

Endotoxin- and Inflammation-Induced Depression of the Hepatic Drug Metabolism in Rats

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Received October 25, 1990 Accepted March 4, 1991

ABSTRACT—Carrageenan-induced inflammation and exposure to endotoxin considerably decreased the content of cytochrome P-450 and activities of ethylmorphine N-demethylase and meperidine N-demethylase, but did not decrease the activities of aniline hydroxylase or NADPH-cytochrome *c* reductase, compared with the respective activities in rats treated with carrageenan alone. These results suggest that under these experimental conditions, the two host-related environmental factors interact and enhance a decrease in rat hepatic microsomal drug metabolizing enzymes depending on the substrate used.

Inflammation generally results in a depression of the mixed function oxidase (MFO) enzyme activities of hepatic microsomes (1). Pathological and abnormal physiological states in animals and man have also been associated with a reduction in the capacity of the host to resist infections. Bacterial infections, particularly gram-negative infections with accompanying endotoxemia, represent life-threatening situations which are being encountered with increasing frequency, especially in children, women, and the elderly (2). During gram-negative sepsis, endotoxins, such as the lipopolysaccharides obtained from *Escherichia coli*, are released into body fluids, with resultant endotoxemic shock. Several investigators have demonstrated liver function abnormalities and diffused necrotic lesions on endotoxin administration in several species of animals, as well as in humans (3).

Recently, some investigators have reported that endotoxin inhibited hepatic microsomal MFO enzyme activities in rats and mice (4, 5). The present studies were undertaken to ex-

amine the interaction of carrageenan-induced inflammation and endotoxin exposure on *in vitro* rat hepatic drug metabolism.

Male Wistar rats (120–140 g) were used throughout the study. Animals were maintained in stainless steel cages in a room at constant temperature on a 12 hr light-dark cycle. Animals were kept in our animal-care facilities for a minimum of 5 days after arrival before being used. Food and water were provided *ad libitum*, but all animals were fasted for the 24 hr before killing. Endotoxin, lyophilized lipopolysaccharide from *Escherichia coli*, serotype 026:B6 Westphal extracted (Difco Lab., Detroit, MI), was dissolved in sterile pyrogen-free isotonic saline solution (Ohtsuka Pharmaceutical Co., Tokyo, Japan). Endotoxin was injected intraperitoneally immediately after administration of carrageenan or saline 48 hr prior to sacrifice. Corresponding control animals received saline.

Carrageenan solution for induction of hind paw edema was prepared as previously described (6). One tenth milliliter of a 1.0%

solution of carrageenan (PICNIN-A, Lot No. P-9, Zushi Chemical Laboratory Inc., Zushi, Japan) was dissolved in sterile saline solution and injected subcutaneously into the plantar surface of the left hind paw of the rat 48 hr prior to sacrifice for determining the drug metabolizing enzyme activity of hepatic microsomes. In preliminary experiments, we established that the drug metabolizing enzyme activities were submaximally depressed on the 2nd day following carrageenan treatment; we therefore used this time point for determining hepatic MFO activity in all subsequent experiments.

Livers were removed, perfused with 0.15% KCl solution and chilled on ice. Microsomes were prepared following homogenization in 0.1 M phosphate buffer (pH 7.4) as described

previously (5). The washed microsomal pellets were stored at -80°C for no longer than 4 days prior to use. The microsomal suspension was used as the source of measurements and made by previously established methods without modification for cytochrome and all the enzymes: cytochrome P-450 (7), aminopyrine N-demethylase, ethylmorphine N-demethylase, meperidine N-demethylase (8), aniline hydroxylase (9) and NADPH-cytochrome *c* reductase (10). Statistical analyses were done by Student's *t*-test. Values were expressed as the mean \pm S.E., and *P* values of >0.05 were considered significant.

Figure 1 shows the effects of acute administration of endotoxin and carrageenan-induced inflammation on the drug metabolizing enzyme activity. Animals injected with car-

