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Acute Ethanol Administration Reduces the Antidote Effect of N-Acetylcysteine after Acetaminophen Overdose in Mice

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1 The combined antidote effect of N-acetylcysteine and ethanol on the toxicity of acetaminophen was investigated.

2 Fed male mice were given acetaminophen i.p. (600 mg kg⁻¹) and after 5 min in addition ethanol i.p. (0.2 ml, 19% v/v), N-acetylcysteine i.p. (1.2 g kg⁻¹, 0.2 ml), N-acetylcysteine + ethanol i.p. (same doses as given individually) or saline i.p. (0.4 ml). Survival rates were determined after 24, 48, 72 and 96 h.

3 In the N-acetylcysteine group the survival rate was 85%. This rate was significantly reduced to 43% in the N-acetylcysteine + ethanol group ($P = 0.0001$). In the groups given ethanol or saline alone only 7% and 3%, respectively, survived 96 h.

4 The data suggest that the protective effect of N-acetylcysteine on acetaminophen-induced toxicity in fed mice is reduced by concomitant administration of ethanol. This may explain the clinical observation that ingestion of ethanol worsens the prognosis after acetaminophen intoxication.

Introduction

Acetaminophen (APAP) self-poisoning is still a major toxicological problem.¹ N-acetylcysteine (NAC) protects humans,²⁻⁴ rats⁵ and mice^{6,7} from the toxic effects of APAP, supposedly by increasing the intracellular availability of glutathione for conjugation of an electrophile APAP intermediate.⁸ It is now generally agreed that NAC is the preferred treatment of APAP intoxication.

A combination of APAP intoxication and ethanol (EtOH) ingestion is common - in a study from 1979 EtOH was found in the blood of 56% of 237 cases of lethal APAP intoxication in England and Wales.⁹ The prognostic influence of concomitant EtOH ingestion has been debated,¹⁰ reflecting the complex role of EtOH in APAP intoxication. Timing seems to be an important factor. Patients chronically abusing EtOH are more susceptible to the hepatotoxic effects of APAP.¹¹ Similar observations have been made in mice, where chronic as well as acute EtOH administration before APAP intoxication enhanced APAP hepatotoxicity.¹² In contrast, acute EtOH administration is reported to reduce APAP toxicity in humans after therapeutic

dosing, denoted by the reduction of toxic metabolites.¹³ If EtOH was administered to mice after APAP intoxication there was a protective effect, denoted by the increase in transaminases¹⁴ and prolonged survival.⁶

A possible beneficial role of EtOH in APAP intoxication is in contrast to the clinical impression that concomitant EtOH ingestion worsens the prognosis. Since NAC at present is administered as an antidote in the treatment of APAP intoxication, we hypothesized that EtOH would reduce the antidote effect of NAC. This hypothesis was studied in mice.

Methods

Male mice, B6D2 hybrids, (25-35 g) were obtained from Bomholtgård Breeding and Research Centre Ltd, Ry, Denmark. One-hundred-and-fifty-eight fed mice were given APAP i.p. (600 mg kg⁻¹) in a volume of 0.5 ml. The animals were then divided into four groups and within 5 min given EtOH i.p. (0.2 ml, 19% v/v, $n = 40$), NAC i.p. (1.2 g kg⁻¹, 0.2 ml, $n = 40$),

EtOH + NAC i.p. (same doses as given individually, $n = 40$) or saline i.p. (0.4 ml, $n = 38$). The EtOH dose corresponds to 23 mmol kg⁻¹, a non-toxic dose in mice.^{6,15}

Survival was determined 24, 48, 72 and 96 h after APAP injection. Survival rates in the treatment groups are reported with 95% confidence limits. Group comparisons were statistically evaluated by Fisher's exact test. A figure of 5% was chosen as the level of significance.

Results

Survival data are illustrated in Figure 1. Treatment with APAP and saline was followed by a 3% survival rate [1/38; 0–14% (95% confidence limits)]. After treatment with APAP and EtOH the survival rate was 7% [3/40; 2–20%]. In both groups all deaths occurred within 24 h. Treatment with APAP and NAC increased the survival rate to 98% [39/40; 87–100%] after 24 h, 95% [38/40; 83–99%] after 48 h, 88% [35/40; 73–96%] after 72 h and 85% [34/40; 70–94%] after 96 h. Treatment with APAP and NAC in combination with EtOH was followed by a 73% survival rate [29/40; 56–85%] after 24 h, 55% [22/40; 38–71%] after 48 h and 43% [17/40; 27–59%] after 72 and 96 h. The APAP + NAC + EtOH treatment was significantly different ($P < 0.005$) from treatment with APAP + NAC, APAP + EtOH or APAP + saline. Survival in the APAP + EtOH group (7%) was higher than in the control group receiving APAP + saline (3%), but this trend

towards a beneficial effect of EtOH was not statistically significant.

