BioMetals 1993, 6, 101–106

Effect of superoxide dismutase, allopurinol and glucocorticoids on liver and lung metallothionein induction by endotoxin in the rat

Mercedes Giralt, Angeles Blanquez, Javier Avila* & Juan Hidalgo

*Departamento de Biología Celular y Fisiología, Unidad de Fisiología Animal, Facultad de Ciencias, Universidad Autónoma de Barcelona, Bellaterra and *Servicio de Medicina Interna, Hospital de Bellvitge Príncep d'Espanya, Hospitalet de Llobregat, Barcelona, Spain*

Received 16 October 1992; accepted for publication 15 December 1992

Liver and lung metallothionein (MT) levels were increased by endotoxin. The administration of superoxide dismutase (SOD) or allopurinol (ALLO) before (30–60 min) or after (24–32 h) the endotoxin treatment either increased or did not affect the effect of endotoxin on MT levels, depending on the particular treatment and tissue. SOD and ALLO also increased liver and lung MT levels in control rats. In contrast, liver MT levels tended to be decreased by the glucocorticoid prednisolone (PRED) when administered before the endotoxin and were significantly decreased when it was administered after endotoxin. The effect of PRED on lung MT levels was completely different, since it decreased the effect of endotoxin when injected before the lipopolysaccharide, but increased it when injected after the endotoxin. Liver lipid peroxidation, as measured by thiobarbituric acid reactants (TBARs), increased after endotoxin in the liver but not in the lung, an effect even potentiated in some cases by the antioxidants studied. As expected, tissue MT and TBARs could not be correlated.

Keywords: allopurinol, glucocorticoids, lipid peroxidation, metallothionein, prednisolone, superoxide dismutase

Introduction

Metallothionein (MT) is a low molecular weight, heavy metal-binding protein. The MT gene is induced directly by heavy metals such as zinc, cadmium and copper, and by glucocorticoids (Kägi & Kojima 1987). Factors that initiate the inflammatory response, such as turpentine oil or endotoxin lipopolysaccharide, are also known to induce liver MT synthesis (Sobocinski *et al.* 1978, Suzuki & Yamamura 1980, Sobocinski & Canterbury 1982). MT induction by endotoxin does not involve either heavy metals or glucocorticoids (Durnam *et al.* 1984). Certain cytokines are released during the inflammatory response initiated by endotoxin, which might be the ones responsible for MT induction during endotoxin shock. This is suggested, in the first place, because several cytokines are known to induce MT synthesis (DiSilvestro & Cousins 1984, Cousins & Leinart 1988, De *et al.* 1990, Schroeder & Cousins 1990) and, in the second place, because low cytokine-producing (C3H/Hej) mice were less responsive to endotoxin than normal mice (Maitani *et al.* 1986, De *et al.* 1990, Liu *et al.* 1991).

On the other hand, it is well known that free radical production is increased during the endotoxin shock (Hammond *et al.* 1985, Brigham & Meyrick 1986). Because MT appears to be a good antioxidant *in vitro* (Thornalley & Vašák 1985, Thomas *et al.* 1986) and possibly *in vivo* in some circumstances (Bakka *et al.* 1982, Shiraishi *et al.* 1983, Hidalgo *et al.* 1988a, Bauman *et al.* 1991, Martins *et al.* 1991), the possibility exists that MT induction by endotoxin is related to free radical processes. Therefore, the aim of the present work was to study the effect of the antioxidants (Halliwell & Gutteridge 1989) superoxide dismutase (SOD) and allopurinol (ALLO) on the MT response to endotoxin. The effect of glucocorticoids was also studied, because these hormones can act as antioxidants (Baud & Ardaillou 1986, Hall *et al.* 1988) in addition to their well known antiinflammatory activity. While this manuscript was in preparation, a report from Min *et al.*

Address for correspondence: J. Hidalgo, Departmento de Biología Celular y Fisiología, Unidad Fisiología Animal, Facultad de Ciencias, Universidad Autónoma de Barcelona, Bellaterra 08193, Barcelona, Spain.

