Acta pharmacol. et toxicol. 1984, 54, 120–123.

From the Department of Medicine A, Division of Hepatology and Laboratories of Experimental Pathology, Rigshospitalet, 9 Blegdamsvej, DK-2100 Copenhagen, Denmark

Quantitative Liver Functions after Administration of Allyl Alcohol to Rats

By

H. E. Poulsen and B. Korsholm

(Received August 9, 1983; Accepted November 2, 1983)

Abstract: In rats a single dose of allyl alcohol (100 µl per kg body weight) was given to produce periportal liver damage. Prothrombin index was reduced to a minimum after 12 hours and reestablished after 24 hours. The galactose elimination capacity was not changed. Hepatic glutathione content was unchanged for the first 24 hours but was then elevated twofold. Microsomal p-nitro-anisole demethylation showed a slight initial increase and a subsequent reduction. The pattern of these changes is similar to that seen after centrilobular liver damage from acetaminophen overdose (Poulsen *et al.* 1981a; Poulsen *et al.*, unpublished results), with the exception that the latter causes glutathione depletion. This indicates that in chemical liver damage ribosomal function, e.g. protein synthesis and drug hydroxylation, is more vulnerable than cytosolic phosphorylation of carbohydrate.

Key words: Quantitative liver functions – allyl alcohol – time course – rats.

Sublethal doses of acetaminophen, corresponding to the LD10, given to rats, reduces prothrombin-proconvertin concentrations to 50 per cent after 12 hours, whereas galactose elimination capacity does not change significantly (Poulsen *et al.* 1981a). This indicates that acetaminophen in this dose primarily interferes with ribosomal protein synthesis in the liver, whereas a cytosolic function like phosphorylation of galactose is affected to a much lesser extent. This is in agreement with the hypothesis that the hepatotoxic effect of acetaminophen is caused by a reactive metabolite, formed in the endoplasmatic reticulum (Mitchell *et al.* 1974), under the assumption that the metabolite is bound to this organelle and that other toxic effects, eventually leading to liver cell necrosis, are secondary to damage to the endoplasmatic reticulum. Alternatively, the lack of observable changes in those cytosolic functions reflected by the galactose elimination capacity might be due to compensatory hyperfunction of liver cells less or not affected by the toxic metabolite of acetaminophen which mainly causes centrilobular necrosis. Such hyperfunction is seen after 70 per cent partial hepatectomy (Yildirim & Poulsen 1981).

In order to distinguish between these possibilities the specificity of the acetaminophen-induced changes was evaluated by comparison with changes produced by allyl alcohol. This hepatotoxin is converted to the reactive metabolite acrolein in the cytosol (Serafini-Cessi 1972) and causes periportal necrosis (Reid 1972) in contrast to acetaminophen.

Materials and Methods

LD10 of allyl alcohol was assessed by giving 6 groups of rats, each consisting of 8 female Wistar rat weighing about 190 g and fasted overnight, doses of 100, 120, 140,

160, 180, and 200 μl allyl alcohol (Merck) in 2 ml of saline orally. On this basis 100 μl of allyl alcohol per kg body weight was chosen as the standard dose corresponding to mortality of about 10%. Groups of rats weighing 190–210 g, fasted overnight, were studied before, and 6, 12, 24, 36, 48, 72, and 96 hours after allyl alcohol administration. At each interval 3 groups were studied, one for determination of galactose elimination capacity (Keiding 1973), one for measurement of prothrombin-proconvertin (Owen & Aas 1951), and one for liver tissue analysis (see below). It was planned to examine 4 animals in each group, but in order to be able to compensate for losses due to lethal allyl alcohol intoxication, 5 animals were initially included in each group. Nevertheless, some groups eventually consisted of but 3 animals. The data from acetaminophen intoxication, reported in detail elsewhere (Poulsen *et al.* 1981, Poulsen *et al.*, unpublished observations) are based on observation of 4 to 8 animals in each group.

Allyl alcohol was measured by GLC (Palo & Ilkova 1970) in portal venous and aortic blood 5, 10, 20, 30, and 60 min. after the standard dose of allyl alcohol in 5 animals at each interval. The detection limit was 10 μmol/l.

