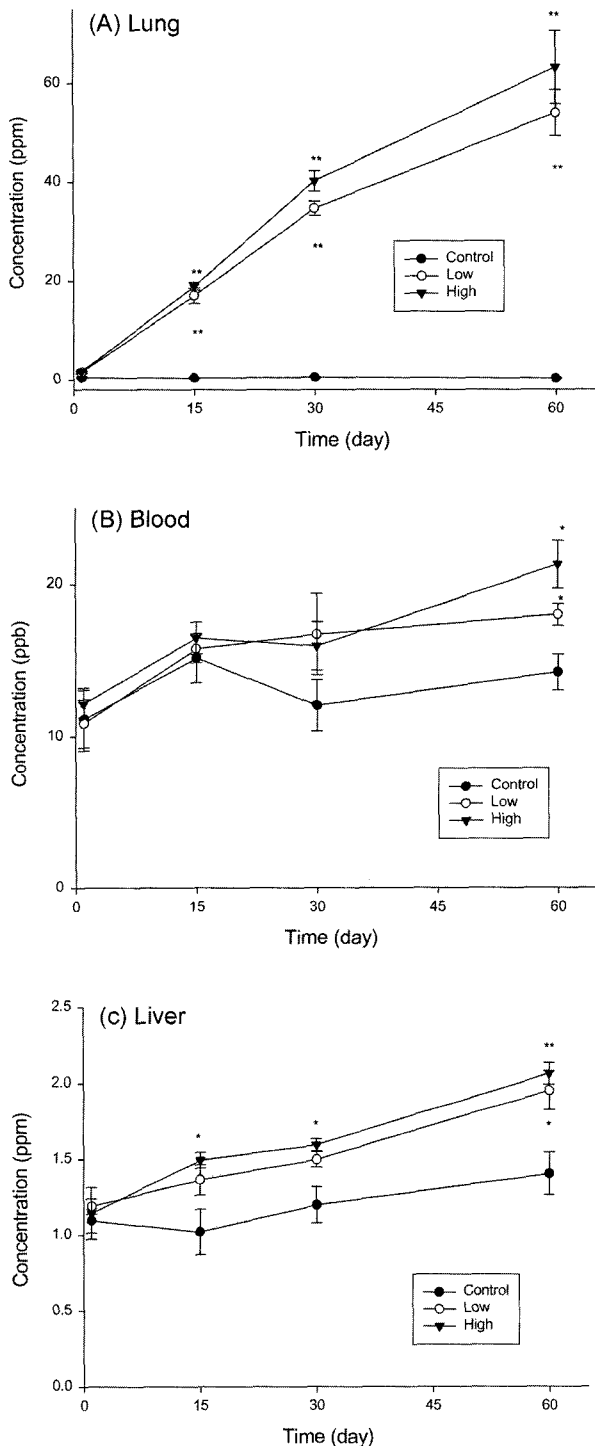
Fig. 2. Manganese concentrations in lungs, liver and blood during 60 days of welding-fume exposure. The errors bars indicate standard error. There were no significant statistical differences in manganese concentrations in lungs, blood, and liver among control groups in different time points. (**$P < 0.01$, *$P < 0.05$ versus control).

