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POTENTIATED HEPATOTOXICITY FROM CONCURRENT ADMINISTRATION OF ACETAMINOPHEN AND ALLYL ALCOHOL TO RATS

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Abstract—Female Wistar rats were treated with acetaminophen 3.0 g/kg BW and allyl alcohol 75 μl/kg BW by gastric tube. Hepatic function, measured as galactose elimination capacity and prothrombin index, was reduced to about 0.40 times control value. Plasma alanine transferase activity was elevated more and earlier after treatment with acetaminophen and allyl alcohol compared to administration of acetaminophen alone. Also prothrombin index was reduced more and earlier from the combination. Hepatic glutathione was depleted to a lower level 3 hr after administration of the combination of toxins, compared to administration of acetaminophen alone, after 6 hr there was no difference. Excretion of acetaminophen metabolites, especially the acetaminophen mercapturate, into urine was not changed from the combination. After administration of the toxic combination histological changes in the liver were minor. The results indicate that the two toxins potentiate each other's action. The potentiation is proposed to be due to prevention of compensatory hyperfunction of non-necrotic liver cells rather than to direct metabolic interaction of the toxins.

Acetaminophen and allyl alcohol are two hepatotoxic agents producing distinctly different histopathological changes [1, 2]. Acetaminophen is activated by cytochrome P-450 [3], mainly localized on the endoplasmatic membranes of centrilobular hepatocytes [4], and allyl alchol is activated to acrolein by the alcohol dehydrogenase [5], mainly localized to the cytosol of periportal hepatocytes [6].

From this one would expect the effect of simultaneous administration of both toxins to be additive. The purpose of the present study was to test this hypothesis.

MATERIALS AND METHODS

Female Wistar rats weighing 172–201 g were fed *ad libitum* with Rostock® rat pellets and tap water. After an overnight fast allyl alcohol was given dissolved in 2 ml isotonic saline and acetaminophen as a suspension (300 mg/ml) in 0.2% tragacanth gum by stomach tube. Animals treated with identical volumes of saline and tragacanth gum were considered control groups.

The time course of functional impairment was investigated 6, 12, 24, 36, 48, 72 and 96 hr after administration of acetaminophen 3 g/kg BW together with allyl alcohol 75 μl/kg BW. In one group of animals, 4 animals at each interval, galactose elimination capacity was investigated as previously described [7]. Another group, similarly treated and examined at the same intervals was anesthesized with diethyl ether, arterial blood was sampled from the aorta for estimation of prothrombin index [8], plasma allyl alcohol concentration [9], and plasma acetaminophen concentration [10] using a C18 μBondapack column (Waters), and the mobile phase described below for analysis of acetaminophen metabolites. Following exsanguination the liver was removed, a piece cut for histological examination. The rest was homogenized for determination of protein [11], and of cytochrome P-450 [12] in homogenate and in a 100,000 G microsomal fraction [13].

Cytochrome P-450 in liver homogenate and in the microsomal fraction was linearly related, showing that microsomal precipitation was reproducible. The content is given per mg microsomal protein.

To compare the effect of acetaminophen and acetaminophen plus allyl alcohol in the same doses as above on prothrombin index, alanine aminotransferase and hepatic glutathione, groups of rats (N = 5–9) were studied after 3 and 6 hr by the same technique.

For histological examination liver tissue was fixed in 10% formaldehyde and paraffin embedded. Five micrometer sections were stained with hematoxylin and eosin (HE) and were examined in random order, without knowledge of treatment group or interval. Five lobules were chosen at random, the radius of the lobule and of the necrotic area was measured by a micrometer ocular. Necrosis was expressed as percent, calculated as radius of the necrosis divided by radius of lobule times 100. Necrotic area was identified by nuclear pyknosis or karyorrhexis, blurring of the cellular membrane, strongly acidophillic cytoplasm, and cellular infiltration. Areas with hydropic degeneration alone were not included.

The effect of allyl alcohol administration on acetaminophen metabolism was studied in two groups of

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seven animals given acetaminophen alone or together with allyl alcohol. They were housed individually in metabolic cages. Urine was collected over dry ice for four periods of 24 hr and stored at −20° until analysis for acetaminophen and its metabolites was performed on a high performance liquid chromatography system consisting of a Waters M6000 pump, a Waters M440 u.v.-detector with fixed wavelength of 254 nm and a 30 cm μBondapack C18 reverse phase column. The flow rate was 2 ml/min and the mobile phase was methanol, water and glacial acetic acid (12.5%, 86.5%, 1%, v/v/v) in 0.05 M KH_2PO_4 [14]. Quantification of the metabolites was done from standard curves of reference substances kindly donated by Sterling Winthrop, Sweden.

