Increased lipid peroxidation and decreased hepatic aminopyrine metabolism during carrageenin-induced granulomatous inflammation in the rat

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Hepatic drug metabolism is decreased during adjuvant arthritis in the rat (Beck & Whitehouse, 1973). We observed increased levels of the stable lipid peroxidation product malondialdehyde (MDA) in plasma and liver of rats with kaolin granuloma pouch inflammation (Bragt, Schenkelaars & Bonta, 1979). We have now investigated both hepatic aminopyrine demethylation and lipid peroxidation during carrageenin-induced granuloma in the rat. Wistar rats weighing 200–250 g were used. Lipid peroxidation was estimated by determining MDA in plasma and livers from rats with carrageenin-induced (10 mg) granulomata growing around subcutaneously implanted teflon chambers (Bragt, Bonta & Adolfs, 1979). Drug metabolism was measured *in vivo* by the expiration of $^{14}CO_2$ after the i.p. injection (120 mg/kg, 0.4 μCi/kg) of [^{14}C]-aminopyrine (Radiochemical Centre, Amersham), as described by Lauterburg & Bircher (1976), in rats which had either polyether sponge implants, carrageenin-soaked (10 mg/sponge) sponge implants (Bonta, Adolfs & Parnham, 1979), or which were sham-operated.

The injection of carrageenin resulted in a significant rise in the MDA concentrations in plasma and liver after 6 h (340% and 64%, respectively, $P < 0.01$ vs day 0, Mann-Whitney U test) a change which was still present after 7 days (700% and 54% respectively, $P < 0.01$). The increase in liver MDA was associated with a decrease in hepatic reduced glutathione (GSH) content (~40%) throughout the entire period of investigation (data not shown). The plasma MDA concentration showed a correlation with the severity of inflammation expressed as wet granuloma weight ($r_s = 0.53$, $n = 25$, $P < 0.01$ Spearman rank test). Five days after operation (i.e. peak of granuloma formation), the half-life ($T_{\frac{1}{2}}$) of aminopyrine was longer in rats with a stronger inflammatory response (Table 1); the $T_{\frac{1}{2}}$ correlated significantly with the amount of granuloma formed ($r_s = 0.76$, $n = 18$, $P < 0.01$). The extended $T_{\frac{1}{2}}$ during inflammation might be due to the reduced hepatic levels of GSH, since the binding of demethylated aminopyrine metabolites would also be reduced. Consequently, these metabolites would compete with the aminopyrine for the active site of cytochrome P-450. We, thus, injected GSH (400 mg/kg i.v.) into rats implanted with carrageenin-soaked sponges, 45 min prior to the administration of aminopyrine. The $T_{\frac{1}{2}}$ was not altered (Table 1). Therefore, GSH depletion *per se* may not be responsible for the decreased drug metabolism during inflammation.

As the two sets of experiments indicate that increased lipid peroxidation and a reduction in drug metabolism occur in parallel during carrageenin-induced granuloma, it is not inconceivable that the two phenomena are interrelated.

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Table 1 Extension of the *in vivo* half-life ($T_{1/2}$) of [^{14}C]-aminopyrine in rats 5 days after subdermal sponge implantation

	Granuloma formed (wet weight g)	*Aminopyrine $T_{1/2}$ (min)*
Sham-operation	0	62 ± 4
Sponge implant without carrageenin	1.45 ± 0.03	71 ± 3*
Carrageenin-soaked sponge implant	5.06 ± 0.47	103 ± 26**
Carrageenin-soaked sponge implant + GSH	n.d.	106 ± 9**

Values represent the means ± s.e. mean of 6 rats. $^{14}CO_2$ was trapped in ethanolamine and collected every 15 minutes. $T_{1/2}$ values were calculated from trapped radioactivity during the elimination phase of aminopyrine, starting 30 min after drug injection and continuing for up to 150 minutes. *: $P < 0.05$, **: $P < 0.01$, one-tailed Mann Whitney U test vs sham-operated rats. n.d. = not determined.

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Determination of lymphokine induced histamine release *in vitro*

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Lymphokines (LK) are produced following the interaction of sensitized lymphocytes with specific antigen and by virtue of the immunological circumstances of their generation and the nature of their action are considered to be mediators of cellular immunity (Dumonde, Wolstencroft, Panayi, Matthew, Morley & Howson, 1969).

It has been previously reported (Morley & Williams, 1972) that the early phase (0 to 30 min) of the LK mediated inflammatory skin reaction in the guinea pig is abolished by pretreatment of animals with mepyramine maleate. These observations suggest that histamine is involved in the early part of LK mediated inflammation, which is consistent with reports of histamine involvement in reactions of delayed hypersensitivity. The present work was undertaken to determine whether LK would release histamine from rat mast cells and/or guinea-pig skin slices *in vitro*.

Mixed peritoneal mast cells were harvested from male rats (250–300 g) as described by Johnson & Moran (1969) and incubated at 37°C for 15 min or more with partially purified LK preparations, compound 48/80 and melittin. The LK preparations, at doses up to 125 μg/ml, did not release detectable amounts of histamine from rat mast cells. However compound 48/80 and melittin produced a dose-dependent release of histamine: compound 48/80 (25 μg/ml) released 85% of the total histamine and melittin (25 μg/ml) released 90% of total histamine.

Skin slices (1 mm thick) were prepared from guinea-pigs (300–350 g) using a hand microtome as described by Greaves, Fairley & Yamamoto (1971). The guinea-pig skin slices were incubated at 37°C for 15 min or more with LK preparations, melittin and compound 48/80. LK preparations produced a dose-dependent release of histamine: at a dose of 125 μg/ml, LK released 20% of the total histamine. By comparison, melittin (100 μg/ml) induced 50% of the histamine to be released while no detectable amounts of histamine were released with compound 48/80 (100 μg/ml).

The results show that LK preparations release histamine from guinea-pig skin slices but not from rat mast cells, whilst melittin produces histamine release in both systems. Compound 48/80 released histamine from rat mast cells but not from guinea-pig skin. These results suggest that more than one mechanism of histamine release may be involved.

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