(1992a) demonstrated that glucocorticoids decrease the liver MT response to endotoxin in mice, which is relevant for the present results.

Materials and methods

Animals

Male Sprague-Dawley rats approximately 3 months old were maintained under standard conditions (lights on from 07.00 to 19.00 h, 22 °C, food and water *ad libitum*) and housed in groups of four per cage.

Reagents

Endotoxin (lipopolysaccharide from *Escherichia coli* 0127:B8), ALLO and Tris were purchased from Sigma (St Louis, MO). Prednisolone (PRED) was from Upjohn (Alcalá de Henares, Spain), and SOD from Laboratorios Andrómaco (Barcelona, Spain). Cadmium, zinc, mercaptoethanol and malondialdehyde were purchased from Merck (Darmstadt, Germany). Thiobarbituric acid (TBA) and sodium azide were purchased from Fluka AG (Buchs, Switzerland). The other reagents were of analytical grade. Laboratory food was from Panlab (Barcelona, Spain).

Experiment 1: treatment with SOD, ALLO and PRED before endotoxin

In this experiment, some rats were pretreated with SOD (5 or 25 mg kg^{-1}, s.c.), ALLO (25 or 250 mg kg^{-1}, oral) or PRED (5 or 25 mg kg^{-1}, s.c.) before treating them with endotoxin (1 mg kg^{-1}, i.v. into the tail vein). SOD and PRED were administered 30 min before endotoxin, whereas ALLO was administered 60 min before endotoxin. All rats were killed 24 h after the endotoxin administration. Some rats were treated with SOD, ALLO and PRED alone to identify the effect of these factors by themselves. Control rats received saline. These rats were killed along with the endotoxin-treated rats.

Experiment 2: treatment with SOD, ALLO and PRED after endotoxin

The rats were injected i.v. with endotoxin (1 mg/kg). Twenty four hours later, some rats received SOD, ALLO or PRED at the doses stated in Experiment 1, once or twice (in this case 8 h apart). All the rats were killed 48 h after the endotoxin administration. Control rats received saline.

Assays

Rats were killed by decapitation. The trunk blood was collected in plastic tubes maintained at 4 °C and centrifuged at the same temperature to obtain the serum, which was stored at −80 °C. Lungs and livers were immediately removed and frozen at −80 °C. Homogenates and cytosols were prepared essentially as previously described (Hidalgo *et al*. 1988a). Lipid peroxidation in the tissues was assessed by measuring malondialdehyde formation, using the TBA assay (Uchiyama & Mihara 1978). Lipid peroxidation in the serum was assessed by fluorescence as described by Yagi (1984). MT levels were measured with a cadmium-saturation method previously described (Hidalgo *et al*. 1988b). Cadmium and zinc were measured by atomic absorption spectrophotometry. Results were analyzed with the Student's *t*-test (for two means) or with one-way ANOVA followed by the SNK test.

Results

Experiment 1

Figure 1 shows liver MT levels. Endotoxin increased them significantly in all cases regardless of the additional treatments (previous administration of SOD, ALLO or PRED); however, rats treated with ALLO (250 mg kg^{-1}) plus endotoxin had higher MT levels than those treated with endotoxin alone. The other SOD, ALLO and PRED treatments did not modify the effect of endotoxin. When these factors were given to rats not treated with endotoxin, only ALLO had a significant effect, increasing liver MT levels (see Figure 1 for significances).

Figure 2 shows lung MT levels. Again, endotoxin increased lung MT levels regardless of the additional treatment; in agreement with the results for liver MT, the administration of ALLO to endotoxin-treated rats further increased lung MT levels, as was the case in rats not treated with endotoxin. Whereas liver MT levels induced by endotoxin only tended to be decreased by the previous administration of

