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EVIDENCE FOR THE INVOLVEMENT OF A REPERFUSION INJURY IN GALACTOSAMINE/ENDOTOXIN-INDUCED HEPATITIS IN MICE

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Abstract—Simultaneous intraperitoneal administration of 700 mg/kg galactosamine and 33 μg/kg *Salmonella abortus equi* endotoxin to male NMRI albino mice resulted in fulminant hepatitis as assessed after nine hours by measurement of serum transaminases as well as sorbitol dehydrogenase activities. Intraperitoneal pretreatment of animals with 2 × 100 mg/kg allopurinol, or intravenous pretreatment with 33 kU superoxide dismutase or 1 MU catalase fully prevented hepatitis. Administration of 10 μg/kg of the prostacyclin analogue iloprost antagonized liver injury when given simultaneously with galactosamine/endotoxin but did not protect when given 90 min later. Tocopherol or desferal pretreatment of the animals had no significant protective effect. Together with our recent finding that hepatic leukotriene D_4 production is likely to be responsible for galactosamine/endotoxin-induced hepatitis we interpret these results as evidence for a leukotriene-induced hepatic ischemia followed by a reperfusion syndrome.

Galactosamine is an established hepatoxin in experimental animal studies which is thought to act primarily via a transient period of uridine triphosphate deficiency and its resulting inhibition of protein synthesis [1]. Likewise, liver injury by GalN* is greatly potentiated by simultaneous administration of endotoxin [2]. Based on earlier work [3, 5] we recently showed that in a combined GalN/endotoxin-induced hepatitis model in the mouse, intravenously leukotriene D_4 can substitute for endotoxin [6]. We concluded from this and further experiments that peptido-leukotrienes represent the actual mediators which are likely to trigger the development of fulminant hepatitis in GalN-sensitized mice. Although this finding further extends the biochemical knowledge on GalN/E hepatitis, the primary pathogenic event still remains open.

There is increasing evidence in the literature that in organs such as the heart, ischemia and reperfusion represent a fundamental mechanism in the production of cellular necrosis [7, 8]. The basic assumptions are that catabolism of ATP during the anoxic ischemia phase gives rise to formation of hypoxanthine. Simultaneously, xanthine dehydrogenase is thought to be converted to an oxidase which produces superoxide once the tissue is reoxygenated during reperfusion. This pathophysiological mechanism was supported by pharmacological interventions by agents interfering with the described causal sequence at defined sites. The most carefully studied agents are the xanthine oxidase inhibitor allopurinol [9] and the enzymes superoxide dismutase and catalase which act upon the steady state concentrations of O_2^- or H_2O_2, respectively.

We report here experiments of this intervention type which suggest that leukotriene-mediated hepatic injury is caused by a reperfusion syndrome.

METHODS

Male NMRI mice received 700 mg/kg galactosamine (Serva, Heidelberg) together with 33 μg/kg *Salmonella abortus equi* endotoxin (Sigma) at 8 a.m. Liver injury was assessed by measurement of serum transaminases and sorbitol dehydrogenase activities nine hours later as described in detail elsewhere [5]. Allopurinol (Sigma) was dissolved by titrating an aqueous suspension to pH 11.5 by addition of 2N NaOH. This solution was intraperitoneally injected in a volume of 200 μl at a concentration of 15 mg/ml 24 hr and 1 hr before the GalN/E experiment (final dose: 2 × 100 mg/kg). Control animals received phosphate-buffered saline made up to pH 11.5. Bovine superoxide dismutase was a gift by Dr. L. Flohé, Grünenthal GmbH, Aachen, W. Germany, and was injected into the tail vein as a solution of 1.5 mg/kg with a specific activity of 33 kU/mg as defined in [10]. Catalase was purchased from Boehringer-Mannheim and analogously administered with a specific activity of 65 kU/mg. Bovine serum albumin of the highest purity grade from Sigma was used for the control experiments. Iloprost was a gift by Schering A. G., Berlin. It was given in phosphate-buffered saline as an intraperitoneal single injection at a dose of 10 μg/kg at the times indicated. Desferioxaminemethanesulfonate (Ciba-Geigy, Desferal®), was injected in two daily doses of 500 mg/kg (s.c.) for three consecutive days. The last dose was administered 2 hr prior to GalN/E. Tocopherolacetate (Serva) was given by gavage in vegetable oil 2 h prior to GalN/E. Lipid peroxidation was assessed by measuring exhaled ethane and pentane over a period of up to nine hours [11] as well as the hepatic *post mortem* malondialdehyde content [12].

