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Effect of Endotoxin on Rat Serum, Lung and Liver Lipid Peroxidation and on Tissue Metallothionein Levels*

M. Giralt, T. Gasull, A. Blánquez and J. Hidalgo**

Departamento de Biología Celular y Fisiología
Unidad de Fisiología Animal, Facultad de Ciencias
Universidad Autónoma de Barcelona
08193 Bellaterra/Barcelona (Spain)

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The effect of endotoxin on serum and lung and liver lipid peroxidation, as measured by thiobarbituric acid reactants (TBARs), as well as on lung and liver metallothionein (MT) has been studied in the rat. Endotoxin consistently increased serum and liver TBARs in a time-response manner. The increase in the serum preceded that in the liver, with peaks 3-6 h and 24 h after endotoxin administration, respectively. In contrast, lung TBARs levels did not increase regardless of the experimental approaches studied, suggesting that the rat is not a good model for the adult respiratory distress syndrome. Endotoxin increased both lung and liver MT levels in a time-response manner, although to a lesser degree in the former than in the latter tissue, indicating that this protein may have a significant role in the response of the organism to a septic insult.

Key words: Adult respiratory distress syndrome, Endotoxin, Metallothionein, Thiobarbituric acid reactants.

Gram-negative sepsis is the most common setting responsible for the development of the adult respiratory distress syndrome (ARDS) (11). Lipopolysaccharide components of gram negative bacteria, the so-called endotoxins, are presumably the bacterial components causing injury to the lungs as well as to other organs (1). Tissue injury caused by endotoxin probably is mediated at least in part by the excessive production of oxygen free radicals by inflammatory cells, especially neutrophils

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** To whom correspondence should be addressed. (Phone: 3-581 20 37; Fax: 3-581 20 03.)

(1, 5). In the sheep, as a result of the endotoxin-induced free radicals production, increased lung lipid peroxidation levels are observed as measured by thiobarbituric acid reactants (TBARs) (13). Increased arterial TBARs after endotoxin has also been reported in sheep (3), rabbits and mice (9). In rat, the liver rather than the lung appears to be the organ with a significant increase in lipid peroxidation after a septic insult (16).

On the other hand, it is well-known that endotoxin increases liver metallothionein (MT) levels in the liver (4,14, 15). Lung MT mRNA levels are also increased by endotoxin but to a much lower extent than in the liver (2). This MT induction by endotoxin, likely mediated by several cytokines (2, 10), may reflect the putative antioxidant function of this protein (6).

The aim of this report was to further characterize the effect of endotoxin on serum, lung and liver TBARs and on tissue MT levels in rat.

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.

Materials. — Endotoxin (lipopolysaccharide from *E. coli* 0127:B8) and Tris were purchased from Sigma. Cadmium, mercaptoethanol, and malondialdehyde were purchased from Merck, and thiobarbituric acid and sodium azide from Fluka AG (Buchs, Switzerland). The other reagents were of analytical grade. Laboratory food was from Panlab (Barcelona, Spain).

Experiment 1: Rats were injected with endotoxin (0.5, 1, 2 or 5 mg/kg, i.p.) and killed 48 h later. Control rats received saline and were killed at the same time. Food consumption and body weight gain were measured daily.

Experiment 2: Rats were injected with endotoxin (3 mg/kg, i.p.) and killed 3, 6, 10, 24 or 48 h later. Control rats received saline and were killed at the same time as endotoxin-treated rats.

Experiment 3: Rats were injected with endotoxin (5 mg/kg) intravenously, intraperitoneally or subcutaneously, and were killed 24 h later. Control rats received saline i.v.

Experiment 4: Rats were injected with endotoxin (0.5 mg/kg, i.v.) once (at 9.00 h) or twice (at 9.00 and 10.30 h), and were killed 3 hours after the first injection. Control rats received saline.

Experiment 5: Rats were injected with endotoxin (10 mg/kg, i.v.) once (at 9.00 h) and killed 1, 3 or 8 hours later, or twice (at 9 and 12 h) and killed 8 hours after the first injection. Control rats received saline.