Discussion

The clinical impression that the prognosis of APAP intoxication is worsened by the concomitant ingestion of alcohol is apparently in conflict with survival data from studies on laboratory animals and metabolic studies in humans taking non-toxic doses of APAP, suggesting a protective effect of EtOH. Since NAC is now generally administered as the treatment of choice after APAP overdose it was hypothesized, that EtOH would reduce the antidote effect of NAC, and that this was the reason for the clinically observed deleterious effect of EtOH on APAP intoxication. In the present study this was investigated in mice.

The 96-h survival rate after APAP overdose was 3% in the control group, treated with saline, and increased more than 20-fold to 85% in the group of mice treated with NAC. This reproduces the known protective effect of NAC on APAP intoxication. When a combination of NAC and EtOH was given, the 96-h survival rate was only 43%, significantly lower than after NAC treatment alone. Theoretically, the observed effect of EtOH could be due to a local effect of EtOH (reducing the uptake of NAC from the peritoneal cavity), an additional toxic effect of EtOH, or an interaction of EtOH with NAC. Since amino acids, and amino acid analogues, like NAC, are known to be rapidly absorbed over the peritoneal membrane, which covers a large area in the abdomen of the mouse, it would seem unlikely, if EtOH was able to dilute or exert an osmotic effect that reduced the absorption of NAC in any significant degree. In other studies, the dose EtOH given (0.2 ml 19% v/v) is reported to be non-toxic in mice,^{6,15} so an additional toxic affect of EtOH would not be expected to be responsible for the substantial difference between survival in the APAP + NAC group and the APAP + NAC + EtOH group. Therefore the most reasonable interpretation of our data, is that EtOH interacts with NAC by reducing its antidote effect. The mechanism for such an interaction could be explained by EtOH inhibiting the metabolism of NAC to GSH¹⁶ combined with an increased loss of GSH, also caused by EtOH.¹⁷ The result would be reduced synthesis of GSH for APAP detoxification.

In contrast to other studies,^{6,14} EtOH alone (96-h survival rate 7%) was not significantly more protective than saline (96-h survival rate 3%). However, a minor protective effect of EtOH could be overlooked due to a type-2 error.

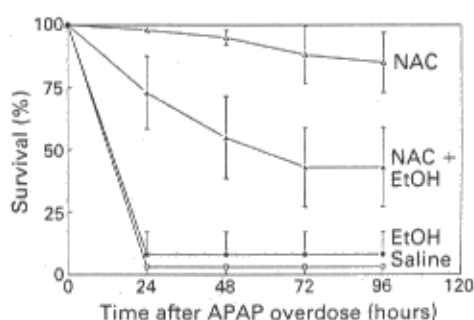


Figure 1 Survival rate of mice treated with APAP i.p. (600 mg kg⁻¹) and NAC ip (1.2 g kg⁻¹, 0.2 ml, Δ), EtOH i.p. (23 mmol kg⁻¹, 0.2 ml, \bullet), NAC + EtOH i.p. (same doses as given individually, \blacktriangle) or saline i.p. (0.4 ml, \circ). APAP + NAC vs APAP + saline ($P < 0.00005$), APAP + NAC vs APAP + EtOH ($P < 0.00005$), APAP + NAC + EtOH vs APAP + NAC + EtOH ($P = 0.0001$), APAP + NAC + EtOH vs APAP + saline ($P < 0.00005$), APAP + NAC + EtOH vs APAP + EtOH ($P = 0.0005$), APAP + saline vs APAP + EtOH ($P = 0.66$).

Considering the number of experimental animals employed, this risk was calculated to be 58%. The difference between survival rates were 4% in favour of EtOH (3% vs 7%, respectively) and with the sample size chosen, the 95% confidence limits of this difference was -5% to 14%. Therefore a difference of more than 14% in favour of EtOH is unlikely. In the discussion of the differences in the survival rates the feeding status of the experimental animals may be important. The toxic effect of APAP is caused by an electrophile metabolite generated by cytochrome P450 catalysed oxidation dependent on a cytosolic NADPH regenerating system.¹⁸ The reactive metabolite is detoxified by glutathione.²⁻⁸ In fasted rats and mice concomitant EtOH administration protects against APAP hepatotoxicity.^{6,8,19} The results obtained in fasted rats suggest that the antidote effect of EtOH is related to a decreased availability of NADPH in the cytosol secondary to inhibition of the mitochondrial/cytosol substrate shuttle systems. The tricarboxylate and maleate/pyruvate shuttles are the primary means for transferring

NADPH-reducing equivalents from mitochondria to cytosol.²⁰ These shuttles are dependent on Krebs' cycle intermediates which are depleted when Krebs' cycle is inhibited by NADH produced by dehydrogenation of EtOH and acetaldehyde.¹⁹ In the fed mice used in the present study, sufficient cytosolic NADPH may be available for generation of the toxic APAP metabolite, despite EtOH administration. Thus, a potential protective effect of EtOH against APAP hepatotoxicity in fed mice may be reduced compared to fasted animals.

