Biochemical Pharmacology, Vol. 27, pp. 531–533. Pergamon Press, 1978. Printed in Great Britain.

ADJUVANT-INDUCED AND CARRAGEENIN-INDUCED INFLAMMATION AND LIPID PEROXIDATION IN RAT LIVER, SPLEEN AND LUNGS

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(*Received* 4 *May* 1977; *accepted* 29 *July* 1977)

Abstract—Lipid peroxidation in homogenates of spleen, liver and lungs of rats was tested by the thiobarbituric acid method in the presence or in the absence of exogenous ferrous ions and ascorbic acid. A mild increase of stimulated lipid peroxidation was observed in liver of rats with carrageenin hind paw edema only 3 hr after the injection. In the Freund's adjuvant treated rats a dramatic suppresion of liver but not spleen and lungs lipid peroxidation was found at days 1–21 after the injection. This phenomenon was not correlated with the observed splenohepatomegaly. It is probably the result of the damage of an enzymic or non-enzymic mechanism which induces lipid peroxidation in the liver.

Enzymic peroxidation of lipids by animal lipoxygenases and by microsomal dioxygenase in prostaglandin synthetase system [1] is supplemented by co-oxygenation of lipids in various enzymic systems [2–5] as well as by non-enzymic autooxidation of lipids which is vigorously stimulated by ferrous ions and ascorbic acid [4, 5]. An increased lipid peroxidation occurs during intoxication with ethanol and carbon tetrachloride [6], after exposure to ionizing irradiation [7] and as the results of aging [8] and acute inflammation [9]. The thiobarbituric acid test [10] is most frequently used to assay lipid peroxidation, though that it cannot differentiate between various types of lipid peroxides and it cannot indicate the source of their origin. Here we report the influence of carrageenin edema and adjuvant arthritis on the lipid peroxidation in some rat tissues.

MATERIALS AND METHODS

Animals. Wistar rats (about 2 months old) both sexes weighing 150–200 g were killed by a blow on the neck. Livers, spleen and lungs were quickly removed and placed in a dry container on ice.

Carrageenin edema. Carrageenin edema of hind paw in rats was produced by the method of Winter *et al.* [11]. Animals were killed 3 or 24 hr after carrageenin injection.

Adjuvant arthritis. This was produced in rats by the method of Sofia *et al.* [12]. Animals were killed at day 1, 5, 10 or 21 after Freund's adjuvant injection. Rats which did not develop generalized arthritis at day 21 after Mycobacterium injection were not taken to lipid peroxide estimation.

Lipid peroxides. These were estimated by the Utley's procedure [10]. Tissue samples were homogenized at 4° in 0.067 M phosphate buffer pH 7.4 and made up to the final concentration of 60 mg wet weight per ml (spleen and lungs) or 30 mg of wet weight per ml (liver). The homogenates (2 ml) in the presence or in the absence of ferrous sulfate (30 μM) and ascorbic acid (850 μM) were incubated with shaking at 37° for 90 min and then 1 ml of 20% (w/v) trichloracetic acid was added. After the centrifugation at 20,000 *g* for 10 min, the supernatant was separated, 1 ml of 0.67% sodium thiobarbiturate solution was added and the mixture was heated in boiling water for 10 min. The developed colour was read using a Spekol colorimeter at 530 nm against a sample treated with trichloracetic acid before the incubation. The amount (nmoles) of malondialdehyde formed was quantified from the standard graph drawn for malondialdehyde tetraethyl acetal and calculated for 1 g of tissue.

Total lipid content in tissues. Lipids were extracted with a mixture of chloroform and methanol [13] separated and weighed. The lipid content in tissues was expressed in mg of lipid extract per 1 g of tissue.

Reagents. Malondialdehyde tetraethyl acetal was obtained from K and K Laboratories Inc., Plainview, U.S.A., Carrageenin from Marine Colloids.