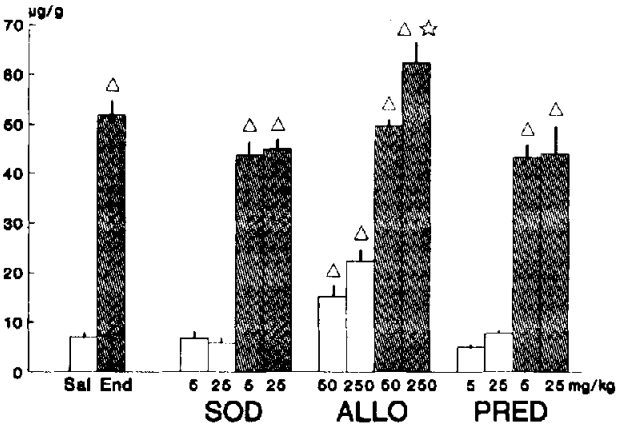

Figure 1. Effect of the previous administration of SOD, ALLO and PRED on endotoxin-induced changes of liver MT levels. The rats were treated 30 min (SOD and PRED, s.c.) or 60 min (ALLO, oral) before the endotoxin (End, shaded bars, 1 mg/kg, i.v.) injection and were killed 24 h later. Some rats received SOD, ALLO and PRED alone (open bars). Control rats received saline (Sal). Results are means ± SE (n = 4–6). $^{\triangle}P < 0.05$ versus saline rats. $^{*}P < 0.05$ versus endotoxin rats.

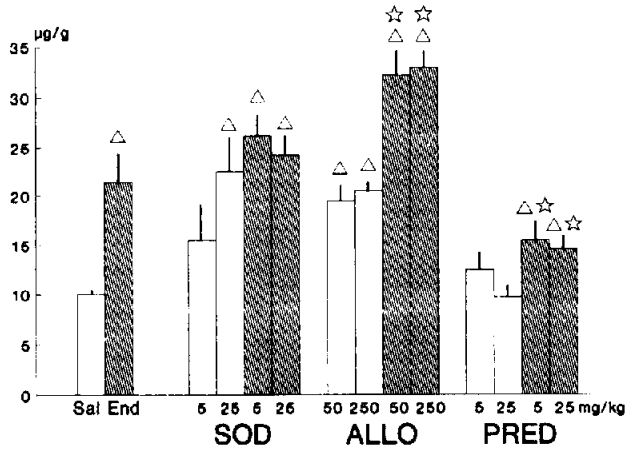

Figure 2. Effect of the previous administration of SOD, ALLO and PRED on endotoxin-induced changes of lung MT levels. For further details, see legend of Figure 1. Results are means ± SE ($n = 4$–6). $^{\triangle}P < 0.05$ versus saline rats. $^{*}P < 0.05$ versus endotoxin rats.

PRED, there was a significant reduction in the case of lung MT.

Table 1 shows body weight gain, liver cytosolic zinc, liver and lung TBA reactants (TBARs), and serum TBARs. Endotoxin decreased body weight gain regardless of the previous treatments, although the administration of SOD (25 mg kg^{-1}) further decreased it. PRED (25 mg kg^{-1}) was the only factor with a significant effect on body weight gain in rats not treated with endotoxin. In contrast, little effects were seen in liver and lung weights, since only in rats treated with ALLO and endotoxin a significant decrease of liver weight was observed (not shown). Liver cytosolic zinc levels were increased by endotoxin in most cases. Rats treated with endotoxin plus ALLO (50 mg kg^{-1}) or PRED (5 mg kg^{-1}) had higher liver TBARs levels than those treated with endotoxin alone. The other SOD, ALLO and PRED treatments did not modify the effect of endotoxin. In contrast, lung TBARs levels were not affected by endotoxin. Serum TBARs levels were only slightly affected by the different treatments.

Experiment 2

Figure 3 shows liver MT levels. Endotoxin increased liver MT regardless of the additional treatments (SOD, ALLO or PRED, one or two doses at two different concentrations, given one day after the endotoxin injection). However, the effect of endotoxin was reduced by the administration of PRED (25 mg kg^{-1}, one or two doses).

Figure 4 shows lung MT levels. In contrast to liver MT, lung MT levels were further increased by SOD, ALLO and PRED when given to endotoxin-treated rats.

Table 2 shows body weight gain, liver cytosolic zinc, liver and lung TBARs, and serum TBARs.

