In vitro acetaldehyde formation by human colonic bacteria

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Abstract

Incubation of human colonic contents with various ethanol concentrations (2·75–44 mM) in vitro at 37°C resulted in significant accumulation of acetaldehyde – a toxic and highly reactive compound. At pH 9·6, all samples produced notable acetaldehyde concentrations (58 (13) μM; mean (SEM)) even from the lowest (2·75 mM) ethanol concentration, and the production of acetaldehyde increased linearly with rising ethanol concentration ($r=0·97$; $p<0·005$), reaching a peak concentration of 238 (37) μM at 44 mM ethanol. The formation of acetaldehyde took place rapidly, as almost 50% of acetaldehyde formed during the total eight hour incubation was detectable after one hour, and 75% of the total after four hours. Maximal acetaldehyde production from 22 mM ethanol occurred at pH 9·6 (160 (35) μM) but appreciable concentrations were also seen at pH 7·4 (110 (38) μM) and pH 6·0 (63 (19) μM). At pH 4·0, by contrast, acetaldehyde formation was negligible (17 (5) μM). 4-Methylpyrazole, a potent inhibitor of alcohol dehydrogenase, showed a decreasing effect on acetaldehyde production in vitro but first at a concentration of 100 mM. Considerable acetaldehyde production by human colonic bacteria – if it occurs also in vivo – could constitute a risk factor for rectal cancer in heavy drinkers and also provide a pathogenetic mechanism for alcohol induced diarrhoea.

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Alcohol consumption has been incriminated as a risk factor for oropharyngeal,[1] [2] laryngeal,[3] [4] oesophageal[5] [6] as well as rectal[7-9] cancer. Furthermore, excessive ethanol consumption has also been related with rapid intestinal transit time and diarrhoea.[10] [11] The pathogenetic mechanisms behind the ethanol associated gastrointestinal morbidity have, however, not been fully elucidated.

Acetaldehyde produced by colonic flora has been suggested to participate in ethanol associated carcinogenesis of the colon in rats.[12] Furthermore, incubation of human faecal homogenates with ethanol has been reported to lead to accumulation of higher alcohols and other unidentified metabolites.[13] Theoretically, individual differences in the susceptibility to ethanol induced organ injury might, consequently, result from differences in the metabolic products of human colonic bacteria.[13] Significant in vitro production

of acetaldehyde occurs also when human bronchopulmonary washings are incubated with ethanol. This acetaldehyde is thought to be formed by micro-organisms in the bronchopulmonary tract, and is suggested to participate in the pathogenesis of upper respiratory cancer.[14]

This study was aimed at investigating the kinetics of acetaldehyde production by human colonic bacterial flora in vitro, by incubating colonic contents obtained during colonoscopy in various ethanol concentrations at different pH values and for different time periods. Moreover, the effect of a well known alcohol dehydrogenase inhibitor, 4-methylpyrazole, on acetaldehyde formation by colonic contents was tested.

Methods

4-Methylpyrazole was obtained from Aldrich Chemical Co (Milwaukee, MI, USA). Colonic contents were collected from 26 patients (11 females, 15 males) undergoing colonoscopy for lower gastrointestinal symptoms. The age of the patients ranged from 24 to 67 years. Before the procedure bowel preparation was carried out in a conventional manner including a 48 hour full liquid diet, total abstinence from food on the procedure day, and colonic cleaning by drinking of four to six litres of Colonsteril, a balanced electrolyte solution (Medipolar, Oulu, Finland). During the colonoscopy, about 5–10 ml of colonic content was aspirated through the fibrescope from the sigmoid, transverse colon, and the caecum. The samples were then stored at +4°C (maximum five hours) in closed vials until they were pooled and the pH was measured immediately before the incubation procedure. A 250 μl aliquot of the colonic contents (pH ranging from 7·47 to 8·43) was then incubated in closed vials for two hours at 37°C with 250 μl glycine buffer (pH 9·6) containing different ethanol concentrations (final concentrations varying from 0 to 44 mM). The effect of pH on acetaldehyde formation was evaluated by incubating colonic contents with 22 mM ethanol prepared either in a 1 M citrate (pH 4·0 and 6·0), a 1 M potassium phosphate (pH 7·4) or a 1 M glycine (pH 9·6) buffer. In addition, the velocity of acetaldehyde formation was studied by incubating three of the colonic suspensions with 22 mM ethanol in a similar fashion for different time periods (one to 8 hours). The effect of 4-methylpyrazole on acetaldehyde production by the colonic contents in vitro was tested by incubating colonic contents with 1 M glycine buffer (pH 9·6) containing 22 mM ethanol and