Tissue analysis. During diethyl ether anaesthesia animals were bled, the liver removed, blotted on filter paper, cut with scissors in ice cold 1.5% KCl and homogenized in a Potter-Elvehjelm homogenizer with 10 ml of isotonic KCl. Microsomes (Andreasen *et al.* 1974) were assayed for cytochrome P-450 (Omura & Sato 1964) and p-nitroanisole demethylation (Schoene *et al.* 1972) and liver homogenate for cytochrome P-450 and total glutathione (Tietze 1969). Protein was measured by the method of Groves (Groves *et al.* 1968).

Calculations. P-nitro-anisole demethylation was calculated as the activity of microsomes from 1 g liver × cytochrome P-450 (homogenate)/cytochrome P-450 (microsomes) × liver weight to express the capacity of the whole liver.

Comparison between several means was performed with one way analysis of variance and comparison of two means with a two tailed t-test with correction for mass significance (Duncan 1955). P-values less than 0.05 were considered statistically significant.

Results

Fig. 1 and 2 show the changes in prothrombin index and galactose elimination capacity (GEC) with time after administration of allyl alcohol compared with the changes after acetaminophen (shaded area). The initial fall in prothrombin index ($P < 0.01$) is similar after allyl alcohol and acetaminophen, but recovery is more rapid after allyl alcohol, normal levels being reached after 24 hours. The change in GEC after allyl alcohol is not significant ($P > 0.05$), as it is after acetaminophen.

The glutathione content of the liver, which is strongly reduced after acetaminophen in all animals during the first 12 hours of the intoxication, shows no immediate change after allyl alcohol, but increases to about twice the initial values after 24 to 48 hours ($P < 0.05$). During the late phase there is no clear difference between the allyl alcohol and the acetaminophen data (fig. 3).

Microsomal drug metabolizing function, as

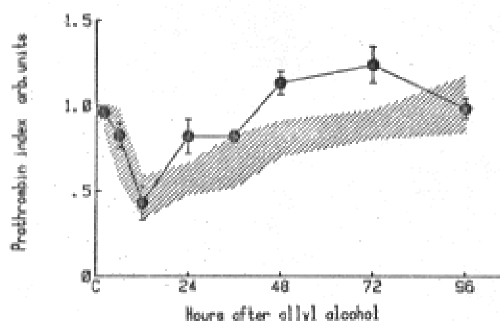

Fig. 1. The time course of prothrombin index after administration of 100 μl allyl alcohol per kg body weight. Symbols indicate mean ± S.E.M. of 3–5 animals. The hatched area indicate 95% confidence limits for the time course after acetaminophen, adapted from Poulsen *et al.* (1981).

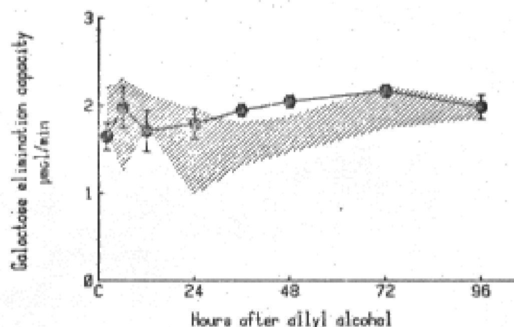

Fig. 2. The time course of the galactose elimination capacity after administration of 100 μl allyl alcohol per kg body weight. Symbols indicate mean ± S.E.M. of 3–5 animals. The hatched area indicate 95% confidence limits for the time course after acetaminophen adapted from Poulsen *et al.* (1981).

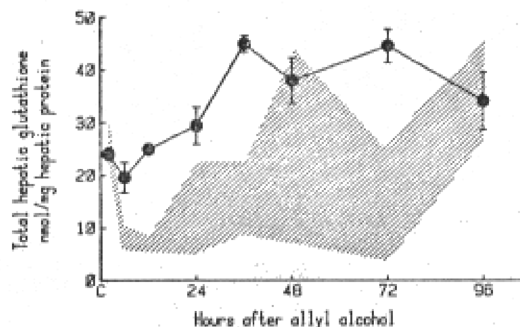

Fig. 3. The time course of hepatic glutathione, reduced + oxidized, after administration of 100 μl allyl alcohol per kg body weight. Symbols indicate mean ± S.E.M. of 3–5 animals. The hatched area indicate the 95% confidence limits of acetaminophen, data adapted from Poulsen *et al.* (unpublished observations).

