Bull. Environ. Contam. Toxicol. (1990) 44:686–691

Bulletin of Environmental Contamination and Toxicology

In vivo Effects of Nickel and Cadmium in Rats on Lipid Peroxidation and Ceruloplasmin Activity

J. Solé, J. Huguet, Ll. Arola, and A. Romeu

Department of Chemical Engineering and Biochemistry, University of Barcelona, 43005 Tarragona, Spain

Before Ni(II) and Cd(II), or any other metallic ion, can interact intracellulary, it must penetrate the cell membrane (McCay 1981). The latter, therefore, is a primary target for toxic metals (Christie and Costa 1984). Damage to cell membranes may allow a greater uptake of metal and thus injury may extend to more critical targets (Slater 1984), although loss of plasmatic membrane functionality may be a crucial factor to explain the interactions of these metals with cellular components (Younes 1984). In this sense the present study has been carried out. Factors that have been investigated in order to prove the membrane response of nickel and cadmium toxicity include lipid peroxidation, since divalent ions of transition metals can promote lipid peroxidation and this evidently contributes to the toxicity of certains metals (Slater 1982, Sunderman 1986) and to metal interaction with ceruloplasmin, as its ferroxidase and scavanger of superoxide radicals activities (EC 1.16.3.1) are important protective mechanisms *in vivo* against peroxidative damage (Gutteridge et al., 1980, Cousins 1985).

MATERIALS AND METHODS

The experimental animals were female virgin Wistar rat (180-200 g body wt) kept under standard conditions of light (on from 08.00 to 20.00) and temperature 21-22 °C with constant (75-85%) relative humidity, and fed with rat chow pellets (Panlab, Barcelona, type A04) and tap water *ad libitum*. $NiCl_2$ (Merck) a dossage of 7 mg Ni(II)/kg, and $CdCl_2$ (Merck) a dossage of 1 mg Cd(II)/kg respectively were i.p. administered. The animals were killed at 1 and 24 hours respectively after i.p. metal-administration. Control rats were treated with NaCl (Merck) physiological saline, by the same i.p. route and at the same time that test rats were treated with the test substances.

Rats were sacrified by an overdose of ether at the beginning of light cycle, carcasses were dissected and samples of serum, liver, kidney and brain were obtained.

Send reprint request to A. Romeu at the above address.

One gram aliquots of the samples were homogenized with 9 ml of KCl 150 mM, duplicate aliquots of each homogenate were processed for determination of malondialdehyde (MDA) and related chromogens concentration by the method given by Sunderman et al. (1985). The analytical results are expresed as nmol of malondialdehyde per g of dry weight of tissue. Serum ceruloplasmin activity wa assayed as EC 1.16.3.1 acting on the oxidation of p-phenylenediamine (PPD) as described by Oosthuizen et al. (1985). Student's T test was used to establish the degree of statistical significance of the differences between groups.

Table 1. Serum ceruloplasmin activity in control, Ni-, and Cd-treated rats.

Treatment	Enzymatic activity (μkat/l) Time post-(ip)injection: 1hour	24hour
NaCl ip (control)	10.7±0.6	10.5±0.5
$NiCl_2$ ip (7mg Ni(II)/kg)	13.6±0.5*	13.9±1.0*
$CdCl_2$ ip (1mg Cd(II)/kg)	9.5±0.4	12.6±0.9

Each value is the mean±S.D. of 8-10 animals. *: $p<0.05$ vs control. Ceruloplamin activity measured as EC 1.16.3.1 acting on the oxidation of PPD.