Data from a time course was tested with Bartlett's test for homogeneity of variance prior to one way analysis of variance [15]. Difference between two means was tested with a two-tailed Student's *t*-test. P-values of less than 0.05 were considered statistically significant.

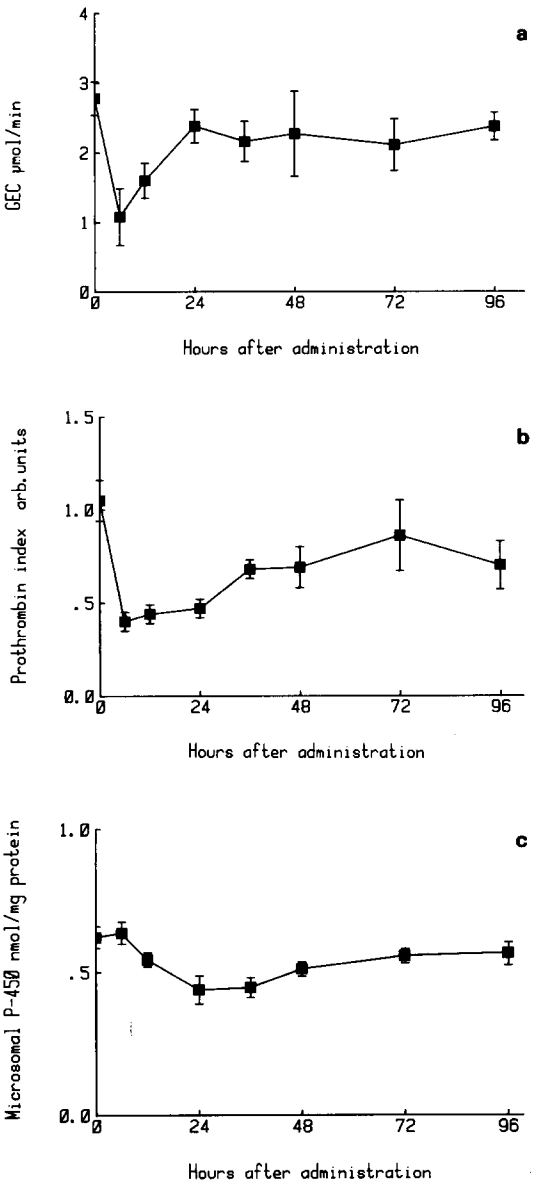

Fig. 1.(a) Galactose elimination capacity at various periods after administration of acetaminophen and allyl alcohol. Symbols indicate mean ± S.E.M. of four animals. (b) Prothrombin index, in arbitrary units, at various periods after administration of acetaminophen and allyl alcohol. Symbols indicate mean ± S.E.M. of four animals. (c) Microsomal cytochrome P-450 at various periods after administration of acetaminophen 3 g and allyl alcohol 75 μl per kg BW by gastric tube after an overnight fast. The values are given as nmole per mg microsomal protein. Symbols indicate mean ± S.E.M. of four animals.

RESULTS

It was previously found under similar experimental conditions that LD10 of acetaminophen (A) was about 4.25 kg BW [16] and of allyl alcohol (AA) about 100 μl/kg BW [17]. Simultaneous administration of these doses resulted in a mortality of 71% (N = 28) and reduction of these doses to 3.0 g/kg BW plus 75 μl/kg BW, respectively, reduced the mortality to 14% (N = 28). The latter doses therefore were chosen for the present experiments to permit comparison.

The time course of galactose elimination capacity (GEC) is depicted in Fig. 1(a), showing that the combined treatment with acetaminophen and allyl alcohol decreased GEC from 2.78 ± 0.24 μmole/min (mean ± S.E.M.) to 1.08 ± 0.41 6 hr later ($P < 0.05$). After longer periods, GEC recovered and was restored to control values after 24 hr.

Figure 1(b) shows that prothrombin index was reduced from 1.05 ± 0.11 arbitrary units (mean ± S.E.M.) to 0.40 ± 0.16 after 6 hr. After longer periods the prothrombin index gradually increased but did not recover within 96 hr. Microsomal cytochrome P-450 (Fig. 1c) showed a slight elevation after 6 and 12 hr and decreased after longer periods.

Serum alanine aminotransferase was elevated 3 hr after administration of A together with AA compared to 3 hr after administration of A alone (Table 1, $P < 0.05$). The elevation was more pronounced after 6 hr, and higher in the group treated with AA compared with animals treated with A, the difference not significant.