Discussion

The current results show that the manganese distribution in the brain regions after welding-fume inhalation exposure behaved differently from other exposure regimens using Mn-only exposure via oral, inhalation, and peritoneal routes. Other inhalation exposure studies using pure Mn or Mn compounds found increased Mn concentrations in lung and brain tissue of rats and monkeys (Moore *et al.*, 1975; Coulston & Griffin, 1977; Ulrich *et al*, 1979; Newland *et al.*, 1989 & 1992; St-Pierre *et al.*, 2001, Normandin *et al.*, 2002), and the globus pallidus was found to have the highest increase in brain Mn in monkey, human and rats (Bird *et al.*, 1984;, Newland *et al;*, 1989 & 1992; Nelson *et al.*, 1993; St-Pierre *et al.*, 2001). Although the current study also found clear dose-dependent increases of manganese in the lung and liver tissue, along with a certain increase in the blood manganese level, the main accumulation site of manganese in the brain was the cerebellum, not the basal ganglia. A recent 13-week manganese dust (less than 1 (m diameter) exposure study showed a high accumulation in the globus pallidus and putamen compared to a low accumulation in the cerebellum (St-Pierre *et al.*, 2001). Another 14-day manganese phosphate inhalation study revealed increased manganese concentrations in the olfactory bulb, striatum, and lungs (Vitarella *et al.*, 2000). Both of these previous studies along with the current study showed the blood manganese concentration to be a poor biomarker for the manganese exposure. The poor correlation between air and the blood manganese concentration may be related to the short half-life of manganese in the blood following acute inhalation exposure (Smargiassi and Mutti, 1999) or intra-peritoneal injection (Dastur *et al.*, 1969). Yet neither of the above two studies found an increase in the manganese concentration in the liver in contrast to the time- and dose-dependent manganese increase in the liver revealed in the current study. The liver plays a significant role in the homeostasis of manganese concentrations in the body. Although tissue manganese levels generally remain relatively constant despite a large fluctuation in the oral manganese intake (Schroeder *et al.*, 1966), the current welding fume inhalation study demonstrated a dose- and time-dependent increase in the manganese concentration in the liver. Accordingly, the manganese metabolism resulting from a mixture
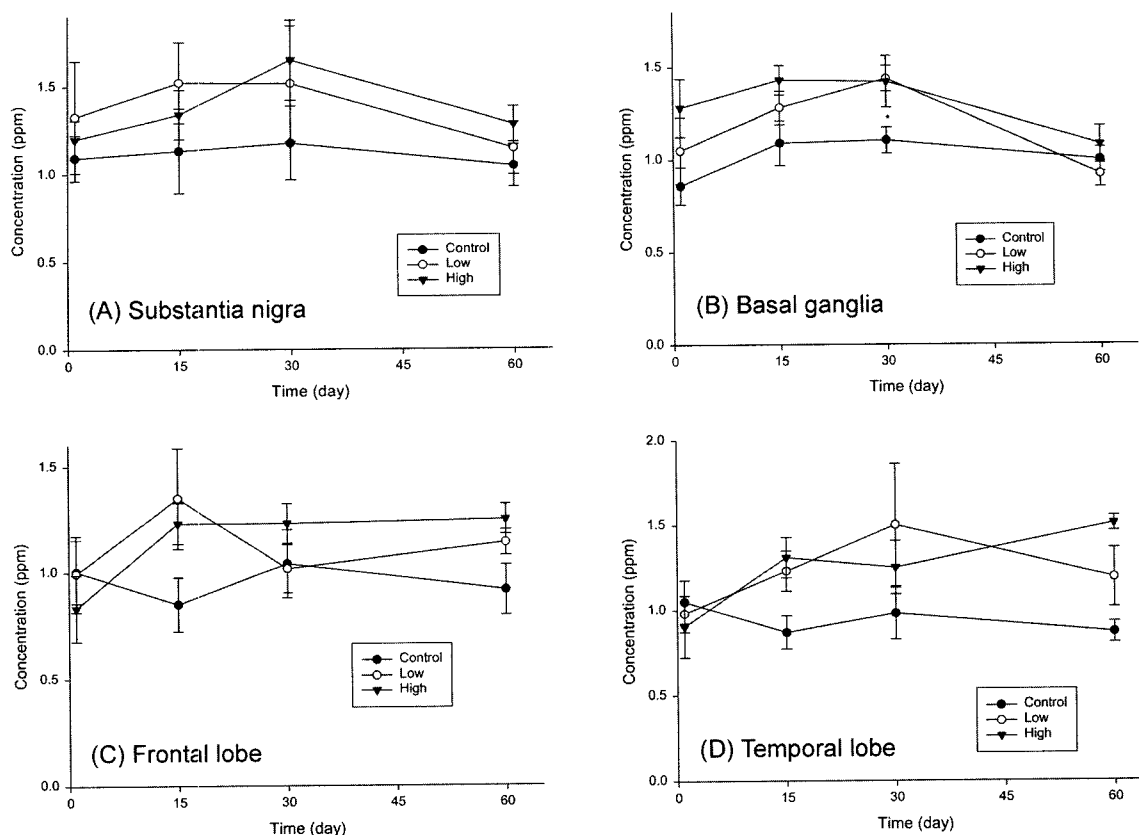
Fig. 3. Manganese concentrations in cerebellum during 60 days of welding-fume exposure. The errors bars indicate standard error. (* P < 0.05 versus control). There were no significant statistical differences in manganese concentrations in the cerebellum among control groups in different time points.

inhalation exposure situation would appear to behave differently from single substance inhalation or oral administration.

The lung manganese concentration exhibited both dose- and time-dependent increases following welding-fume inhalation exposure. No evaluation of the manganese levels was conducted for an exposure period of longer than 60 days, as interstitial fibrosis (Yu et al., 2001), which could affect manganese absorption and metabolism in lung tissue, appeared in the animals exposed to the high dose after 60 days. Histopathological examination of lungs conducted concomitantly showed that the interstitial fibrosis appeared at day 60 and became prominent by day 90 in the present study, along with the additional appearance of pleural fibrosis (pictures not shown).

Although histological evaluation was not conducted in this experiment due to the limitation of brain samples to use for Mn concentration in different brain regions, the

histological examinations combined with immuno-histochemistry using specific antibodies against glial fibrillary acid protein (GFAP) and S100 beta did not show any significant features in previous another 90 days welding fume exposure study (57-67 mg/m^3 for low dose and 105-118 mg/m^3 for high dose) (data not shown). Since there were no specific behavioral changes or symptoms due to welding fume exposure except some progressive lung fibrosis during 60 days of exposure period, it may be difficult to observe metal ion influx induced brain pathology. Further studies need to be planned to investigate the effect of metal ion influx into the CNS.