Table 1. Effect of the previous administration of SOD, ALLO and PRED on endotoxin-induced changes of some physiological variables

	Body weight gain (g day^{-1})	Liver cytosolic zinc (μg g^{-1})	Liver TBARs (nmol g^{-1})	Lung TBARs (nmol g^{-1})	Serum TBARs (μM)
Control					
saline	1.9 ± 1.5	18.1 ± 0.9	68.7 ± 8.2	95.3 ± 3.8	1.14 ± 0.05
SOD 5	4.0 ± 2.7	18.7 ± 1.8	52.0 ± 0.1	98.0 ± 2.6	1.17 ± 0.08
SOD 25	−0.7 ± 2.4	19.9 ± 0.9	59.0 ± 2.5	102.0 ± 6.7	1.11 ± 0.02
ALLO 50	1.6 ± 1.9	20.8 ± 0.3	67.0 ± 8.2	100.0 ± 4.3	1.20 ± 0.07
ALLO 250	−4.3 ± 2.6	23.0 ± 1.4†	78.0 ± 11.0	112.1 ± 3.6†	1.37 ± 0.05†
PRED 5	−2.5 ± 0.8	17.8 ± 0.5	80.0 ± 10.5	89.3 ± 9.9	1.09 ± 0.05
PRED 25	−8.1 ± 1.9†	18.7 ± 0.5	86.0 ± 16.0	105.5 ± 3.7	1.17 ± 0.07
Endotoxin					
+ saline	−26.0 ± 1.7†	22.2 ± 1.2†	122.7 ± 10.5†	92.7 ± 4.1	1.18 ± 0.04
+ SOD 5	−28.2 ± 2.2†	23.2 ± 1.1†	120.0 ± 11.5†	101.3 ± 2.2	1.34 ± 0.08
+ SOD 25	−35.0 ± 2.9$^{\dagger *}$	24.0 ± 0.8†	155.3 ± 15.2†	87.3 ± 5.8	1.38 ± 0.06†
+ ALLO 50	−22.4 ± 1.1†	23.5 ± 0.5†	164.0 ± 5.6$^{\dagger *}$	100.3 ± 3.3	1.26 ± 0.09
+ ALLO 250	−18.4 ± 3.8†	25.2 ± 0.7†	125.3 ± 13.9†	98.5 ± 3.8	1.46 ± 0.14
+ PRED 5	−24.9 ± 1.4†	22.7 ± 1.0†	168.7 ± 7.7$^{\dagger *}$	94.2 ± 3.8	1.25 ± 0.10
+ PRED 25	−25.3 ± 1.3†	21.1 ± 1.8	129.3 ± 6.2†	81.3 ± 8.2	1.37 ± 0.10

Data are means ± SE ($n = 4$–6). The doses stated are mg kg^{-1}. $^{\dagger}P$ at least < 0.05 versus control, saline rats. $^{*}P < 0.05$ versus endotoxin + saline rats.

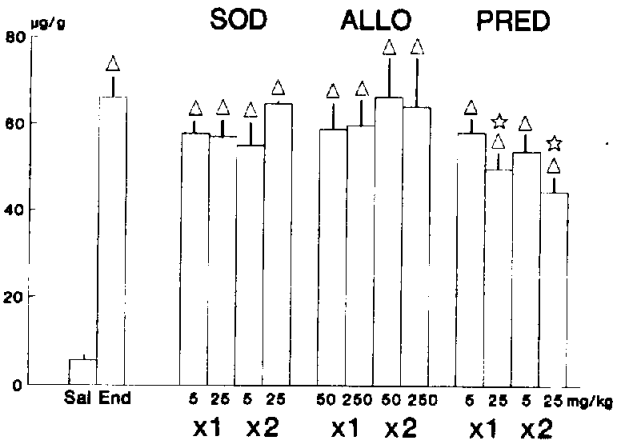

Figure 3. Effect of SOD, ALLO and PRED on endotoxin-induced changes of liver MT levels when administered 24 h after the endotoxin. The rats received one or two (8 h apart) doses and were killed 48 h after the endotoxin injection. Control rats received saline (Sal). Results are means ± SE (n = 4–6). $^{\triangle}P < 0.05$ versus saline rats. $^{*}P < 0.05$ versus endotoxin rats.