Depletion of hepatic glutathione 3 and 6 hr after AA and after A is given in Table 2. Glutathione was depleted more by AA after an interval of 3 hr. After 6 hr there was no difference.

After an interval of 3 hr, prothrombin index was more decreased after treatment with AA than 3 hr after treatment with A ($P < 0.05$, Table 1). After a 6 hr interval there was a similar difference, although not statistically significant.

Light microscopy revealed centrilobular and periportal hepatic necrosis. Periportal necrosis appeared within 6–12 hr after treatment, while centrilobular necrosis appeared from 24 to about 96 hr (Fig. 2). The maximum extent of the total necrotic part, i.e. periportal plus centrilobular necrosis, comprised less than 20% of the lobule.

Allyl alcohol was not detectable in aortic blood obtained 6 hr after treatment. The arterial acetaminophen plasma concentration was at a maximum after 6 hr, 91.0 ± 54.1 μmole/l (mean ± S.E.M.), but

Table 1

Time after dosing	Serum alanine transferase (IU) Control value	Treatment A	Treatment AA	Prothrombin index (arbitrary units) Control value	Treatment A	Treatment AA
3 hr	19.4 ± 6.1 (N = 9)	14.1 ± 1.6 (N = 9)	43.6 ± 12.7* (N = 7)	1.05 ± 0.11 (N = 4)	0.49 ± 0.04 (N = 9)	0.38 ± 0.02* (N = 7)
6 hr	19.4 ± 6.1 (N = 9)	129 ± 89 (N = 6)	200 ± 70 (N = 7)	1.05 ± 0.11 (N = 4)	0.42 ± 0.05 (N = 5)	0.31 ± 0.03 (N = 7)

A, Acetaminophen 3 g/kg BW.
AA, Acetaminophen 3 g/kg BW and allyl alcohol 75 μl/kg BW.
* Indicate $P < 0.05$ (A vs AA).

Table 2. Total hepatic glutathione (μmole/g liver)

Time after dosing	Control value	Treatment A	Treatment AA
3 hr	4..74 ± 0.23 (N = 4)	2.11 ± 1.6 (N = 9)	1.07 ± 0.26* (N = 6)
6 hr	4.74 ± 0.23 (N = 4)	1.14 ± 0.24 (N = 7)	1.40 ± 0.17 (N = 8)

A, Acetaminophen 3 g/kg BW.
AA, Acetaminophen 3 g/kg BW and allyl alchol 75 μl/kg BW.
* Indicates $P < 0.05$ (A vs AA).

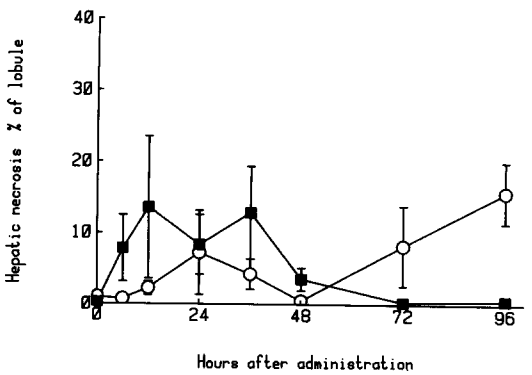

Fig. 2. Hepatic necrosis at various intervals after administration of acetaminophen and allyl alcohol. The values are the mean of five readings of the radius of centrilobular and periportal necrosis expressed as per cent of the lobule radius in five randomly chosen lobules in each biopsy. Open circles indicate periportal necrosis, closed squares indicate centrilobular necrosis. Symbols represent mean ± S.E.M. of four animals.

concentrations of about 50% of this were found after the longer intervals.

There was no difference in the urinary excretion of acetaminophen, acetaminophen-glucuronide, -sulfate, or -mercapturate between the animals treated with acetaminophen and with acetaminophen given together with allyl alcohol during the four

Table 3. Urinary excretion of acetaminophen and acetaminophen metabolites after treatment with acetaminophen (A) and after treatment with acetaminophen plus allyl alcohol (A + AA)

Metabolite	0–24 hr	24–48 hr	48–72 hr	72–96 hr	Total
Glucuronide					
A + AA	615 ± 60	579 ± 95	702 ± 181	74.5 ± 42	1970 ± 163
A	716 ± 100	653 ± 148	268 ± 98	113 ± 52	1751 ± 171
Sulfate					
A + AA	249 ± 14	231 ± 36	204 ± 46	48 ± 14	733 ± 76
A	233 ± 21	262 ± 28	139 ± 35	41.9 ± 35	674 ± 53
Mercapturate					
A + AA	97.6 ± 9	100 ± 21	140 ± 25	29.2 ± 8	363 ± 28
A	85 ± 12	131 ± 17	55 ± 13	29.7 ± 12	297 ± 22
Acetaminophen					
A + AA	190 ± 27	104 ± 26	210 ± 60	24.6 ± 9	528 ± 38
A	253 ± 51	147 ± 30	137 ± 30	35.3 ± 12	572 ± 82