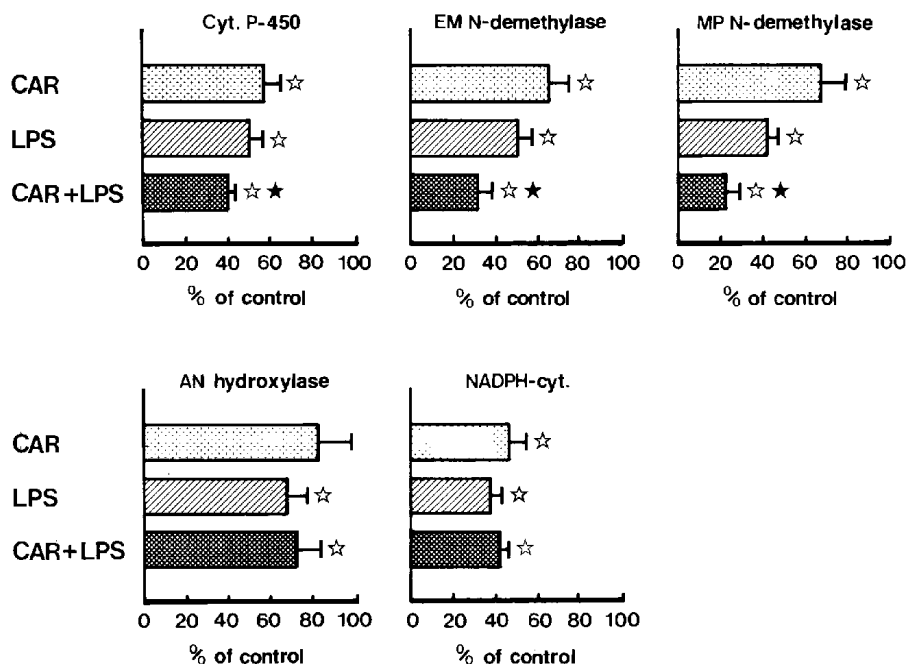


Fig. 1. Effect of the combination of carrageenan-induced inflammation and endotoxin on hepatic drug metabolizing enzyme activity in rats. Each point represents the mean \pm S.E. of the percentage of control enzyme activity for 5–6 rats. Control values were as follows: cytochrome P-450 (Cyt. P-450): 0.62 ± 0.032 nmol/mg protein, ethylmorphine (EM) N-demethylase: 229.6 ± 13.23 $\mu\text{mol}/20$ min/mg protein, meperidine (MP) N-demethylase: 187.0 ± 21.21 $\mu\text{mol}/20$ min/mg protein, aniline (AN) hydroxylase: 77.3 ± 5.29 $\mu\text{mol}/20$ min/mg protein, and NADPH-cytochrome *c* reductase (NADPH-cyt.): 0.19 ± 0.024 units/min/mg protein. Significant differences from the control or carrageenan-treated group are indicated as ☆ or ★ ($P < 0.05$), respectively. For additional information, see the letter of present paper on the methods. Abbreviations: CAR, carrageenan; LPS, endotoxin.

rageenan did not show any significant change from the control in aniline hydroxylase activity. However, the activities of ethylmorphine N-demethylase, meperidine N-demethylase and NADPH cytochrome *c* reductase, and the content of cytochrome P-450 were significantly decreased by 30–50% in carrageenan-treated rats. In the normal rat, acute treatment with endotoxin (0.5 mg/kg, i.p.) induced a significant decrease, 50–60%, in the activities of ethylmorphine N-demethylase, meperidine N-demethylase and NADPH-cytochrome *c* reductase, and in the content of cytochrome P-450.

The combination of carrageenan-induced inflammation and endotoxin significantly decreased the content of cytochrome P-450 and the activities of ethylmorphine N-demethylase and meperidine N-demethylase, but not the activities of aniline hydroxylase and NADPH-cytochrome *c* reductase, compared with the respective activities in rats treated with carrageenan alone. However, all the parameters of drug metabolizing enzymes were not decreased in comparison with those of rats treated with endotoxin alone. Thus, it appears that the effect of carrageenan exposure on acute endotoxin-treated animals is exacerbative in nature in comparison with the effect of carrageenan exposure alone.

The inflammation status predisposes the host to a variety of infections, especially to those of gram-negative bacteria. The effects of the combination of these two host-related environmental factors, i.e., inflammation and endotoxin, on the processes of drug metabolism are unknown. Many different factors are known to change the activities of drugs and exogenous chemicals by increasing or decreasing their rate of biotransformation by cytochrome P-450 dependent MFO enzymes in the liver. The data presented here confirm earlier observations on the effects of endotoxin (4, 5) and inflammation on drug-metabolizing enzymes (1). However, in this paper, we have further demonstrated that these two environmental factors interact to enhance the decrease in hepatic microsomal MFO enzymes.

The mechanism responsible for the depression of hepatic cytochrome P-450 by endotoxin has been suggested by some investigators (11–13). They observed marked enhancement of the degradation of cytochrome P-450 heme by endotoxin which also blocked the induction of delta-aminolevulinic acid synthetase activities. The same authors raised some doubts about whether the response to endotoxin was a specific physiological effect or rather, a secondary reaction to a pharmacologic challenge. Therefore, the possible mechanism, assuming that endotoxin may enhance the degradation or turnover of heme protein or heme enzymes in the liver, could account for our observations of decreased levels of hepatic microsomal cytochrome P-450 or dependent monooxygenase activities induced by endotoxin.

While the reason for the difference in substrate inhibition is unknown, it may involve the multiplicity of the forms of cytochrome P-450 that exist in rat liver microsomes. It is well-known that these different forms of the hemoprotein exhibit differential specificities for substrates and inhibitors (14). Thus, carrageenan exposure may alter the cytochrome P-450 composition in hepatic microsomes and preferentially affect the cytochrome P-450 species responsible for the type I (ethylmorphine and meperidine) binding. These results are consistent with earlier studies by Ishikawa et al. (6, 15), who showed that carrageenan-exposure increased the duration of hexobarbital hypnosis and decreased the hepatic drug biotransformation in male rats but not in female ones, and the activity of aminopyrine N-demethylase is impaired in carrageenan-exposed rats while the activity of aniline hydroxylase are not affected.

The pharmacological and toxicological implications for understanding the interactions of inflammation and its accompanying infections are clearly evident. Further studies on the interactions of altered inflammation status and diseases may be important, not only for the rational therapeutic management of patients, but also because such studies may lead to an explanation for many of the clinically apparent

dangers of using normal recommended drug dose regimens under conditions of infection and inflammation.

Acknowledgment

This work was supported by a grant from The Science Research Promotion Fund, Japan Private School Promotion Foundation.

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