Assays. — Rats were sacrificed 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 (7). Lipid peroxidation in the tissues were assessed by measuring malondialdehyde formation, using the thiobarbituric acid (TBA) assay (19). Lipid peroxidation in the serum was assessed by fluorescence as described by YAGI (20). MT levels were measured with a cadmium-saturation method (7). Cadmium was measured by atomic absorption spectrophotometry.

Statistical analysis. — When two means were compared, the Student's *t* test or the Mann-Whitney's «U» test were used. When more than two means were com-

pared, one- or two-way ANOVA were used. Individual comparisons between means were made by means of the Duncan's test.

Results

Experiment 1. — Figure 1 shows lung and liver lipid peroxidation levels, as thiobarbituric acid reactants (TBARs), as well as MT levels. One-way ANOVA revealed that liver but not lung TBARs were significantly increased by endotoxin ($p < 0.001$). In contrast, both lung and liver MT levels were increased by endotoxin (p at least <0.01).

Serum TBARs levels were not affected by endotoxin at any dose studied (1.42 ± 0.16, 1.22 ± 0.08, 1.24 ± 0.11, 1.33 ± 0.18 and 1.35 ± 0.20, mean±SE for control and 0.5, 1, 2 and 5 mg/kg endotoxin, respectively).

Experiment 2. — Figure 2 shows lung, liver and serum TBARs levels. Two-way ANOVA revealed that liver and serum, but not lung, TBARs were significantly increased by endotoxin ($p < 0.001$).

Two-way ANOVA revealed that both lung and liver MT levels were significantly increased ($p < 0.001$) by endotoxin (fig. 3).

Experiment 3. — Table I shows serum and lung and liver TBARs as well as liver MT levels. One-way ANOVA indicated that lung TBARs were not modified by endotoxin regardless of the route of administration. In contrast, liver TBARs were significantly increased ($p < 0.001$). The Duncan procedure indicated that endotoxin administered i.v. further increased liver TBARs compared to the i.p. and s.c. routes. Serum TBARs were also increased by endotoxin ($p < 0.01$), but Duncan procedure revealed that only the

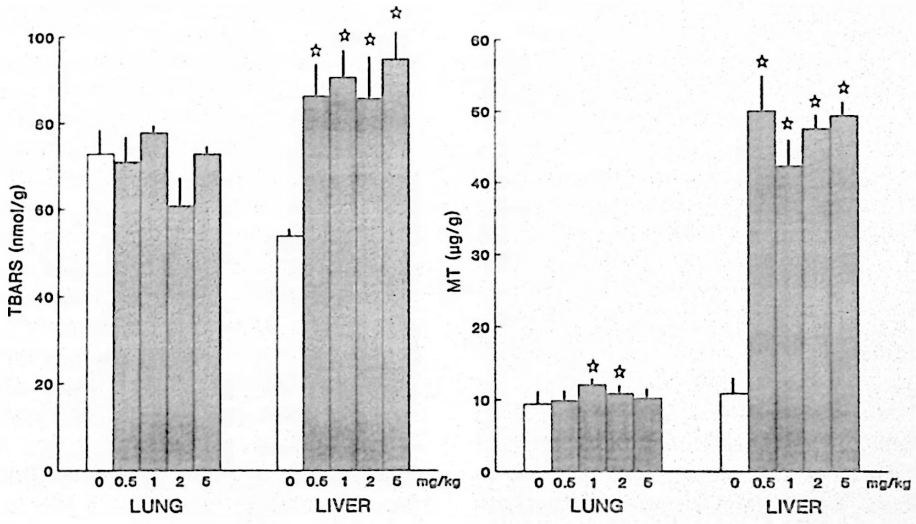

Fig. 1. *Effect of endotoxin on lung and liver lipid peroxidation measured as thiobarbituric acid reactants (TBARs) and on metallothionein (MT) levels.*
Rats were injected i.p. with endotoxin (0.5, 1, 2 or 5 mg/kg) and killed 48 h later. Results are mean ± SE (n = 8–9). The differences between the means (Duncan procedure) are shown. *$p < 0.05$ vs saline rats.