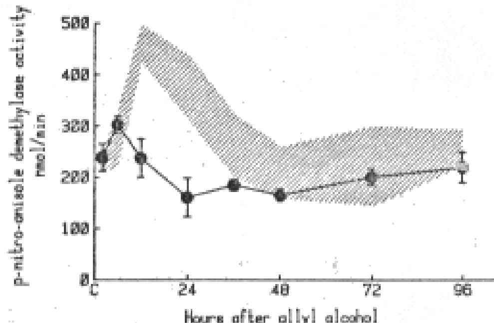

Fig. 4. P-nitro-anisole demethylation after administration of 100 μl allyl alcohol per kg body weight. Symbols indicate mean ± S.E.M. of 3–5 animals. The hatched area indicate the 95% confidence limits of the activity after administration of actaminophen, data adapted from Poulsen *et al.* (unpublished observations). The demethylase activity is given per one liver, calculated as stated under Material and Methods.

expressed by p-nitro-anisole demethylase activity (fig. 4), changes less dramatically after allyl alcohol than after acetaminophen, but the pattern with an initial increase and a subsequent reduction is the same. The lowest value is seen 24 hours earlier than after acetaminophen.

Allyl alcohol was rapidly eliminated from the blood. Five minutes after administration it was 544 ± 84, (mean ± S.E.M.) μmol/l in portal blood and 117 ± 91 μmol/l in aortic blood. After 20 min. it was 45 ± 9 μmol/l in the aorta and detectable in 4 of 5 animals in the portal vein. Thirty minutes after ingestion the recovery of allyl alcohol in whole gut homogenate (8 animals) was 7 ± 3 per cent of the dose.

Macroscopically the liver appeared normal in animals sacrified after six hours. At later intervals more or less confluent haemorrhagic areas indicated widespread necrosis. Light microscopy of these areas showed predominantly periportal necrosis.

Discussion

The change of prothrombin index and galactose elimination capacity (GEC) after allyl alcohol were very similar to those seen after acetaminophen intoxication, indicating that the primary role of the liver, whether in the microsomes or the cytosol, or centrilobular or periportal, makes little difference concerning these fundamental variables.

The experiments therefore give no clue as to the specific mechanism of the acetaminophen-induced change in prothrombin index (and absence of change in GEC), but indicate that in chemical liver damage ribosomal protein synthesis is more vulnerable than cytosolic phosphorylation of carbohydrate, possibly because the latter is more rapidly compensated for by an increase in the capacity of non-injured liver cells *in vivo* (Yildrim & Poulsen 1981), as well as *in vitro* (Bauer *et al.* 1976).

The only difference between these two variables in allyl alcohol and acetaminophen intoxication is a trend to a more rapid rebound in the former group (not statistically significant concerning GEC). This is consistent with the more rapid elimination of allyl alcohol and indicates that the toxic metabolite has a shorter life.

Allyl alcohol does not reduce hepatocellular glutathione, which thus can be considered as a "specific" acetaminophen effect. Fig. 3 indicates a slight decrease in hepatic glutathione. As glutathione is regulated by a feed-back mechanism (Lauterburg & Mitchell 1981), a decrease should markedly stimulate glutathione synthesis and result in a temporarily increased glutathione

level. The effect might be quantitatively similar to that of acetaminophen in the presence of acetylcystein (Lauterburg *et al.* 1983). It is noteworthy that 36 hours after allyl alcohol, glutathione is almost doubled, which may be regarded as another sign of "postinjury" hyperfunction. In acetaminophen intoxicated animals there was a large variation in glutathione during the late phases, resulting from high levels in some animals.

From this it is assumed to be an unspecific phenomenon which is also seen in man following toxic and viral hepatitis (Poulsen *et al.* 1981).