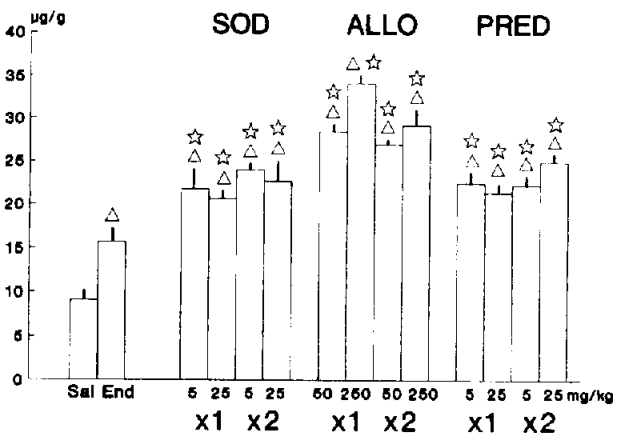

Figure 4. Effect of SOD, ALLO and PRED on endotoxin-induced changes of lung MT levels when administered 24 h after the endotoxin. The rats received one or two (8 h apart) doses and were killed 48 h after the endotoxin injection. Control rats received saline (Sal). Results are means ± SE (n = 4–6). $^{\triangle}P < 0.05$ versus saline rats. $^{*}P < 0.05$ versus endotoxin rats.

Endotoxin decreased body gain and increased liver cytosolic zinc, with no effect in general of the additional treatments. Liver and lung weights were not altered (not shown). Liver TBARs were increased in general by endotoxin, with no clear effects of either SOD, ALLO or PRED, whereas lung TBARs were not affected by any treatment. PRED increased serum TBARs when administered to endotoxin-treated rats in all cases, whereas SOD and ALLO did it only in some cases.

Discussion

As stated above, endotoxin causes the release of both cytokines and free radicals, both of which may be related to MT induction by this lipopolysaccharide. The contribution of the latter has not been previously studied.

Endotoxin increased liver MT and also liver TBAR levels, suggesting that liver MT induction by

Table 2. Effect of the administration of SOD, ALLO and PRED 24 h after the endotoxin injection

	Body weight gain (g 2 days^{-1})	Liver cytosolic zinc (μg g^{-1})	Liver TBARs (nmol g^{-1})	Lung TBARs (nmol g^{-1})	Serum TBARs (μM)
Control	10.1 ± 1.0	19.0 ± 0.4	68.7 ± 7.7	88.3 ± 10.0	1.01 ± 0.02
Endotoxin	−18.6 ± 2.5†	22.4 ± 1.1†	101.3 ± 6.7†	100.7 ± 4.3	0.97 ± 0.03
One dose					
SOD 5	−14.3 ± 6.0†	22.5 ± 0.7†	104.7 ± 19.7	90.5 ± 3.4	1.22 ± 0.09$^{\dagger *}$
SOD 25	−16.2 ± 1.7†	22.4 ± 0.8†	136.7 ± 23.1†	97.3 ± 3.7	1.00 ± 0.04
ALLO 50	−7.7 ± 8.6	22.7 ± 0.8†	148.2 ± 30.5†	96.8 ± 3.4	1.08 ± 0.05
ALLO 250	−33.9 ± 9.5†	23.1 ± 1.9†	162.7 ± 25.5†	94.2 ± 3.3	1.15 ± 0.08
PRED 5	−22.0 ± 2.3†	21.5 ± 1.1†	120.2 ± 14.7†	75.6 ± 11.5	1.17 ± 0.05$^{\dagger *}$
PRED 25	−20.1 ± 3.2†	21.5 ± 0.8†	138.7 ± 24.0†	80.6 ± 2.9	1.14 ± 0.05$^{\dagger *}$
Two doses					
SOD 5	−24.3 ± 2.1†	23.5 ± 0.7†	132.8 ± 19.9†	96.4 ± 2.8	1.07 ± 0.04
SOD 25	−21.8 ± 4.1†	22.7 ± 0.3†	91.3 ± 8.0	93.1 ± 3.4	1.05 ± 0.02
ALLO 50	−17.3 ± 3.5†	23.6 ± 1.5†	104.8 ± 21.3	93.6 ± 3.4	1.02 ± 0.04
ALLO 250	−27.4 ± 4.2†	25.2 ± 2.0†	121.5 ± 10.0†	87.3 ± 5.1	1.30 ± 0.03$^{\dagger *}$
PRED 5	−16.9 ± 3.2†	21.1 ± 0.7†	118.4 ± 26.6†	82.4 ± 6.3	1.24 ± 0.11$^{\dagger *}$
PRED 25	−28.1 ± 7.4†	20.1 ± 1.1	117.6 ± 19.4†	80.7 ± 6.6	1.25 ± 0.11$^{\dagger *}$

