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In Vitro and *In Vivo* Responses of Rat Tissues to Cadmium-Induced Lipid Peroxidation

Dino Manca, Anne C. Ricard, Bertin Trottier, and Gaston Chevalier

Environmental Toxicology Research Laboratory, Department of Biological Sciences, University of Quebec in Montreal, C.P. 8888, Suc. A., Montreal, H3C 3P8, Canada

Oxidative destruction of polyunsaturated fatty acids of membrane phospholipids, a phenomenon generally termed lipid peroxidation (LPO), is considered to be an important mechanism of toxicity for a wide variety of chemicals. Among these, cadmium (Cd), a pollutant of industrial and environmental importance, induces LPO in various tissues despite its apparent inability to directly generate free radicals under physiological conditions (Ochi et al. 1987). Consequently, although LPO is not the primary mechanism of Cd toxicity, it represents an early intracellular response of tissues following exposure to Cd compounds (Muller and Ohnesorge 1982) and its occurence as a consequence of damages may be important in view of the cytotoxicity of the end-products of the peroxidation process (Halliwell and Gutteridge 1985). Although Cd-induced LPO has been observed in numerous tissues as well as in in vitro systems (Vincent et al. 1989; Hussain et al. 1987; Stacey et al. 1980), information on the relative susceptibility of these tissues, and hence on the identity of the primary target organs to Cd-induced LPO, are still lacking. Consequently, comparison of specific tissue responses to Cd-induced LPO is important to determine which ones are the most affected and to what extent.

Recently, we reported the in vitro specific response to LPO of liver, lung, heart, kidney, testes and brain tissues incubated with various concentrations of $CdCl_2$ (Manca et al. 1990). LPO was assessed by the measurement of thiobarbituric acid reactive substances (TBARS) which include malondialdehyde and lipid hydroperoxydes, and by gaz chromatographic analysis of evolved hydrocarbons, namely ethane and pentane (TEP: total ethane plus pentane). These classes of compounds result from the polyunsaturated fatty acids breakdown process (Halliwell and Gutteridge, 1985). In order to adequately compare

Address correspondence to Dr. G. Chevalier

the results obtained by both methods, we standardized TEP and TBARS values against incubated controls after subtracting endogenous levels of TBARS (time 0 values) because the measurement of endogenous levels of TEP in tissue incubates is not practicable. Thus, data were standardized against autoxidation levels (spontaneous LPO) wich occurs during incubation of tissues under air atmosphere. By using this approach, results showed that both methods were comparable when measuring the net release of breakdown products from tissues incubated with Cd. It was demonstrated that liver was the most responsive organ, while major increases of LPO were also observed in heart and testes incubates. In order to compare the in vitro LPO responses to those measured in the same tissues of Cd-intoxicated animals TBARS are more suitable than volatile hydrocarbons as they permit the identification of target organs. However, a different standardization procedure must be employed as in vivo data are generally expressed against endogenous levels (control groups). Consequently, autoxidation levels must be subtracted from in vitro data. The objective of the present report is to clarify this concept in order to adequately compare the data obtained from in vitro and in vivo studies. This should permit a better assessement of the relative importance of tissue responses to LPO following exposure to Cd.

MATERIALS AND METHODS

The in vitro TBARS data were obtained from previous studies (Manca et al. 1990). For in vivo studies, similar animals (12 week-old male Long Evans rats; 336 ± 35 g.) were injected with 25, 125, 500, and 1250 ug Cd/kg I.P. as $CdCl_2$. Each dose was given twice at one hour interval. Twenty four hours (24h) after the last injection, animals were sacrificied with an intraperitoneal overdose of sodium pentobarbital and whole body perfusion was then performed with a 37°C saline solution through the abdominal aorta. Lungs were reperfused via the pulmonary artery. Liver, testes, lungs, kidneys, brain and heart were excised, rinsed in ice-cold saline, blotted dry and weighed. Tissues were homogenized in 4 volumes of ice-cold 10 mM Tris buffer (pH 7.6) using a motor-driven Teflon homogenizer. Heart was homogenized in 9 volumes of the same buffer. Thiobarbituric acid reactive substances (TBARS) were quantified in tissue homogenates according to Ohkawa et al. (1979) as previously adapted (Manca et al. 1990a). The Cd tissue content was determined by graphite furnace atomic absorption spectrometry. The method has been described in detail elsewhere (Manca et al., Submitted). Statistical analysis of the in vivo data was carried out at a preset probability level of ≤ 0.05 by using the two-tailed Student t-test.

