Investigación Clínica 26(2): 117-124, 1985

THE NEUROPATHOLOGY OF EXPERIMENTAL CHRONIC MANGANESE POISONING IN RATS. A PRELIMINARY REPORT

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ABSTRACT

Adult male-Sprague-Dawley rats were exposed to manganese for a period of eight months by giving them drinking water containing 0.1, 1.0 and 5.0 mg Mn/ml, as $MnCl_2$. This experimental animal model for chronic manganese poisoning shares neuropathological features with the human disease such as cortical and striatal alterations. Our results suggest that relatively low concentrations of manganese (1.0 mg/ml) orally ingested are capable of inducing morphological alterations as evidenced by light microscopy studies.

INTRODUCTION

The recent advent of manganese as an antiknock gas additive (methycyclopentadienyl manganese tricarbonyl, MTT) has made this metal one of the atmospheric pollutants in the form of several manganese oxides and even though their concentrations appear safe, the susceptibility to manganese poisoning varies with the individuals depending on several factors

such as age (5, 8) nutritional defficiency and/or impaired intestinal absorption (11), period of exposure (12, 14), etc.

The present paper stresses the fact that manganese administered orally is capable of inducing morphological alterations, even at relatively low concentrations, suggesting that manganese might eventually become a public health problem specially among susceptible individuals living in densely populated areas with significant atmospheric pollution.

MATERIAL AND METHODS

Animals:

Twelve adult male Sprague-Dawley rats, average body weight 200 gr. were fed ad-libitum with Protinal rat chow (Protinal-Zulia, Venezuela).

Manganese Exposure:

The animals were divided into four groups of three rats each.

Group 1 (control group) consisted of rats receiving distilled, demineralized water. Groups 2, 3 and 4 received 0.1. 1.0 and 5.0 mg of $MnCl_2$/ml of water respectively. The daily water intake was approximately 35 - 40 ml per animal. The exposure period lasted 8 months.

At the end of the exposure period the animals were anesthetized with ether and decapitated. The brains were rapidly removed and placed in 10% formalin, remaining in fixation for one week. The brains were then sectioned coronally at 5 mm intervals and sampled. Samples included striatum, frontal cortex, hippocampus, midbrain, pons, medulla and cerebellum. The samples were embedded in paraffin, sectioned at 6 microns and stained with hematoxylin and eosin, phosphotungstic acid hematoxylin, and Holzer stains.

RESULTS

Gross examination of brains did not reveal significant changes.

Microscopic examination of sections of brains in groups 1 and 2 did not show alterations. The brains of animals in groups 3 and 4 revealed the following:

Neurons:

Alterations detected in the neuronal volume and stainability were seen at the following levels: conspicuosly in frontal cortex, neostriatum, midbrain and pons. The neurons appeared: scalloped, eosinophilic, with shrunken nuclei (Figs. 1 and 2). Scattered neuronal loss was observed in the frontal cortex, hippocampus, and neostriatum.