Data are means ± SE (n = 4–6). The doses stated are mg kg^{-1}. $^{\dagger}P$ at least < 0.05 versus control rats. $^{*}P < 0.05$ versus endotoxin rats.

endotoxin might be related to free radical processes. An increase of TBARs (an index of lipid peroxidation) levels is usually considered a reflection of increased free radical production (Aust 1985), although it must be acknowledged that, in tissues, TBA may measure more than lipid peroxidation (Janero & Burghardt 1989). It is now clear, however, that MT levels are not directly related to lipid peroxidation levels in the liver (Hidalgo *et al.* 1988a, 1990, Sato & Sasaki 1991, Min *et al.* 1992b), which is also clear in the present study.

The results found for SOD and ALLO were quite surprising. Both are well known antioxidants, SOD causing the dismutation of the superoxide radical, and ALLO causing the inhibition of xanthine oxidase and therefore decreasing superoxide and hydroxyl radical formation and scavenging the latter and myeloperoxidase-derived oxidant HOCL through oxypurinol formation (Halliwell & Gutteridge 1989). However, neither SOD nor ALLO decreased the effect of endotoxin on liver TBARs regardless of the pattern of treatment. Furthermore, ALLO potentiated it significantly in some cases, which does not seem to be due to different food intakes (Hidalgo *et al.* 1988a) as suggested by the body weight losses observed. The glucocorticoid PRED, similarly, did not reduce the effect of endotoxin on liver TBARS levels and even potentiated it in some cases. These hormones are potent antioxidants in a number of pathological processes (Baud & Ardaillou 1986, Hall *et al.* 1988), but their effects on liver TBARs in different situations remain conflicting (Hidalgo *et al.* 1991). Although we have no explanation for these unexpected effects of SOD, ALLO and PRED on liver TBARs, the conclusion is clear that liver MT and TBARs levels are not directly correlated. The results are even more evident for the lung, since lung TBARs were mostly unaffected by any of the treatments including endotoxin, whereas lung MT levels were clearly increased.

SOD and PRED tended to decrease the effect of endotoxin on liver MT levels when administered before endotoxin, although it was not statistically significant. ALLO, in contrast, further increased liver MT levels, likely through a different mechanism, since ALLO had a significant effect on its own in control rats. The results for PRED are in agreement with those of Min *et al.* (1992a), since they found a slight but significant effect in mice with the same dose of PRED; as might be expected, they also found that dexamethasone was more potent than PRED. Perhaps the effect of PRED was not statistically significant in our experiment because we administered PRED 30 min before the endotoxin, whereas Min *et al.* (1992a) injected it 6 h before. Also, the dose of endotoxin we used was twice that used by those authors. The present results extend those of Min *et al.* (1992a) because we found that PRED significantly reduced the effect of endotoxin on lung MT levels. Therefore, it seems that glucocorticoids, in spite of the fact that they are direct inducers of the MT gene (Mayo & Palmiter 1981), decrease the effect of endotoxin on tissue MT levels when administered previously to it, at least in the liver and lung. Min *et al.* (1992a) have proposed that this effect is caused by the glucocorticoid-induced inhibition of cytokines release, since cytokines are well-known MT inducers (DiSilvestro & Cousins 1984, Cousins & Leinart 1988, De *et al.* 1990, Schroeder & Cousins 1990). Given the absence of any effect of the antioxidants SOD and ALLO (and even the additive effect of ALLO with endotoxin), that hypothesis is reasonable.