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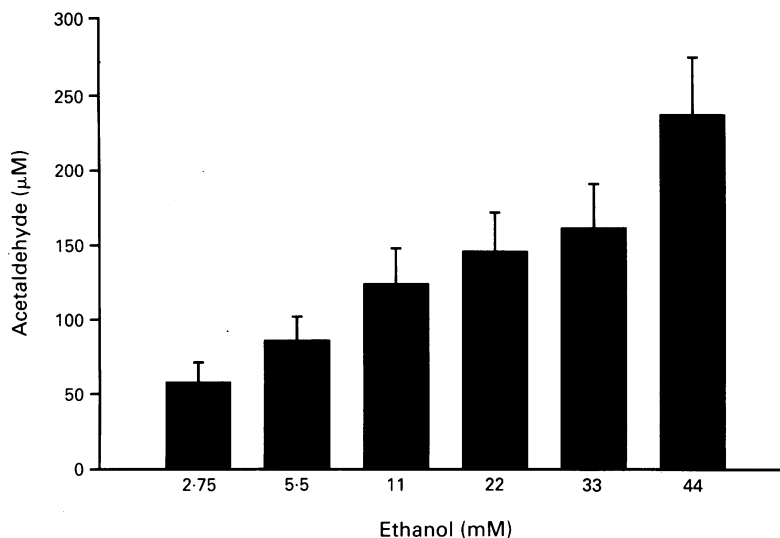
Figure 1: Acetaldehyde formation (mean (SEM)) by colonic contents (n=21) incubated at various ethanol concentrations at pH 9·6 at 37°C for two hours.

Figure 3: The velocity of acetaldehyde production by colonic contents at 22 mM ethanol at pH 9·6 at 37°C, expressed as percentage (mean (SEM) of total acetaldehyde detected after eight hours incubation.

increasing drug concentrations (0—100 mM) at 37°C for two hours. The acetaldehyde formed during the incubation was immediately analysed by head space gas chromatography by heating the vials to a temperature of 37°C as reported earlier.[15] Conditions for analysis were: Column 60/80 Carbopack B/5% Carbowax 20 M, 2 m×1/8" (Supelco Inc, Bellefonte, PA, USA); oven temperature, 85°C; transfer line and detector temperature, 200°C; carrier gas flow rate (N_2), 20 ml/min. The recovery of acetaldehyde with this method was tested and it was found to be 100%.

STATISTICS

All values are expressed as mean (SEM). The dependency between ethanol concentrations and acetaldehyde formation was tested by simple linear regression analysis.

Results

At pH 9·6, all samples of colonic contents produced significant acetaldehyde concentrations (58·4 (12·7) μM, mean (SEM)) even from a low (2·75 mM) ethanol concentration and the amount of acetaldehyde formed increased linearly with increasing ethanol
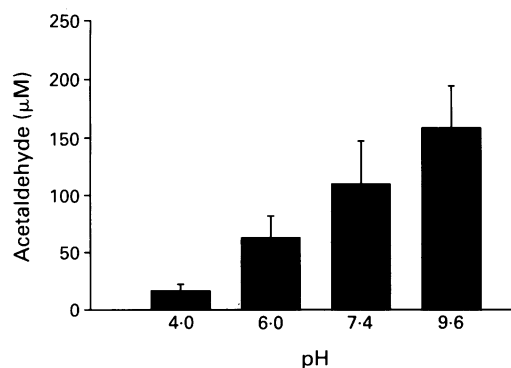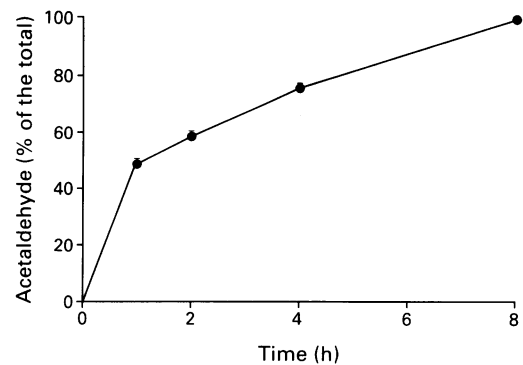

Figure 2: Acetaldehyde formation (mean (SEM)) by colonic contents (n=10) incubated at 22 mM ethanol at various pH values at 37°C for two hours.

concentration ($r=0·97$; $p<0·005$), reaching a peak of 238 (37) μM of acetaldehyde at 44 mM ethanol (Fig 1).