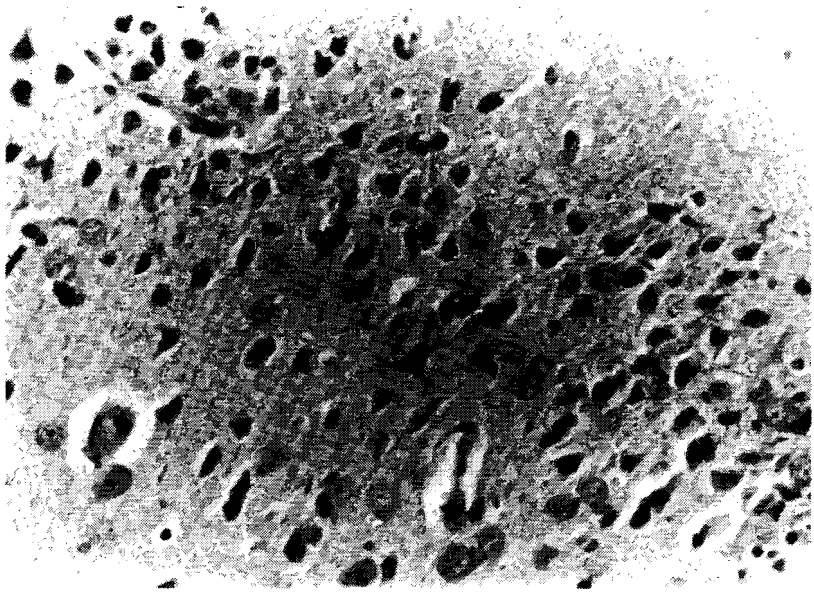

Fig. 1.– Frontal cortex in a chronic manganese poisoned rat. Numerous scalloped neurons with shrunken eosinophilic nuclei are present. H & E x 400.

Glia:

Mild reactive astrocytosis was seen surrounding the lateral ventricles and the cerebral aqueduct. Occasional satellitosis was seen in the neostriatum.

Other:

Moderate mononuclear infiltrate was observed in the choroid plexus of the temporal horns of the lateral ventricles (Fig. 3) in the interventricular foramina; mononuclear cells and a few isolated neutrophils were also seen surrounding the third ventricle (Fig. 4).

The intensity of the changes was similar in both groups.

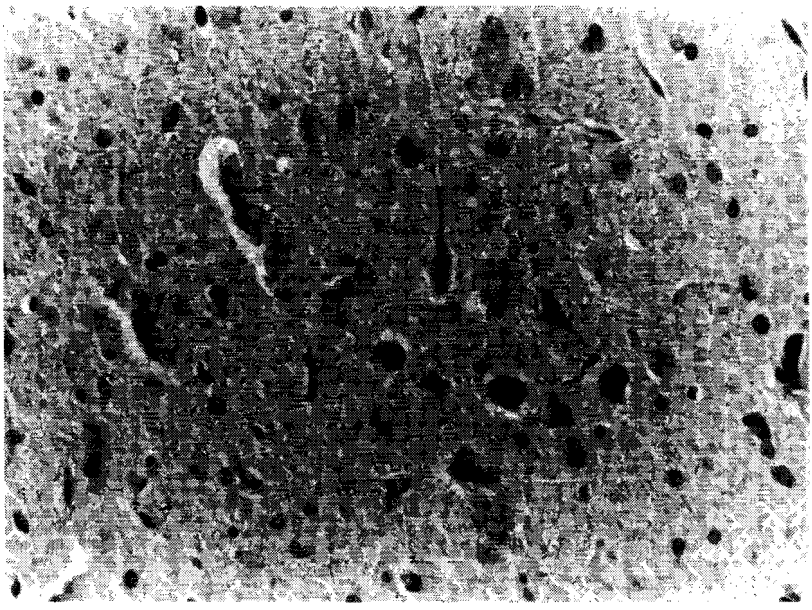

Fig. 2.– Head of the caudate nucleus in a chronically manganese poisoned rat. Shrunken neurons are evident. H & E x 450.