The effect of PRED is not limited to administration of the hormone previous to the endotoxin injection. In rats treated with PRED 24 h after the endotoxin, a significant decrease was also observed in liver MT levels, indicating that even well after the processes engaged by endotoxin have started (presumably cytokines release and action), glucocorticoids are still effective in decreasing their effects on liver MT levels. However, the situation is completely different for lung MT. Glucocorticoids, but also the antioxidants SOD and ALLO, not only did not decrease the effect of endotoxin when administered 24 h after the lipopolysaccharide, but potentiated it. Whereas this effect may be explained in the case of SOD and ALLO because they had a significant effect on lung MT levels on their own, and therefore producing an additive effect, no explanation is obvious for glucocorticoids.

In sum, the present results suggest that although it is clear that cytokines induced tissue MT synthesis in many tissues, including the liver and the lung (De *et al.* 1990), it is also clear that other factors such as glucocorticoids may modulate, positively or negatively depending on the tissue, the MT regulation. Further work is clearly needed to establish the mechanism(s) through which these effects are taking place.

Acknowledgments

These studies were supported by a grant of the Fondo de Investigaciones Sanitarias de la Seguridad Social (90/0065-2-D)

References

Aust SD. 1985 Lipid peroxidation. In: Greenwald RA, ed. *Handbook of Methods for Oxygen Radical Research*. Boca Raton: CRC Press.

Bakka A, Johnsen AS, Endresen L, Rugstad HE. 1982 Radioresistance in cells with high content of metallothionein. *Experientia* **38**, 381–383.

Baud L, Ardaillou R. 1986 Reactive oxygen species: production and role in the kidney. *Am J Physiol* **251**, F765–F776.

Bauman JW, Liu J, Liu YP, Klaassen CD. 1991 Increase in metallothionein produced by chemicals that induce oxidative stress. *Toxicol Appl Pharmacol* **110**, 347–354.

Brigham KL, Meyrick B. 1986 Endotoxin and lung injury. *Am Rev Resp Dis* **133**, 913–927.

Cousins RJ, Leinart AS. 1988 Tissue-specific regulation of zinc metabolism and metallothionein genes by interleukin 1. *FASEB J* **2**, 2884–2890.

De SK, McMaster MT, Andrews GK. 1990 Endotoxin induction of murine metallothionein gene expression. *J Biol Chem* **265**, 15267–15274.

DiSilvestro RA, Cousins RJ. 1984 Mediation of endotoxin-induced changes in zinc metabolism in rats. *Am J Physiol* **247**, E436–E441.

Durnam DM, Hoffman JS, Quaife CJ, *et al*. 1984 Induction of mouse metallothionein-I mRNA by bacterial endotoxin is independent of metals and glucocorticoid hormones. *Proc Natl Acad Sci USA* **81**, 1053–1056.

Hall ED, Braughler JM, McCall JM. 1988 New pharmacological treatment of acute spinal cord trauma. *J Neurotrauma* **5**, 81–89.

Halliwell B, Gutteridge JMC. 1989 *Free Radicals in Biology and Medicine*. Oxford: Clarendon Press.

Hammond B, Kontos HA, Hess ML. 1985 Oxygen radicals in the adult respiratory distress syndrome, in myocardial ischemia and reperfusion injury, and in cerebral vascular damage. *Can J Physiol Pharmacol* **63**, 173–187.

Hidalgo J, Campmany L, Borras M, Garvey JS. 1988a Metallothionein response to stress in rats: role in free radical scavenging. *Am J Physiol.* **255**, E518–E524.

Hidalgo J, Giralt M, Garvey JS, Armario A. 1988b Physiological role of glucocorticoids on rat serum and liver metallothionein in basal and stress conditions. *Am J Physiol* **254**, E71–E78.