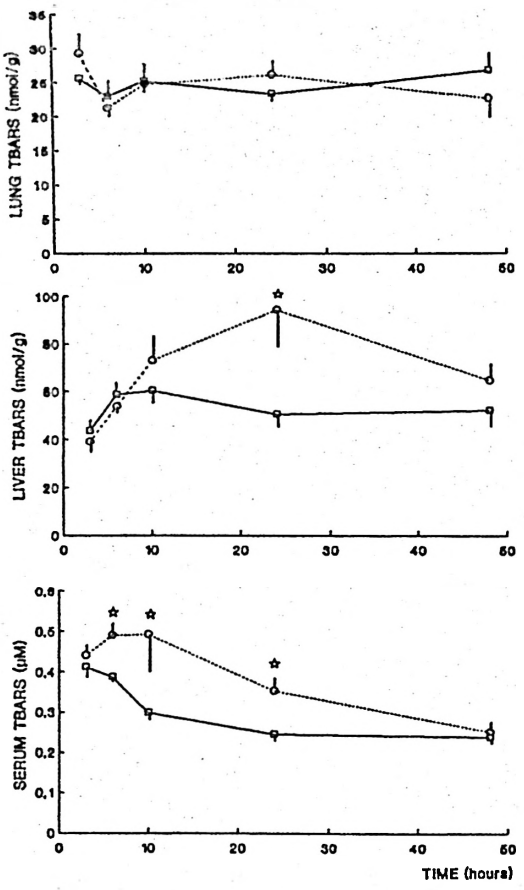

Fig. 2. *Effect of endotoxin on lung, liver and serum TBARs levels.*
Rats were injected with endotoxin (3 mg/kg) and killed at the indicated times. Results are means ± SE (n = 3–6). Squares, saline rats. Circles, endotoxin rats. The differences between the means *t* test are shown. *p < 0.05 vs saline rats.

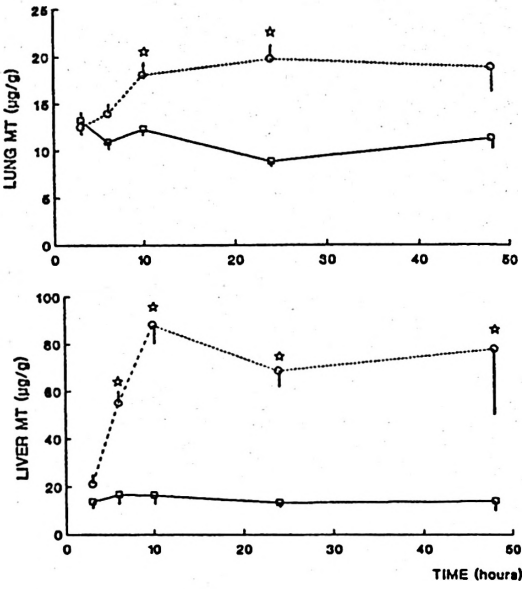

Fig. 3. *Effect of endotoxin on lung and liver MT levels.*
Legend as in figure 2.

i.v. route (as measured 24 h after the administration of endotoxin) had a significant effect. The i.p. rats had intermediate levels. Liver MT levels were similarly increased by endotoxin by the three routes of administration ($p < 0.001$).

Experiments 4 and 5. — Serum and lung and liver TBARs were not significantly modified by endotoxin in the experimental conditions described for experiment 4 (data not shown). Experiment 5 was performed as a final attempt to increase lung TBARs. Three rats out of 6 receiving a single dose of endotoxin (10 mg/kg, i.v.) died before arriving at their sacrifice schedule (8 hours), whereas 4 out of 6 receiving 2 doses died, indicating that this was a very toxic dose. However, neither lung nor liver TBARs were significantly increased by endotoxin (data not shown). In agreement with Exp. 2, serum TBARs were significantly increased ($p < 0.05$) 8 hours after the endotoxin administration (not shown).

Discussion

The administration of endotoxin is considered a model of ARDS in species such

Table I. *Effect of different routes of administration of endotoxin on TBARs and MT levels*
Rats were injected with endotoxin (5 mg/kg) and killed 24 h later. Results are means ± SE (n = 8–11).