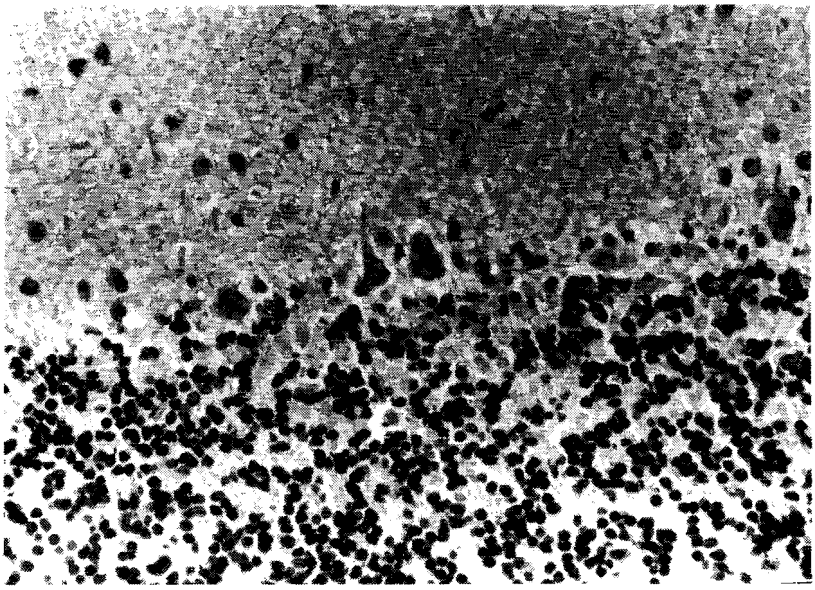

Fig. 3.– Choroid plexus of the temporal horn of the right lateral ventricle in a chronically manganese poisoned rat. The transmural inflamatory infiltrate is composed of mononuclear cells. H & E x 450.

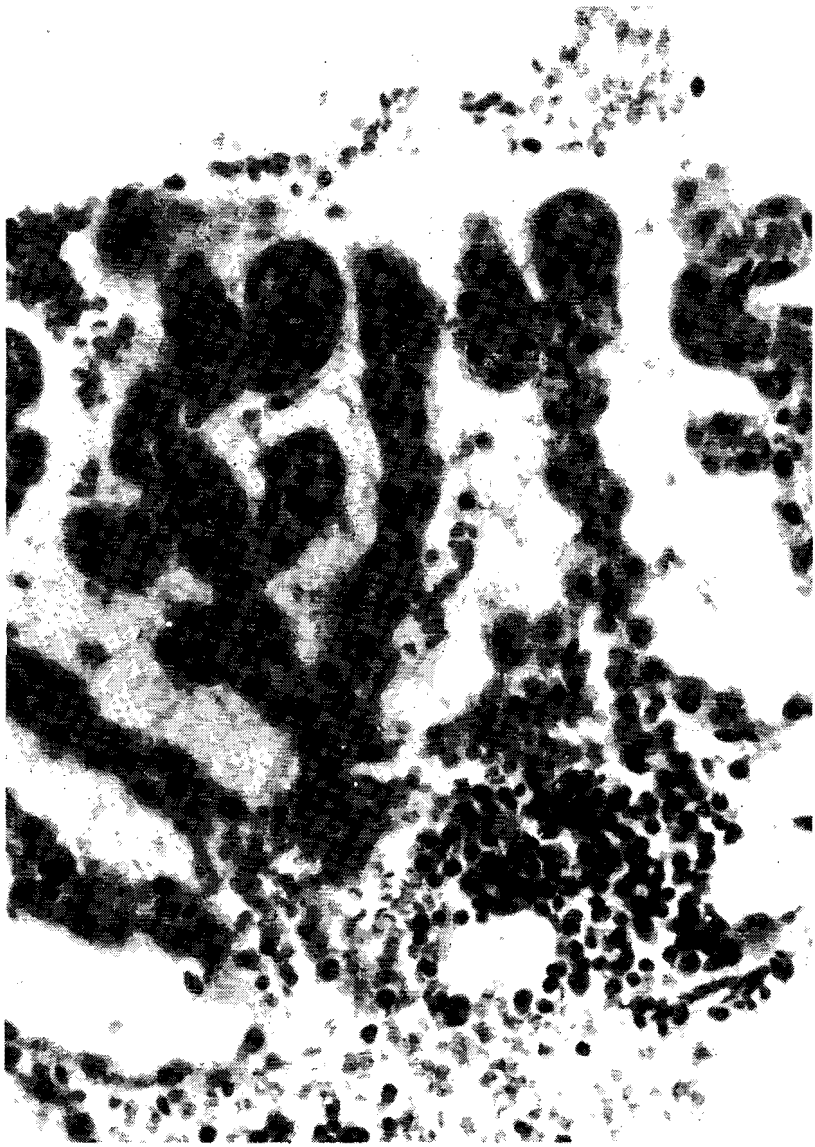

Fig. 4.– Lateral wall of the rostral Third Ventricle. Mild inflammatory infiltrate composed of mononuclears and few scattered polimorphonuclears. H & E x 250.