The current results conflicted several observations made by other manganese only inhalation studies using rats. Ninety days of manganese phosphate inhalation exposure to rats showed increased manganese concentrations in all tissue of brain. The increase was dose-dependent in olfactory bulb and caudate/putamen

(Normandin *et al.*, 2002). Fourteen day of manganese sulfate and manganese tetroxide inhalation exposure to rats also showed increased manganese concentrations in striatum and olfactory bulb (Vitarella *et al*, 2000; Dorman *et al.*, 2001). Thirteen weeks of manganese dust inhalation exposure to rats also indicated that manganese concentrations were increased in brain regions including globus pallidus, frontal cortex, putamen, and cerebellum (St-Pierre *et al.*, 2001). In primate studies, inhalation exposure to manganese chloride aerosol for five months resulted in manganese accumulation in the globus pallidus and pituitary gland with little effect in gray and white matter (Newland *et al.*, 1989). All these inhalations studies to rats showed the increased concentrations of manganese in the striatum and globus pallidus, whereas our welding fume inhalation study showed a significant increase in cerebellum not basal ganglia or striatum, indicating different pharmacokinetics from manganese only exposure studies.

Lung depots of manganese showed different uptake and elimination from other routes. Lung depots of manganese prolonged exposure to other tissues by releasing manganese slowly and eliminated slower than other routes of exposure (Newland *et al.*, 1987; Newland, 1999). Thus lung stored manganese and the lung depots continued to supply manganese into the brain long after exposure terminated. Since dietary manganese intake does not affect brain manganese concentrations in rats following manganese tetroxide or manganese sulfate inhalation in rats maintained on either a manganese-deficient or high manganese diet (Dorman *et al.*, 2001 & 2002), the oral intake of manganese many not affect brain manganese concentrations in our welding fume inhalation study.

The difference in the absorption and transportation of the manganese from the welding fumes may have been due to the influence of the various other metals contained in the welding fumes, i.e. mainly Fe, and Cr and Ni. Since globus pallidus was known to accumulate more iron than any other brain tissue (Drayer *et al.*, 1986; Rutledge *et al.*, 1987), there could be some interaction between manganese and iron affinity. Other evidences also suggested that manganese and iron tend to accumulate in the same organs (Scheuhammer & Cherian, 1982 & 1983). Since transferrin has already been implicated in the brain uptake of manganese (Malecki *et al.*, 1999) and DMT1 is expressed in neurons (Gunshin *et al.*, 1997), it is possible that the manganese

and iron may compete for the transferrin binding site or DMT1. The increased signal intensities observed in the globus pallidus and subcortical frontal white matter on the T1-weighed images among asymtomatic manganese-exposed welders by Kim *et al.* (1999) were not observed in our experiment. This difference of may have been due to the species difference, type of welding employed, and exposure duration. The different pharmacokinetcs of metal components in the welding fumes need to be investigated further.

The doses were selected based on actual exposure monitoring data and previous published experiments. Several domestic monitoring studies have reported that welding fume concentrations can be higher than TLV 5 mg/m^3 and lower than the high dose used in the current study. Several studies on the monitoring of exposure to welding fumes in the shipbuilding industry have reported on concentrations ranging from 6-73 mg/m^3 (geometric mean (GM) 16.6 mg/m^3) to 0.3-91.16 mg/m^3 (GM 5.59 mg/m^3) (Kwag and Paik 1997; Choi *et al.*, 1999). In addition, the current exposure duration was only 2 hrs compared with 6 hrs in most inhalation study or 8 hrs in actual workplace monitoring. In the present study it was presumed that the actual exposure to welding fumes in the workplace could be 20-30 mg/m^3 for longer than five years in a severe case. Thus, in terms of a 6 hr exposure, a low and high dose would be 20 and 40 mg/m^3 of TSP, respectively. Our results can be interpreted that if welders are exposed long-term to welding fumes at high concentrations several fold over TLV in a confined space, as in the shipbuilding industry or other occupational settings, could have a risk of increasing manganese in the CNS.

Manganese and other metals are known to be taken up via the olfactory pathways and pass transneuronally to other parts of the brain. As such, the occupational neurotoxicity of inhaled manganese may be related to the uptake of the metal into the brain via the olfactory pathways (Tjälve and Henriksson, 1999). The welding fume particles in the current study were less than 0.5 ìm in diameter (Yu *et al*., 2001), plus MMA-SS welding fume particles are soluble in water (Antonini *et al.*, 1997), therefore, they could have been easily absorbed by the olfactory epithelium and transported to the olfactory bulbs of the brain. However, our previous study light microscopic examination of the nasal pathway did not reveal any adsorption of the welding fume particles by the nasal epithelial cells (Yu *et al.*,

2000). Further studies need to identify the role of olfactory pathways in manganese transport to CNS.

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