In conclusion, this study demonstrated that EtOH administration reduced the hepatoprotective effect of NAC after APAP overdose. We could not demonstrate a statistically significant hepatoprotective effect of EtOH alone, a result which might be related to the feeding status of the experimental animals. If EtOH also reduces the antidote effect of NAC after APAP overdose in humans, concomitant EtOH ingestion worsens the prognosis after APAP intoxication; this is consistent with the clinical observations.

References

- Meredith TJ, Prescott LF & Vale JA. Why do people still die from paracetamol poisoning? *British Medical Journal* 1986; **293**: 345-6.
- Prescott LF, Ballantine GR, Park J, Adriaenssens P & Proudfoot AT. Treatment of paracetamol (acetaminophen) poisoning with N-acetylcysteine. *Lancet* 1977; **ii**: 432-4.
- Rumack BH. Acetaminophen overdose. *American Journal of Medicine* 1983; **5**: 104-12.
- Prescott LF. Paracetamol overdosage. Pharmacological considerations and clinical management. *Drugs* 1983; **25**: 290-314.
- Piperno E & Berssenbruegge DA. Reversal of experimental paracetamol toxicosis with N-acetylcysteine. *Lancet* 1976; **ii**: 738-9.
- Banda PW & Quart BD. The effect of alcohol on the toxicity of acetaminophen in mice. *Research Communications in Chemical Pathology and Pharmacology* 1984; **43**: 127-38.
- Massey TE & Racz WJ. Acetaminophen toxicity and its prevention in isolated mouse hepatocyte suspension. In: *Isolation, Characterization and Use of Hepatocytes*, eds Harris RA, Connoll NW, pp. 379-84. Amsterdam: Elsevier Science Publishing Co., Inc., 1983.
- Lauterburg BH, Corcoran GB & Mitchell JR. Mechanism of action of N-acetylcysteine in the protection against the hepatotoxicity of acetaminophen in rats *in vivo*. *Journal of Clinical Investigation* 1983; **71**: 980-91.
- Vale JA, Buckley BM & Meredith TJ. Deaths from paracetamol and dextropropoxyphene (Distalgesic) poisoning in England and Wales in 1979. *Human Toxicology* 1984; **3**: 135-43.
- Gomolin IH. Ethanol in paracetamol poisoning (Letter to the editor). *Lancet* 1983; **ii**: 617-8.
- McClain CJ, Kromhout JP, Peterson FJ & Holtzman JL. Potentiation of paracetamol hepatotoxicity by alcohol. *Journal of the American Medical Association* 1980; **244**: 251-3.
- Carter EA. Enhanced acetaminophen toxicity associated with prior alcohol consumption in mice: Prevention by N-acetylcysteine. *Alcohol* 1987; **4**: 69-71.
- Critchley JAJH, Dyson EH, Scott AW, Jarvie DR & Prescott LF. Is there a place for cimetidine or ethanol in the treatment of paracetamol poisoning. *Lancet* 1983; **i**: 1375-6.
- Wong LT, Whitehouse LW, Solomonraj G & Paul CJ. Effect of a concomitant single dose of ethanol on the hepatotoxicity and metabolism of acetaminophen in mice. *Toxicology* 1980; **17**: 297-309.
- Banda PW & Quart BD. The use of N-acetylcysteine long after an acetaminophen overdose in mice. *Toxicology Letters* 1987; **36**: 89-94.
- Lauterburg BH, Davies S & Mitchell JR. Ethanol suppresses hepatic glutathione synthesis in rats *in vivo*. *Journal of Pharmacology and Experimental Therapy* 1984; **230**: 7-11.
- Speisky H, MacDonald A, Giles G, Orrego H & Israel Y. Increased loss and decreased synthesis of hepatic glutathione after acute ethanol administration. *Biochemical Journal* 1985; **225**: 565-72.
- Dahlin DC, Miwa GT, Lu AYH & Nelson SD. N-acetyl-p-benzoquinone imine: A cytochrome P-450-mediated oxidation product of acetaminophen. *Proceedings of the National Academy of Science USA* 1984; **81**: 1327-31.
- Thummel KE, Slattery JT & Nelson SD. Mechanism by which ethanol diminishes the hepatotoxicity of acetaminophen. *Journal of Pharmacology and Experimental Therapy* 1987; **245**: 129-36.
- Weigl K & Sies H. Drug oxidations dependent on cytochrome P-450 in isolated hepatocytes. *European Journal of Biochemistry* 1977; **77**: 401-8.

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