DISCUSSION

The neurotoxicity of manganese has been established by numerous reports (5, 6, 7, 9, 13, 14). Manganese poisoning leads to a chronic disabling

disease that resembles parkinsonism in its clinical symptoms (9, 10) with an initial psychiatric phase known as manganese madness (12).

It has been found that animals exposed to Manganese (0.1 and 5.0 mg Mn/ml of drinking water) displayed an altered behavioral pattern as determined by measurement of spontaneous motor activity which revealed hyperactivity during the first month and hypoactivity during the 7th and 8th month as compared to the control group (3). The histopathological changes observed in this study were evident only in the groups exposed to 1.0 and 5.0 mg Mn/ml, whereas no lesions were observed in the brains of the group receiving 0.1 mg Mn/ml. It is possible that the neuropathological lesions induced by the lowest concentration of the metal are at the ultrastructural level and thus cannot be detected by light microscopy, or that the alterations are biochemical rather than structural. Therefore, further studies by electron microscopy in pre-determined areas of brain such as striatum, frontal cortex, and tegmental areas of midbrain and pons of animals receiving the minimal concentration of manganese capable of inducing behavioral changes (0.1 mg Mn/ml) would be of great significance to determine if there are any changes, and if so, to elucidate the reversibility of such for it has been demonstrated that when the structural changes appear the damage to the extrapyramidal system becomes irreversible (10). Nevertheless the intermediate dose used in our study (1.0 mg/ml) was capable of inducing morphological changes, not dose dependant as evidentiated by the similarity of such changes in the 1.0 mg/ml and 5.0 mg/ml groups; thus we believe that this should make health authorities aware of the potential threat posed by manganese acting as an atmospheric pollutant specially among susceptible individuals such as children, individuals with impaired nutritional absorption, living in densely populated areas with significant atmospheric pollution, as previously reported (5, 8, 11, 12, 14).

As for some of the microscopic lesions we observed, neuronal alterations in the neostriatum had not been previously reported, to the best of our knowledge, in rats affected with manganese encephalopathy even though striatal alterations are a common feature of the disease in humans (1, 2, 4, 12). The presence of inflammatory cells in the choroid plexuses of the affected animals appears to be similar to the findings reported by Cavanan et al in their report of a human case of chronic manganese poisoning: "The choroid plexus showed cells on the outside of the tufts, they were mostly low and single but occasionally heaped up" (4). We believe our findings represent an early phase of what appears to be a chronic change in the case reported by Cavanan.

Acknowledgments

This work was partially supported by grants from Fundación para el Desarrollo de la Ciencia y la Tecnología, Región Zuliana (FUNDACITE-ZULIA) y por el Consejo de Desarrollo Científico y Humanístico de la Universidad del Zulia (CONDES-LUZ).

RESUMEN

La neuropatología de la intoxicación crónica experimental por manganeso en ratas. Reporte preliminar. *Cardozo J. (INBIOMED-FUNDACITE. Apartado Postal 376. Maracaibo 4010, Venezuela), Bonilla E.* **Invest Clín** *26(2): 117-124, 1985.*– Ratas machos adultas Sprague-Dawley fueron intoxicadas con manganeso a través del agua de bebida. Los animales recibieron 0.1, 1.0 y 5.0 mg Mn/ml, en forma de $MnCl_2$ durante ocho (8) meses. Este modelo experimental de intoxicación crónica por manganeso, comparte rasgos neuropatológicos con la enfermedad humana, tales como alteraciones corticales y estriatales. Nuestros resultados sugieren que el manganeso administrado por vía oral, a dosis bajas, puede inducir alteraciones, como se evidenció a través de los estudios por microscopía de luz.

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