*Abbreviations: SGOT, serum aspartate aminotransferase (EC 2.6.1.2); SGPT, serum alanine aminotransferase (EC 2.6.1.1); SDH, sorbitol dehydrogenase (EC 1.1.1.14); GalN, galactosamine; E, endotoxin.

Table 1. Prevention of galactosamine/endotoxin hepatitis in mice by pretreatment with the xanthin oxidase inhibitor allopurinol or with reactive oxygen scavenging enzymes

Pretreatment	SDH	SGOT	SGPT	N	m
Untreated control	40 ± 10	75 ± 40	40 ± 10	10	0
Disease control	3700 ± 4170	2330 ± 2130	4970 ± 5280	19	3
Allopurinol†	80 ± 40*	250 ± 110*	120 ± 25*	6	0
Superoxide dismutase‡	70 ± 40*	80 ± 50*	90 ± 30*	8	0
Catalase§	80 ± 100*	150 ± 50*	170 ± 210*	8	0
Bovine serum albumin‖	3050 ± 3440	3890 ± 4150	6550 ±8440	4	1

* $P < 0.05$.
† 100 mg/kg i.p. 24 and 1 hr prior to GalN/E.
‡ 33000 U/kg i.v. in PBS 1 hr prior to GalN/E.
§ 1 million U/kg in PBS 1 hr prior to GalN/E.
‖ 15 mg/kg i.v. in PBS 1 hr prior to GalN/E.

Statistics. Results are expressed as mean values ± standard deviation (SD). Data were analyzed by Student's *t*-test. $P < 0.05$ was considered to be significant.

RESULTS

Mice intoxicated with GalN/E did not exhale significant amounts of ethane or pentane compared to controls within an observation period of nine hours. Likewise, no thiobarbituric acid-reactive material was found in liver homogenates analysed after this time. These findings indicate that lipid peroxidation is unlikely to be associated with GalN/E-hepatitis in mice. However, we observed that dimethyl sulfoxide protected the animals in this model. Since DMSO has radical-scavenging potential and is able to ameliorate cardiac ischemia-reperfusion injury [8] we wondered whether a mechanism of this type might be associated with the experimental hepatitis under investigation.

When mice had been pretreated with allopurinol, they did not develop any significant signs of liver injury as judged by enzyme release into the circulation up to nine hours. Likewise, pretreatment of the animals one hour before intoxication by either superoxide dismutase or catalase resulted in highly significant protection against GalN/E-hepatitis (Table 1). The control experiments indicate that neither the solvent (not shown) nor inert protein in comparable concentration accounted for this effect.

The data in Table 2 show that oral pretreatment with vitamin E, even in high doses and different regimens of application failed to protect significantly against GalN/E hepatitis. Also, the iron chelating agent desferal did not render any protection under conditions where this pretreatment was shown to be highly effective against e.g. allyl alcohol- or carbontetrachloride-induced liver injury [12, 13].

Finally we examined the effect of a vasodilator on GalN/E hepatitis at different times of intervention. The results in Table 3 indicate that the stable prostacylin analogue iloprost is able to antagonize our experimental liver injury when given simultaneously with GalN/E. 90 min after intoxication iloprost was no longer protective.

DISCUSSION

Leukotrienes have been implicated as mediators of ischemia and shock and evidence is reviewed that this class of eicosanoids is able to promote shock-like states [14]. The lungs, kidneys and the heart were considered as the main target organs of leukotriene actions. For the liver, it was shown that this organ is able to produce peptido-leukotrienes upon endotoxin administration and releases them into the bile

Table 2. Lack of efficacy of pretreatment with vitamin E or with the iron depletor desferal against galactosamine/endotoxin-induced hepatitis in mice

	SDH	SGOT	SGPT	N	m
Disease control	2380 ± 2130	1990 ± 2280	3200 ± 2790	13	6
Solvent control (oil)	5670 ± 1770	2820 ± 1170	6430± 1650	6	2
2 mg/kg α-tocopherolacetat†	7060 ± 8660	2580 ± 2510	7850 ± 8670	8	3
20 mg/kg α-tocopherolacetat†	3860 ± 5750	2070 ± 3210	4110 ± 5720	8	1
200 mg/kg α-tocopherolacetat†	590 ± 680	460 ± 400	700 ± 840	8	1
20 mg/kg α-tocopherolacetat‡	6470 ± 7030	4790 ± 5970	6820 ± 5900	6	5
200 mg/kg α-tocopherolacetat‡	1820 ± 1410	1010 ± 410	2300 ± 1260	7	4
Desferal§	1910 ± 2000	1330 ± 1110	1700 ± 1730	6	4
Desferal without GalN/E	100 ± 60	80 ± 30	90 ± 50	3	0

† 2 hr before GalN/E p.o. in oil.
‡ 3 times p.o. for three days prior to study.
§ 6 times 500 mg/kg s.c. twice daily for three days.