Hidalgo J, Garvey JS, Armario A. 1990 On the metallothionein, glutathione and cysteine relationship in rat liver. *J Pharmacol Exp Ther* **255**, 554–564.

Hidalgo J, Gasull T, Garcia A, Blánquez A, Armario A. 1991 Role of glucocorticoids and catecholamines on hepatic thiobarbituric acid reactants in basal and stress conditions in the rat. *Horm Metab Res* **23**, 104–109.

Janero DR, Burghardt B. 1989 Thiobarbituric acid-reactive malondialdehyde formation during superoxide-dependent, iron-catalyzed lipid peroxidation: influence of peroxidation conditions. *Lipids* **24**, 125–131.

Kägi JHR, Kojima Y. 1987 *Metallothionein*. Basel: Birkhäuser Verlag.

Liu J, Liu YP, Sendelbach LE, Klaassen CD. 1991 Endotoxin induction of hepatic metallothionein is mediated through cytokines. *Toxicol Appl Pharmacol* **109**, 235–240.

Maitani T, Saito Y, Fujimaki H, Suzuki KT. 1986 Comparative induction of hepatic zinc-thionein and increase in tissue calcium by bacterial endotoxin in endotoxin-sensitive (C3H/HeN) and endotoxin-resistant (C3H/HeJ) mice. *Toxicol Lett* **30**, 181–187.

Martins EAL, Chubatsu LS, Meneghini R. 1991 Role of antioxidants in protecting cellular DNA from damage by oxidative stress. *Mutat Res* **250**, 95–101.

Mayo KE, Palmiter RD. 1981 Glucocorticoid regulation of metallothionein-I mRNA synthesis in cultured mouse cells. *J Biol Chem* **256**, 2621–2624.

Min K-S, Mukai S, Ohta M, Onosaka S, Tanaka K. 1992a Glucocorticoid inhibition of inflammation-induced metallothionein synthesis in mouse liver. *Toxicol Appl Pharmacol* **113**, 293–298.

Min K-S, Terano Y, Onosaka S, Tanaka K. 1992b Induction of metallothionein synthesis by menadione or carbon tetrachloride is independent of free radical production. *Toxicol Appl Pharmacol* **113**, 74–79.

Sato M, Sasaki M. 1991 Enhanced lipid peroxidation is not necessary for induction of metallothionein-I by oxidative stress. *Chem-Biol Interact* **78**, 143–154.

Schroeder JJ, Cousins RJ. 1990 Interleukin 6 regulates metallothionein gene expression and zinc metabolism in hepatocyte monolayer cultures. *Proc Natl Acad Sci USA* **87**, 3137–3141.

Shiraishi N, Aono K, Utsumi K. 1983 Increased metallothionein content in rat liver induced by X irradiation and exposure to high oxygen tension. *Radiat Res* **95**, 298–302.

Suzuki KT, Yamamura M. 1980 Induction of hepatic zinc-thionein in rat by endotoxin. *Biochem Pharmacol* **29**, 2260.

Sobocinski PZ, Canterbury WJ. 1982 Hepatic metallothionein induction by inflammation. *Ann NY Acad Sci* **389**, 354–367.

Sobocinski PZ, Canterbury WJ, Mapes CA, Dinterman RE. 1978 Involvement of hepatic metallothioneins in hypozincemia associated with bacterial infection. *Am J Physiol* **234**, E399–E406.

Thomas JP, Bachowski GJ, Girotti AW. 1986 Inhibition of cell membrane lipid peroxidation by cadmium- and zinc-metallothioneins. *Biochim Biophys Acta* **884**, 448–461.

Thornalley, PJ, Vašák M. 1985 Possible role for metallothionein in protection against radiation induced stress. Kinetics and mechanism of its reaction with superoxide and hydroxyl radicals. *Biochim Biophys Acta* **827**, 36–44.

Uchiyama M, Mihara M. 1978 Determination of malonaldehyde precursor in tissues by thiobarbituric acid test. *Anal Biochem* **86**, 271–278.

Yagi K. 1984 Assay for blood plasma or serum. *Methods Enzymol* **105**, 328–341.