RESULTS AND DISCUSSION

In order to adequately compare the *in vitro* and *in vivo* responses, *in vitro* data obtained from the preceeding study (Manca et al. 1990) were transformed:

a) to standardise the results on a per weight tissue basis as great variations in protein tissue content were observed in animals following exposure to Cd (results not shown)

b) to eliminate the contribution of autoxidation which could prevent adequate comparison of *in vitro* and *in vivo* data. This approach is illustrated in Figure 1. Following incubation of tissue homogenates endogenous TBARS, spontaneously formed TBARS during incubation (autoxidation) and TBARS formed as a consequence of Cd exposure contribute to the response. Consequently, for comparison of *in vitro* and *in vivo* data, autoxidation values were substracted from the overall *in vitro* response which was thereafter standardized to endogenous levels.

Modified *in vitro* data shown in Figure 2 indicate that liver was still the most responsive organ to Cd-induced LPO although the amplitude of response was lower than previously reported (6 vs 17 fold increase at 450 uM Cd) (Manca et al. 1990) due to higher endogenous levels of TBARS as compared to autoxidation values (Figure 1) and to standardization on a per weight basis. The amplitude of response in testes incubates was similar when calculated with both approaches due to comparable endogenous and autoxidation levels of TBARS. Brain showed significant increases at each concentration of Cd. On the other hand, TBARS levels in heart tissue were decreased (2 vs 10 fold increase) as a consequence of higher endogenous levels when compared to autoxidation rates.

In vivo experiments showed that liver and kidney contained the greatest amounts of Cd 24 hours after injection of various doses of $CdCl_2$, while lowest levels were observed in brain and testes (Figure 3). However, the increase of Cd in brain tissue was not dose-related and a plateau was attained at 125 ug Cd/kg.

Lipid peroxidation, as measured by the TBARS assay, was significantly increased in the majority of tissues investigated following administration of various doses of Cd. When results were expressed on a nmol TBARS/g tissue basis (results not shown), the level of production among tissues was similar to that reported *in vitro* (Manca et al. 1990). Brain and kidney released the greatest absolute amounts of TBARS while lower levels were observed in lung and testes. However, *in vivo* results indicated that the response was not dose

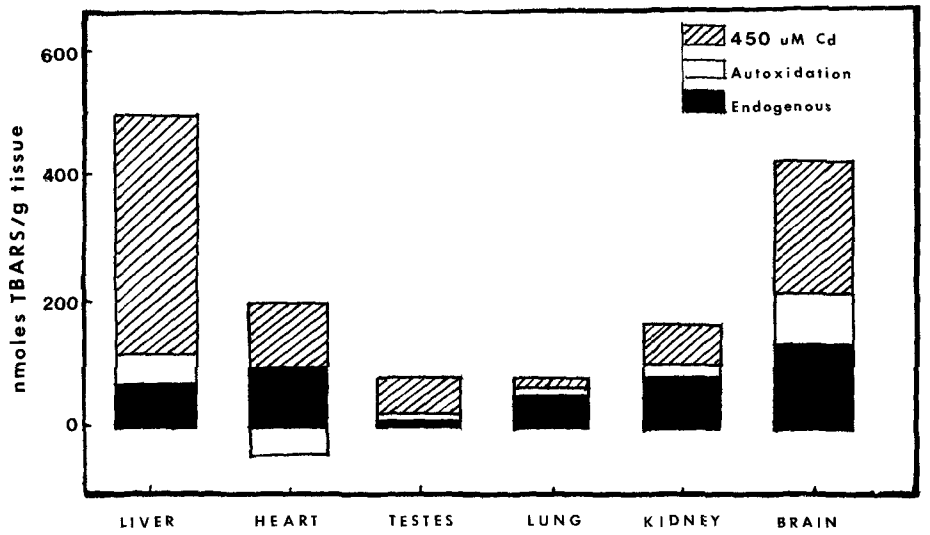

Figure 1. In Vitro Contribution of Endogenous (time Ø), Autoxidation (spontaneously formed during incubation), and Cadmium-Induced TBARS to Total TBARS Values Measured in Tissue Incubates. Homogenates were incubated in Tris buffer (pH 7.6) at 37°C for 90 minutes (see Manca et al. 1990). Cadmium concentration was 450 uM. Results (mean of 4 experiments) are expressed as nmol TBARS/g tissue.