A, Acetaminophen 3.0 g/kg BW.
A + AA, Acetaminophen 3.0 g/kg BW plus allyl alcohol 75 μl/kg BW.
Values are in mmole and are given as mean ± S.E.M. of seven animals.
The animals were housed individually in metabolic cages and their urine collected over dry ice for periods of 24 hr.

periods of 24 hr. The recovery of the acetaminophen dose was 87.2 ± 6.2% (mean ± S.E.M., N = 7) in the group of animals treated with acetaminophen plus allyl alcohol, and 82.5 ± 9.6% in the group treated with acetaminophen alone. The data are given in Table 3.

DISCUSSION

The combined administration of acetaminophen and allyl alcohol in doses that given separately produced a mortality about 10% [16, 17] resulted in a mortality of about 70%, suggesting a potentiating effect of the combined treatment.

For both toxins hepatotoxicity is considered to arise from formation of toxic metabolites by enzymes that are localized exclusively to the liver. Although mortality has been used as a measure of hepatotoxicity [18, 19] extrahepatic toxicity may contribute to increased mortality.

The GEC, considered a quantitative measure of hepatocellular cytosolic function [7], is unchanged after separate administration of both toxins [16, 17], probably due to rapidly occurring compensatory hyperfunction, as seen following 70% partial hepatectomy [20]. The combined intoxication depressed the GEC to 0.39 times control value.

The prothrombin index (PP) is regarded a quantitative measure of hepatic function [16, 17]. It is depressed dependently on the dose of acetaminophen and correlated to the histological changes in the liver [16]. After administration of acetaminophen, in a dose corresponding to LD_{10}, PP is only slightly reduced, and the same is the case after allyl alcohol at LD_{10} [17]. Concomitant administration of the smaller doses of both hepatotoxins which only slightly influences PP depresses PP to 0.38 times control value and it recovers much more slowly than after higher dose of the two toxins which gave a similar reduction of PP [16, 17].

The early and more pronounced elevation of serum alanine amino transferase activity following treatment with acetaminophen and allyl alcohol as compared to treatment with acetaminophen alone (Table 1), a similar pattern in the depression of PP, and the more severe impact on the quantitative measures of hepatic functions indicate that the combined treatment is more hepatotoxic than acetaminophen alone.

Since allyl alcohol is rapidly and almost exclusively metabolized to the aldehyde acrolein [21] increased formation of this toxic metabolite is unlikely. On the other hand only a small portion of acetaminophen is converted to the supposedly toxic metabolite and changes in the toxic pathway are likely to modulate the toxicity [22]. Since the toxic metabolite has not yet been identified it cannot be tested if allyl alcohol produces such a change. However, the toxic metabolite is presumed to arise from hepatic cytochrome P-450 mediated oxidation which is reflected by the amount of acetaminophen mercapturate excreted into the urine [22, 23]. The urinary excretion of acetaminophen mercapturate was identical in animals treated with acetaminophen alone and in animals treated with acetaminophen and allyl alcohol (Table 3). Cytochrome P-450 was reduced slightly by the combined treatment. This makes increased metabolic activation of acetaminophen less likely, but does not exclude it.

Toxic doses of acetaminophen depresses hepatic glutathione synthesis [24]. Whether this also is the case in other kinds of hepatic damage is not known. Hepatic glutathione was depressed earlier after the combined treatment (Table 2), but after 6 hr there was no difference. This indicates that the combined treatment may influence the glutathione detoxification system.

Histological changes after the combined treatment were descreet, consisting of small centrilobular and periportal changes contrasting the marked functional impairment, indicated by GEC and PP.

These observations are interpreted as evidence of a potentiating effect of simultaneous exposure to two toxins in doses which are relatively harmless *per se*. Potentiation may be due to the fact that the toxins preferentially affect different parts of the liver lobule, thereby preventing compensatory hyperfunction of less intoxicated liver cells. This phenomenon is more clearly revealed by functional than by histological studies, because functional impairment preceeds cell death, and probably only causes cell death, when it is severe. This situation may well be relevant to situations, e.g. in occupational medicine, where persons are exposed to several hepatotoxins, even if each of them occurs in amounts assumed to be harmless.

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