RESULTS AND DISCUSSION

In figure 1, the MDA concentration found in the studied tissues of $NiCl_2$-$CdCl_2$- and NaCl-treated rats are presented. This investigation shows that administration of $NiCl_2$ and $CdCl_2$ enhances lipid peroxidation in liver and kidney. MDA liver concentrations were significantlly increased in both Ni(II)- and Cd(II)-treated rats at 1 hour post-injection. However, only the Cd(II)-treated rats showed MDA liver concentration significantly increased at 24 hours after i.p. cadmium administration. Kidney lipid peroxidation, in terms of MDA and related chromogens concentrations in fresh homogenates, became significant only in Ni(II)-treated rats at 1 hour post-injection. The mean MDA concentration in kidney homogenates of Ni(II)- and Cd(II)-treated rats became significantly increased at 24 hours after i.p. test substance administration. Concentrations of MDA and related chromogens were not significantly increased in fresh homogenates of brain of rats killed at 1 and 24 hours after an i.p. injection of $NiCl_2$ or $CdCl_2$ versus control rats killed at the same time.

In table 1, serum oxidase activity of ceruloplasmin from controls and nickel- and cadmium-treated animals are presented. Serum ceruloplasmin levels of cadmium-treated rats do not differ significantly from those of the control. Therefore, ceruloplasmin activity of nickel treated-rats was significantly increased both at 1 and 24 hours after i.p. administration of

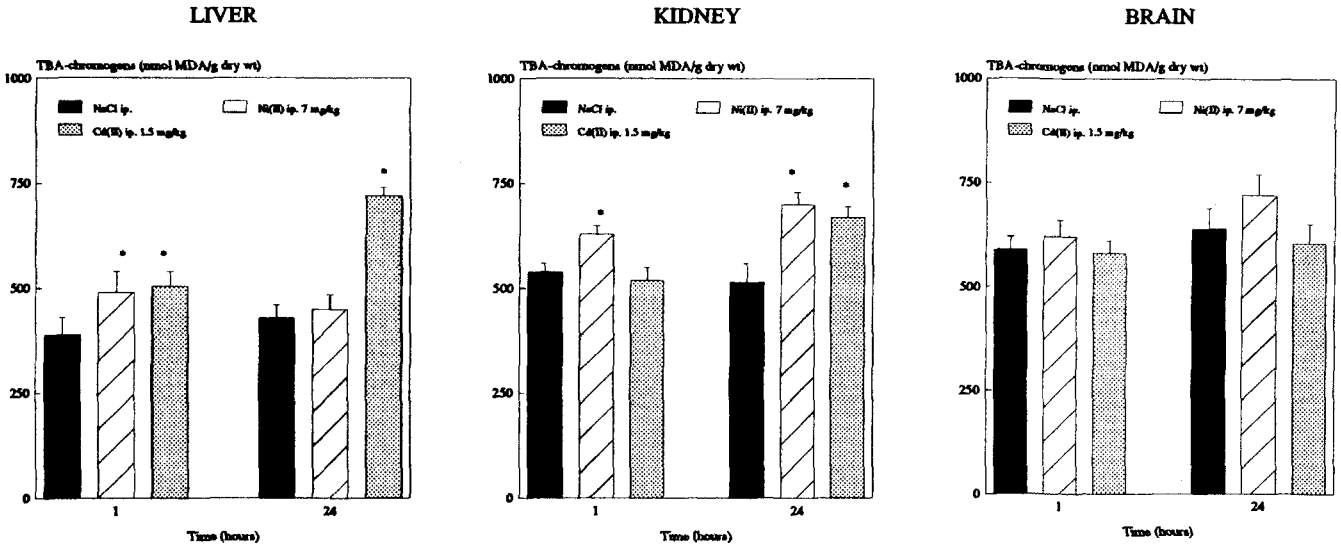

Figure 1. Effect of Ni(II) and Cd(II) versus control (injection of NaCl vehicle) on concentrations of TBA-chromogens, as biochemical indices of lipid peroxidation, in liver, kidney and brain at 1 and 24 hours post-(ip)injection respectively. Each value is the mean ± S.D. of 8-10 animals. *: $P < 0.05$ vs control.