	TBARs			Liver MT
	Lung (nmol/g)	Liver (nmol/g)	Serum (μM)	(μg/g)
Control	58.2 ± 5.4	36.5 ± 1.7	2.74 ± 0.21	3.3 ± 0.37
Intravenous	59.1 ± 5.0	63.4 ± 4.8[a,b]	3.52 ± 0.22[a]	31.4 ± 2.70[a]
Intraperitoneal	50.4 ± 5.9	47.5 ± 3.6[a]	3.21 ± 0.21	26.8 ± 4.92[a]
Subcutaneous	59.2 ± 6.7	44.0 ± 3.1[a]	2.84 ± 0.06	36.6 ± 3.89[a]

[a] $p < 0.05$ vs control rats. [b] $p < 0.05$ vs the other groups.

as the sheep (1, 11). In the present report it was intended to gain insight on the putative use of the rat for ARDS studies. Thus, serum, lung and liver lipid peroxidation levels, measured as thiobarbituric acid reactants (TBARs), have been determined in several experiments. Several conclusions arise from them.

In the first place, lung TBARs were never increased by endotoxin regardless of the schedule of treatment. Even in Exp. 5, where several rats died, lung TBARs were not modified. This is in contrast with the results for sheep, where endotoxin significantly increases lung TBARs (13). In the second place, liver TBARs were significantly increased by endotoxin after a period of about 10 hours, reached a peak at 24 h and tended to return to normal levels at 48 h after the administration of endotoxin. Therefore, several hours are needed to produce lipid peroxidation in the liver, which suggests that it is caused by the inflammatory response induced by endotoxin (1, 5). It is also noteworthy that although all the routes of administration (i.v., i.p. and s.c.) had a significant effect, the i.v. route had the highest effect. These results are in agreement with a previous report that indicated that the liver rather than the lung is the organ with significant lipid peroxidation after a septic insult in the rat (16). Finally, serum TBARs levels were significantly increased by endotoxin in a time-response manner, which was different from that observed in the liver, i.e., they were increased as early as 6 h after the endotoxin administration, returning to control levels 48 h later. The source of serum TBARs is unclear. It has been suggested that they come from the lung in sheep after measuring TBARs in arterial and venous blood (3, 13). However, in the present report this might seem unlikely, since lung TBARs did not increase after endotoxin. It remains possible, however, that increased lipid peroxidation levels in the lung cannot be detected in the rat if, for example, high levels of unspecific (related to lipid peroxidation) molecules such as those derived from arachidonic acid are present (13), which deserves further attention. On the other hand, it also remains possible that serum TBARs come from the liver, since in this organ TBARs levels were clearly increased by endotoxin, although the fact that the serum increase precedes that of the liver is conflicting. However, there may be a releasable pool of TBARs in the liver somewhat separated from the total TBARs levels. The finding that the i.v. administration of endotoxin increased both serum and liver TBARs to a greater extent than the other routes support a relationship between them.

Regarding metallothionein (MT), the results are in very good agreement with the published literature. Thus, liver MT was clearly increased by endotoxin in a temporal manner comparable to previous

results (12). Lung MT levels were also increased by endotoxin although to a lower extent than liver MT, which is in agreement with the MT mRNA data (2). Thus, MT levels are increased by endotoxin in both tissues following a similar temporal pattern. This induction is likely due to the interleukins released by the inflammatory cells activated by endotoxin (1, 2). The function of this protein is unknown, but it may be related to Zn and/or Cu redistribution during the inflammatory response or to free radical scavenging events (6, 8, 17, 18). The putative importance of this protein in the treatment of the ARDS merits attention.

Resumen

Se estudia el efecto de la endotoxina sobre la peroxidación lipídica de suero, pulmón e hígado, medida como reactantes con el ácido tiobarbitúrico (TBARS), así como sobre la metalotioneína (MT) pulmonar y hepática. La endotoxina incrementa claramente los TBARs séricos y hepáticos, siendo el incremento en el suero anterior al del hígado, que presentan picos a las 3-6 h y a las 24 h después de la administración de endotoxina, respectivamente. En cambio, los TBARs pulmonares no se incrementan en ninguna de las situaciones experimentales estudiadas, sugiriendo que la rata no es un buen modelo experimental para el estudio del distrés respiratorio del adulto. La endotoxina provoca aumento de la MT pulmonar y sobre todo de la hepática, indicando que esta proteína puede tener un papel significativo en la respuesta del organismo a la sepsis.

Palabras clave: Síndrome del distrés respiratorio del adulto, Endotoxina, Metalotioneína, Reactantes con el ácido tiobarbitúrico.

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