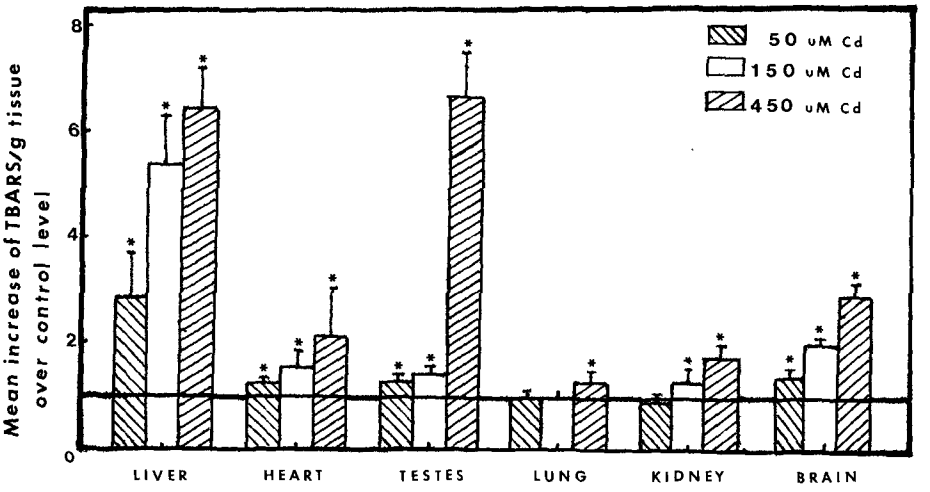

Figure 2. In Vitro Susceptibilities to LPO of Rat Tissues as a Function of Cadmium Concentration. Results and conditions as reported by Manca et al. (1990). Autoxidation levels were substracted and results are expressed as the mean increase over endogenous levels (SEM; n=4) following standardization on a nmol TBARS/g tissue basis. *Significantly different from control levels (P Ø.Ø5).

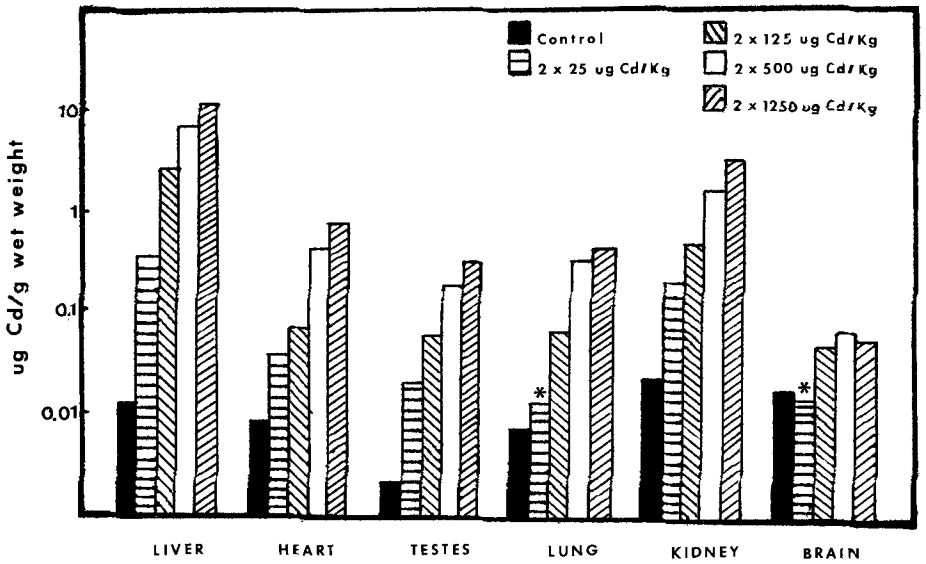

Figure 3. In Vivo Cadmium Content of Rat Tissues 24 Hours After Injection of Various Doses of Cd^{+2} as $CdCl_2$. Results (ug Cd/g wet weight) are expressed as the mean value (SEM) of 5 rats per group. Values are significantly different from control groups (P Ø.Ø5) unless otherwise indicated (*).