$NiCl_2$. *In vitro* additions of appropriate concentration of $NiCl_2$ to the normal (control) serum from ten untreated rats did not have any significant effect upon enzymatic oxidation of PPD, indicating that Ni(II) or any Ni-complex of serum protein(s) does not interfere in the assay procedure of ceruloplasmin.

The outcome of this lipid peroxidation study corroborates partially the finding of Sunderman et al., (1985), who administrated Ni(II) with s.c. injection (43.8 mg Ni/kg body wt), and MDA concentrations in liver and kidney (but not in brain) became significantly increased by nine hours and reached peak values at 48 hours. Renal lipid peroxidation stimulation found in this *in vivo* study is consistent with the actual knowledge that follows the administration of soluble nickel in experimental animals, it is rapidly cleared from the serum excreted primarily in urine and to a lesser degree in faeces (Sunderman 1977, Sarkar 1984). Lipid peroxidation induced by nickel has been reported *in vitro* in primary epithelial cell cultures of rat kidney (Helmut et al. 1985). The significant increase in liver MDA concentration, found in this study, only at 1 hour after i.p. Ni(II) follows the same pattern of hepatic Ni-concentration, that is only increased at the same time post-injection (Marzouk and Sunderman 1985, Donskoy et al 1986). This finding is in substantial agreement with the earlier papers on rat Ni-distribution experiment (Parker and Sunderman 1974). Significant differences are found, however, with respect to brain in which neither nickel nor MDA levels are increased, in agreement with the lowest nickel concentration found in brain, since this is not a target organ for nickel (Parker and Sunderman, 1974). A single parenteral dose of cadmium is cleared rapidly from the blood and distributed amongst most tissues (Samarawickrama 1979, Kelman 1986), the organs of the body which accumulate the largest quantities of Cd(II) are the liver (mainly) and the kidneys (Webb 1986). The relation between lipid peroxidation and cell injury by cadmium has been studied in isolated hepatocites (Stacey et al 1980, Din and Frazier 1985), where there is some indications of a positive correlation between both of them, consistent with cadmium which produces a time-dependent liver formation of thiobarbituric reactants found in the present series of *in vivo* experiments. The effects of cadmium on the kidneys induced ultrastructural and functional changes (Webb 1986) and lipid peroxidation is implicated as a general mechanism of tissue damage in the light of the present results. In agreement with the earlier work by Webb (1979) on distribution of cadmium experiments in the rats, brain Cd-concentration was insignificant, even at a short time after treatment. This aspect of cadmium metabolism is consistent with the observed lack of brain peroxidative damage.

In spite of the above considerations, ceruloplasmin can often prevent lipid peroxidation (Cousins 1985) and ·OH generation in extracellular fluids since they contain transition metals in abundance and can be exposed to $O_2\cdot^-$ generated by white blood cells or by enzymes released from damaged cells (Gutteridge et al. 1980). Ceruloplasmin response *in vivo* in terms of its oxidase activity is quite different in both nickel and cadmium treated rats versus control, this finding found in the present study, together with enhanced MDA in tissue homogenates, from metal-treated rats, suggest that

both nickel and cadmium enhanced lipid peroxidation but following different biochemical mechanisms. The different redox behaviour of Ni(II) and Cd(II) (Cammer et al. 1984, Aylett, 1979) ions should be related to the increase in ceruloplasmin activity only in sera from Ni-treated rats. Whether this significant rise may be related to the possibility that Ni(II)/Ni(III) redox couple generate oxygen free-radicals by one-electron transfer reactions or not remains to be investigated.

REFERENCES

Aylett BJ (1979) The chemistry and bioinorganic chemistry of cadmium. In: Webb M (ed) The chemistry, biochemistry and biology of cadmium. Elsevier/North-Holland, Amsterdam, 1-44.