RESULTS

Carrageenin edema. In control rats native lipid peroxidation was the highest in liver, considerably lower in the spleen and very low in the lungs. The formation of malondialdehyde in lungs (3-fold increase) and in the liver (22-fold increase) but not in the spleen was stimulated by ferrous ions and ascorbic acid. Neither spontaneous nor stimulated lipid peroxidation was essentially influenced by the carrageenin-induced inflammation. A moderate increase of lipid peroxidation (by 34 per cent) was observed only in the liver 3 hrs after the carrageenin injection (Table 1).

Adjuvant arthritis. The injection of Freund's adjuvant into rats was associated with a dramatic suppresion in liver lipid peroxidation. This effect was seen as soon as 1 day after the injection of the Freund's adjuvant, however the peak effect was obtained only at day 10 after the injection. Then the non-stimulated lipid peroxidation was suppresed by 80 per cent (Table 2), while the stimulated liver lipid peroxidation was depressed only by 50 per cent (Table 3). Vari-

Table 1. Lipid peroxidation in organ homogenates of rats with carrageenin paw edema

Organ	Presence of ascorbic acid 850 μM and Fe^{2+} 30 μM	Control	3 hr edema	24 hr edema
		nmoles of malondialdehyde/1 g of tissue		
Liver	0	94 ± 6 $n = 19$	100 ± 19 $n = 4$	43 ± 24 $n = 3$
Spleen	0	25 ± 4 $n = 12$	32 ± 4 $n = 4$	23 ± 3 $n = 3$
Lungs	0	12 ± 2 $n = 4$	12 ± 3 $n = 4$	12 ± 3 $n = 3$
Liver	+	2089 ± 146 $n = 26$	2805 ± 123* $n = 4$	2184 ± 246 $n = 5$
Spleen	+	36 ± 4 $n = 13$	45 ± 4 $n = 4$	35 ± 2 $n = 5$
Lungs	+	38 ± 4 $n = 5$	33 ± 3 $n = 4$	36 ± 8 $n = 5$

The composition of the incubation mixture is described in the Methods. The results were analyzed using Student's t-test.
* $P < 0.001$.

Table 2. Spontaneous lipid peroxidation in internal organs of adjuvant-treated rats

Time after mycobacterium injection (days)	Liver	Spleen	Lungs
	nmoles of malondialdehyde/ 1 g of tissue		
0	94 ± 6 $n = 19$	25 ± 4 $n = 12$	12 ± 2 $n = 4$
1	32 ± 11* $n = 4$	20 ± 1 $n = 4$	NT
5	40 ± 7* $n = 4$	12 ± 2† $n = 4$	NT
10	11 ± 2* $n = 4$	21 ± 1 $n = 4$	NT
21	40 ± 9* $n = 11$	30 ± 1 $n = 8$	16 ± 2 $n = 4$

The composition of the incubation mixture was as described in the Methods. The results were analyzed using Student's t-test.
* $P < 0.001$.
† $0.01 > P > 0.001$.
NT: not tested.

Table 3. Ferrous ions and ascorbic acid-stimulated lipid peroxidation in internal organs of adjuvant treated rats

Time after mycobacterium injection (days)	Liver	Spleen	Lungs
	nmoles of malondialdehyde/ 1 g of tissue		
0	2089 ± 146 $n = 26$	36 ± 4 $n = 13$	38 ± 4 $n = 5$
1	1662 ± 77‡ $n = 4$	28 ± 2 $n = 4$	NT
5	1531 ± 170‡ $n = 4$	22 ± 0† $n = 4$	NT
10	905 ± 88* $n = 4$	29 ± 2 $n = 4$	NT
21	1480 ± 116† $n = 10$	24 ± 4 $n = 8$	27 ± 2 $n = 6$

The composition of the incubation mixture was as described in the Methods. The results were analyzed using Student's t-test.
* $P < 0.001$.
† $0.01 > P > 0.001$.
‡ $0.01 < P < 0.05$.
NT: not tested.