Table 3. Effect of the intraperitoneal administration of the prostacyclin analogue iloprost (10 μg/kg) at different intervention times on galactosamine/endotoxin-induced hepatitis in mice

Treatment	SDH	SGOT	SGPT	N	m
GalN/E	6000 ± 5000	2870 ± 2460	8740 ± 7810	11	7
Iloprost + GalN/E	170 ± 120*	190 ± 100*	230 ± 160*	10	0
Iloprost (90 min after GalN/E)	2380 ± 2340	990 ± 830	3050 ± 3150	8	2
Iloprost	30 ± 10	60 ± 30	30 ± 20	4	0

*$P < 0.05$ compared to disease control.

[3, 5]. This observation led to the suggestion that leukotrienes might be causally involved in the pathogenesis of liver injury [4, 5]. We were able to corroborate this concept by direct demonstration of an LTD_4-induced fulminant hepatitis in GalN-sensitized mice [6]. This finding, however, raises the question by which mechanism the liver cells are actually destroyed. A direct biochemical interaction of peptido-leukotrienes during their passage into the bile with critical hepatocyte structures seemed rather unlikely.

The vasoconstrictive properties of leukotrienes [14] and the sensitivity of the liver to reperfusion injury after mechanical occlusion of the vessels, however, offer a rationale for the patho-mechanism of GalN/E- or GalN/LTD_4-induced hepatitis. Our findings support the view that leukotriene D_4, released by an as yet unknown sequence of signals induced by endotoxin, leads to a transient spasm in hepatic blood flow. Since leukotrienes have half-lives in the order of minutes, this effect is likely to be reversible. Restoration of blood flow and normoxia would then lead to the well-described generation of oxygen radicals during this reperfusion period. The observed protective action of the vasodilator iloprost when given during the initial phase within the pathogenic sequence lends strong support for this hypothesis. Because in this sequence of events no endogenous defence systems, such as the glutathione redox system, are paralyzed by conjugation reactions, the reperfusion-induced oxidative stress need not necessarily lead to such a metabolic decompensation that lipid peroxidation becomes analytically detectable. We have no explanation as to the role of galactosamine in sensitizing the mouse liver for this assumed sequence of events. Also, the lack of protection by vitamin E in our mouse model does not agree with a reported protection in a surgical rat liver ischemia model [15].

Our findings add another type of hepatic primary lesion of endogenous origin to the hitherto discussed pathogenic mechanisms such as covalent binding or peroxidative deleterious reactions of reactive metabolites.

REFERENCES

1. K. Decker and D. Keppler, *Rev. Physiol. Biochem. Pharmac.* **71**, 77 (1974).
2. C. Galanos, M. A. Freudenberg and W. Reutter, *Proc. natn. Acad. Sci. U.S.A.* **76**, 5939 (1979).
3. W. Hagmann, C. Denzlinger and D. Keppler, *FEBS Lett.* **180**, 309 (1985).
4. D. Keppler, C. Forsthove, W. Hagmann, S. Rapp, C. Denzlinger and H. K. Koch, in *Trends in Hepatology*, (Eds. L. Bianchi *et al.*), p. 137. MTP Press, Lancaster (1985).
5. A. Wendel and G. Tiegs, *Biochem. Pharmac.* **35**, 2115 (1986).
6. A. Wendel and G. Tiegs, *Z. Gastroenenterologie* **25**, 32 (1987).
7. G. J. van der Vusse and R. S. Reneman, *Trends pharmac. Sci.* **6**, 76 (1985).
8. J. A. Thompson and M. L. Hess, *Progr. Cardiovasc. Dis.* **28**, 449 (1986).
9. D. E. Chambers, D. A. Parks, G. Patterson, R. Roy, J. N. McCord, S. Yoshida, L. F. Parmley and J. M. Downey, *J. molec. cell. Cardiol.* **17**, 145 (1985).
10. L. Flohé and F. Ötting, *Meth. Enzm.* **105**, 93 (1984).
11. A. Wendel and E. E. Dumelin, *Meth. Enzm.* **77**, 10 (1981).
12. H. Jaeschke, C. Kleinwaechter and A. Wendel, *Biochem. Pharmac.* **36**, 51 (1987).
13. M. Younes and C. P. Siegers, *Chem.-Biol. Interact.* **55**, 327 (1985).
14. A. M. Lefer, *Biochem. Pharmacol.* **35**, 123 (1986).
15. S. Marubayashi, K. Dohi, K. Ochi, T. Kawasaki, *Surgery* **99**, 184 (1986).