Camner P, Casarett-Bruce T, Cursted T, Jarstrand C, Johansson A, Lundborg M, Robertson B, Wiernik A (1984) Toxicology of nickel. In Sunderman FW (ed) Nickel in the human environment. International Agengy for Research on Cancer, Lyon, 267-276.

Christie NT, Costa M (1984) In vitro assessment of the toxicity of metal compounds. IV. Disposition of metals in cells: Interaction with membranes, glutathione, metallothionein, and DNA. Biol Trace Element Res 6:139-158.

Cousins RJ (1985) Adsorption, transport, and hepatic metabolism of copper and zinc: special reference to metallothionein and ceruloplasmin. Physiol Rev 65:238-311.

Din WS, Frazier JM (1985) Protective effect of metallothionein on cadmium toxicity in isolated rat hepatocytes. Biochem J 230:395-402.

Donskoy E, Donskoy M, Forouhar F, Gillies C, Marzouk A, Reid MC, Zaharia O, Sunderman FW (1986) Hepatic toxicity of nickel chloride in rats. Annals Clin Lab Sci 16:108-117.

Gutteridge JMC, Richmond R, Halliwell B (1980) Oxygen free-radicals and lipid peroxidation: inhibition by the protein ceruloplasmin. FEBS Lett 112:269-272.

Helmutz M, Liber C, Sunderman FW (1985) Nickel toxicity in primary epithelial cell cultures of rat kidney. Annals Clin Lab Sci 15:346-347.

Kelman GR (1986) Cadmium metabolism in man. Human Toxicol 5:91-93.

Marzouk A, Sunderman FW (1985) Biliary excretion of nickel in rats. Toxicol Lett 27:65-71.

McCay PB (1981) Physiological significance of lipid peroxidation. Fed Proc 40:173-188.

Oosthuizen MMJ, Nel L, Myburgh JA, Crookes RL (1985) Purification of undegrated ceruloplasmin from outdated human plasma. Anal Biochem 146:1-6.

Parker K, Sunderman FW (1974) Distribution of ^{63}Ni rabbit tissues following intravenous injection of $^{63}NiCl_2$. Res Comn Chem Pathol Pharmacol 7:755-762.

Samarawickrama GP (1979) Biological effects of cadmium in mammals. In Webb M (ed) The chemistry, biochemistry and biology of cadmium. Elsevier/North-Holland, Amsterdam, 341-421.

Sarkar B (1984) Nickel metabolism. In: Sunderman FW (ed) Nickel in the human environment. International Agengy for Research on Cancer, Lyon, 367-384.
Slater TF (1982) Lipid peroxidation. Biochem Soc Transact 10:70-71.
Slaterr TF (1984) Free-radical mechanism in tisue injury. Biochem J 222:1-15.
Stacey NH, Cantilena LR, Klaassen CD (1980) Cadmium toxicity and lipid peroxidation in isolated rat hepatocytes. Toxicol Appl Pharmacol 53:470-480.
Sunderman FW (1977) A review of the metabolism and toxicology of nickel. Annals Clin Lab Sci 7:377-398.
Sunderman FW, Marzouk A, Hopfer SM, Zaharia O, Reid MC (1985) Increased lipid peroxidation in tissues of nickel chloride-treated rats. Annals Clin Lab Sci 15:229-236.
Sunderman FW (1986) Metals and lipid peroxidation. Acta Pharmacol Toxicol 59s:243-255.
Webb M (1979) Interactions of cadmium with cellular components. In Webb M (ed) The chemistry, biochemistry and biology of cadmium. Elsevier/North-Holland, Amsterdam, 285-340.
Webb M (1986) Role of metallothionein in cadmium metabolism. In Foulkes EC (ed) Handbook of experimental pharmacology, vol. 80, Springer-Verlag, Berlin, 281-335.
Younes M, Siegers CP (1984) Interrelation between lipid peroxidation and other hepatotoxic events. Biochem Pharmacol 33:2001-2003.

Received Febreuary 7, 1989; accepted July 25, 1989.