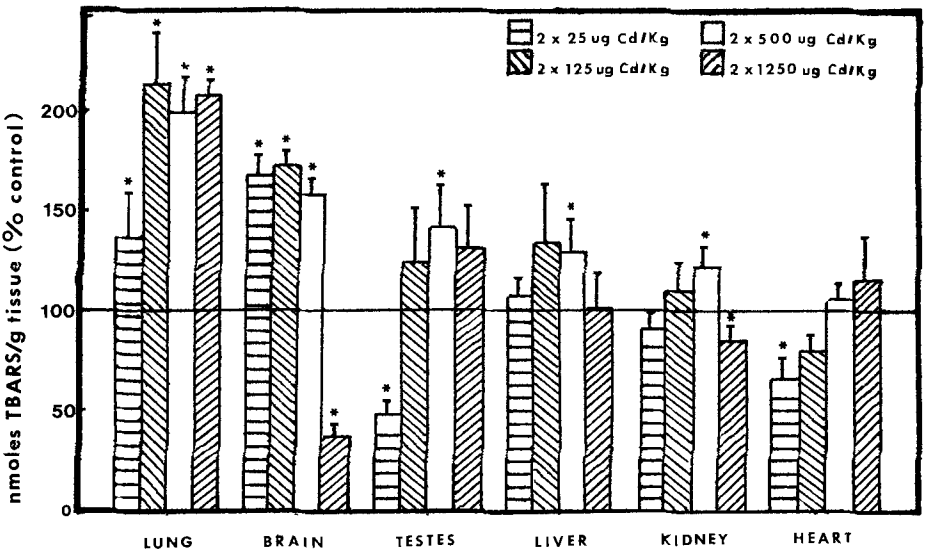

Figure 4. In Vivo Susceptibilities to LPO of Rat Tissues 24 Hours After Cadmium Administration. Results are expressed as the mean percent (SEM; n=5) of control values following standardization on a nmol TBARS/g tissue basis. *Significantly different from control group (P Ø.Ø5).

related. A steep decrease of TBARS content was noted in brain, kidney, liver and testes following administration of 1250 ug Cd/Kg , while a similar trend was observed in heart and testes after 25 ug Cd/Kg. In order to assess the amplitude of response of these tissues, TBARS values were standardized against control levels (endogenous TBARS). Results shown in Figure 4 indicate that lung and brain were the most responsive organs at each dose of Cd, although Cd tissue content was not significantly increased after administration of 25 ug Cd/kg (Figure 3). This indicate that LPO observed at 24 hours could be the result of oxidative phenomenons which occured at earlier times, prior to the clearance of Cd from these tissues. Furthermore, decreased TBARS levels were observed in heart and testes following treatment with low doses of Cd, suggesting that protective mechanisms (e.g. stimulation of the antioxidant defence system, metabolism and/or excretion of TBARS, namely malondialdehyde) could also have been involved at earlier times. Liver and kidney contained the highest amounts of Cd 24 hours after administration of $CdCl_2$; however, small increases in LPO were only observed at 500 ug Cd/kg prior to a decrease at 1250 ug Cd/kg. A similar pattern of LPO response was observed in testes and brain. This suggests that LPO is differently modulated in these tissues as a function of the dose administered. Furthermore, the amplitude of response among various tissues, as measured with the TBARS assay, is not related to the cadmium tissue content.

Comparison of *in vitro* (Figure 2) and *in vivo* data (Figure 4) suggest that although liver has the greatest potential to undergo LPO *in vitro* it also has a great capacity to counteract this phenomenon *in vivo*. One possible explanation for these observations may be attribuable to the higher capacity of liver to synthetize the protective metalloenzyme metallothionein as compared to kidney and lung in response to Cd exposure (Wormser and Nir 1988).

Recent studies by our laboratory have reported the great vulnerability of lung tissues to Cd following inhalation of Cd salts (Vincent et al. 1989; Boudreau et al. 1989; Boudreau et al. 1988). However, the present study indicates that, apart from a direct interaction between aerosolized Cd and lung tissue constituents, parenteral exposure also results in extensive lung damages as reflected by high increases of TBARS at each dose of Cd administered. Kornburst and Mavis (1980) have previously demonstrated that the ratio of vitamin E to polyunsaturated fatty acids in lung tissue was several fold higher than in other organs, which accounted for the relative resistance of lung to LPO *in vitro*. Consequently, in view of the differential responses

obtained in vitro and in vivo and to the Cd lung content which was not significantly elevated 24 hours after injection of 25 ug Cd/Kg, it is possible that LPO observed in lung of intoxicated animals could result from amplification mechanisms following inflammation processes (e.g. activation of infiltrated phagocytes), as already demonstrated following acute pulmonary exposure to Cd compounds (Buckley and Bassett 1987). Consequently, these results suggest that lung and brain could be considered the major target organs to LPO 24 hours after exposure to low and moderate doses of Cd as $CdCl_2$. These findings are of great importance when considering that occupational exposure mainly occurs by the inhalation route and that low amounts of Cd can ellicit significant oxidative phenomenons. As a result, more attention should be given to extrahepatic and extrarenal tissues when assessing adverse effects following Cd exposure. Studies are currently in progress to assess the temporal evolution of LPO in these tissues and its significance as compared to other toxic manifestations.

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