Table 4. The weight and lipid content of liver and spleen of rats treated with Freund's adjuvant

Time after mycobacterium treatment (days)	Weight (g)		Lipid content mg/1 g of tissue	
	Liver	Spleen	Liver	Spleen
0	11.05 ± 0.48 $n = 24$	0.8 ± 0.08 $n = 24$	34.22 ± 1.61 $n = 3$	19.27 ± 2.11 $n = 3$
1	*8.44 ± 0.37 $n = 4$	†0.53 ± 0.064 $n = 4$	NT	NT
5	*8.31 ± 0.88 $n = 4$	1.01 ± 0.113 $n = 4$	NT	NT
10	10.88 ± 0.67 $n = 4$	0.92 ± 0.078 $n = 4$	NT	NT
21	*13.58 ± 0.58 $n = 17$	*1.75 ± 0.217 $n = 17$	39.67 ± 3.3 $n = 3$	22.00 ± 2.36 $n = 3$

Lipid content was estimated by extraction with methanol and chloroform. The results were analyzed using Student's t-test.
* $P < 0.001$.
† $0.05 > P > 0.01$.
NT: Not tested.

ations in the liver weight and lipid content during the experimental period (Table 4) could not contribute to the effect of adjuvant arthritis on lipid peroxidation. A much less pronounced decrease in the spleen lipid peroxidation occurred 5 days after the injection of the Freund's adjuvant.

DISCUSSION

Sharma *et al.* [9] have reported an increase in hepatic lipid peroxidation of mice 3 hr after the animals were injected with carrageenin. We observed the same phenomenon in rats, however this effect was rather mild and reached the border of statistical significance only after the stimulation of lipid peroxidation by the Fe^{2+} and ascorbic acid (Table 1).

The most unexpected finding was that in rats injected with Freund's adjuvant the peroxidation of hepatic and splenic lipids is dramatically reduced. This is a true phenomenon, which does not depend on the variations in weight and lipid content in these organs during the experimental period (Table 4). It may be that the suppression of lipid peroxidation in the adjuvant treated rats is caused by a damage of the ascorbic acid-Fe^{2+} dependent mechanism which is responsible for the lipid peroxidation in the liver. Indeed this suppression of liver lipid peroxidation resulting from the injection of Freund's adjuvant can be partially reversed by ferrous ions and ascorbic acid (Tables 2 and 3).

Another possibility is that a decline of the level of hepatic cytochrome P 450, which has been recently reported to occur in the adjuvant-inoculed arthritis in rats [14], is responsible for a decreased rate of co-oxygenation of lipids in the liver, since this cytochrome is involved in NADPH dependent lipid peroxidation [3, 15].

Since the suppression of lipid peroxidation occurs as early as one day after the injection of Freund's adjuvant we can hardly suggest the immunological background of this phenomenon. The fact that changes in lipid metabolism precede the development of generalized arthritis allows to speculate on the contribution of these changes in etiopathogenesis of arthritis.

Looking for another explanation for a decrease in amount of hepatic malondialdehyde available to the thiobarbituric acid reagent we have thought about the possibility of an increased removal of lipid peroxides from liver into blood of the adjuvant treated rats. There are some indirect evidence that the level of serum lipid peroxides is elevated in adjuvant treated rats. Serum of these animals has a decreased ability to inhibit the Triton X-100 induced lysis of rabbit polymorphonuclear leucocyte granules [16], whereas peroxides labilize cell membranes [8]. In rats with adjuvant arthritis experimental artherosclerosis develops fluminantly [17], and it is known, that an increase of lipid peroxidation is associated with aging [8]. Moreover lipid peroxides were powerful inhibitors of the enzyme from arterial walls that generates PGX (PGI_2, prostacyclin), a vascular hormone with antiaggregatory and vasodilatatory properties, which possibly prevents the development of atherosclerosis [18]. To verify our hypothesis it is necessery to evaluate directly the content of lipid peroxides in serum of the adjuvant-treated rats.

It is interesting that a correlation exists between inhibition of lipid peroxidation in the liver during adjuvant arthritis and the decrease of drug metabolism in the course of this inflammation [19]. Both phenomena may have the same biochemical background.

Acknowledgements—I would like to express my gratitude to Professor Bekemeier from Halle (GDR) for a gift of Mycobacterium. I thank also Mrs. J. Reyman for skilled technical assistance.

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