Also the change in p-nitro-anisole demethylase activity is markedly different after acetaminophen and allyl alcohol intoxication, although a similar pattern may be discussed. In both situations there is an initial rise followed by a decline to or below the control values, but the peak is smaller and reduced earlier in the allyl alcohol experiments. Again this difference may be attributed to the shorter duration of allyl alcohol intoxication, but gives no clue to the mechanism. Possibly it may be regarded as a result of enzyme induction by the toxin, without direct relation to the toxic effect.

Acknowledgements

This work was supported by the Carlsberg Foundation. The Central Laboratory of Rigshospitalet kindly provided the prothrombin-proconvertin analysis. Mrs. Doris Christoffersen is thanked for expert assistance in preparing the animals.

References

Andreasen, P. B., A. Bremmelgaard & B. K. Larsen: Interaction between antipyrine and the microsomal ethanol oxidation in rat liver. *Pharmacology* 1974, **12**, 244–250.

Bauer, C. H., B. F. Hassels & W. G. Reutter: Galactose metabolism in regeneration rat liver. *Biochem. J.* 1976, **154**, 141–147.

Duncan, D. B.: Multiple range and multiple F tests. *Biometrics* 1955, **11**, 1–42.

Groves, W. E., F. C. Jr. Davis & B. H. Sells: Spectrofotometric determination of microgram quantities of protein without nucleic acid interference. *Analyt. Biochem.* 1968, **22**, 195–210.

Keiding, S.: Galactose elimination capacity in the rat. *Scand. J. Clin. Lab. Invest.* 1973, **31**, 319–325.

Lauterburg, B. H., G. B. Corcoran & J. R. Mitchell: Mechanism of action of N-acetylcysteine in the protection against the hepatotoxicity of acetaminophen in rats. *J. Clin. Invest.* 1983, **71**, 980–991.

Lauterburg, B. H. & J. R. Mitchell: Regulation of hepatic glutathione turnover in rats *in vivo* and evidence for kinetic homogeniety of the hepatic glutathione pool. *J. Clin. Invest.* 1981, **67**, 1415–1424.

Mitchell, J. R., S. S. Thorgeirsson, W. Z. Potter, D. J. Jollow & H. Keiser: Acetaminophen-induced hepatic injury: Protective role of glutathione in man and rationale for therapy. *Clin. Pharm. Therap.* 1974, **16**, 676–684.

Omura, T. & R. Sato: The carbon monoxide binding of liver microsomes. *J. Biol. Chem.* 1964, **2390**, 2370–2378.

Owen, P. A. & K. Aas: The control of dicumarol therapy and the quantitative determination of prothrombin and proconvertin. *Scand. J. Clin. Lab. Invest.* 1951, **3**, 201–208.

Palo, V. & H. Ilkova: Direct gas chromatographic estimation of lower alcohols, acetaldehyde, acetone and diacetyl in milk products. *J. Chromatog.* 1970, **53**, 363–367.

Poulsen, H. E., P. Petersen & H. Vilstrup: Quantitative liver function and morphology after paracetamol administration to rats. *Eur. J. Clin. Invest.* 1981a, **11**, 161–164.

Poulsen, H. E., L. Ranek & P. B. Andreasen: The hepatic glutathione in liver diseases. *Scand. J. Clin. Lab. Invest.* 1981b, **41**, 573–576.

Reid, W. D. : Mechanism of allyl alcohol-induced hepatic necrosis. *Experientia* 1972, **28**, 1058–1060.

Serafini-Cessi, F.: Conversion of allyl alcohol into acrolein by rat liver. *Biochem. J.* 1972, **128**, 1103–1107.

Schoene, B., R. A. Fleischmann, H. Remmer & H. F. von Oldershausen: Determination of drug metabolising enzymes in needle biopsies of human liver. *Eur. J. Clin. Pharmacol.* 1972, **4**, 65–73.

Tietze, F.: Enzymic method for quantitative determination of nanogram amounts of total and oxidized glutathione, applications to ammalian blood and other tissues. *Analyt. Biochem.* 1969, **27**, 502–522.

Yildirim, S. I. & H. E. Poulsen: Quantitative liver functions after 70% hepatectomy. *Eur. J. Clin. Invest.* 1981, **11**, 469–472.