Maximal acetaldehyde production at 22 mM ethanol occurred at pH 9·6 (160 (35) μM) but noticeable concentrations were also seen at pH 7·4 (110 (38) μM) and pH 6·0 (63 (19) μM). By contrast, at pH 4·0, acetaldehyde formation was almost negligible (17 (5) μM) (Fig 2).

The formation of acetaldehyde took place rapidly as almost 50% of acetaldehyde formed during the total of eight hours incubation was detectable after one hour, and 75% of the total after four hours at 37°C (Fig 3).

Acetaldehyde production was significantly inhibited by 4-methylpyrazole but first at a high concentration of the compound (at 22 mM ethanol acetaldehyde production at 100 mM 4-methylpyrazole 50 (10)% of control, Fig 4).

Discussion

Acetaldehyde, the first metabolite of ethanol oxidation, is formed in the body mainly by liver alcohol dehydrogenase. To a smaller extent, ethanol is also oxidised to acetaldehyde in other tissues such as kidneys, respiratory tract, intestine, and bone marrow but until recently this metabolism has been thought to be of minor importance.[16 17] The finding that gastric mucosa can metabolise ethanol in significant amounts[18 19] has, however, again recently created interest in other sites of ethanol metabolism as well. This has in part been a consequence of the fact that acetaldehyde, as a highly reactive compound, can be linked to several organ toxic effects of ethanol, although the exact pathogenetic mechanisms behind this toxicity have so far remained obscure.[20 21] Recently, however, acetaldehyde has been shown to bind covalently with macromolecules and proteins thus forming acetaldehyde adducts[22-24] and the formation of these adducts is proportional to the concentration of acetaldehyde.[25] Adducts formed can interfere with normal cellular functions[26] – that is, a human DNA repair protein, O^6-methylguanine transferase, has been shown to be inhibited by acetaldehyde at nanomolar concentrations.[27] Moreover, adducts also act as neoantigens with a consequent immune response.[28 29] Both of
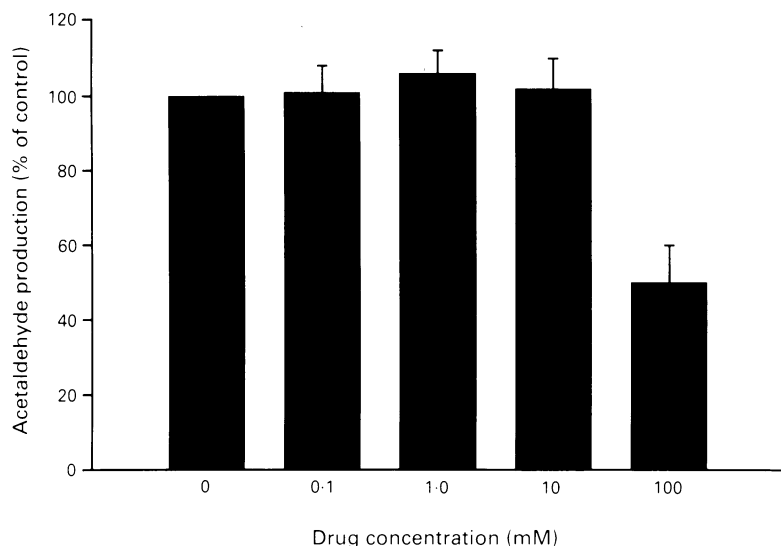
Figure 4: The effect of 4-methylpyrazole on acetaldehyde formation by colonic contents incubated at 22 mM ethanol at pH 9·6 at 37°C for two hours, expressed as percentage (mean (SEM)) of control without the drug.

these phenomena can lead to cellular destruction. In this respect, the probability of alcohol induced organ damage may be proportional to the organ's capacity to metabolise ethanol to acetaldehyde.

Earlier studies have shown that oral ethanol given to rats results in accelerated chemically induced rectal carcinogenesis[30] and also increased mucosal acetaldehyde concentrations in the descending colon compared with the ascending colon.[12] This increase in acetaldehyde concentration was paralleled by a significant increase in the number of anaerobic bacteria.[12] Moreover, rats with a jejunal self filling diverticulum (and consequent bacterial overgrowth) show a noticeable increase in portal acetaldehyde concentrations after intragastric ethanol when compared with controls with a self emptying diverticulum, and incubation of diverticular contents with ethanol leads to fairly high acetaldehyde concentrations.[31] These findings strongly advocate the role of intestinal bacteria in the oxidation of ethanol to acetaldehyde, which in turn could mediate some of the gastrointestinal toxicity of ethanol in laboratory rats.

The concentrations of ethanol in the ileum after alcohol ingestion are similar to those of the vascular space.[32] Our results show that human colonic contents are capable of oxidising ethanol to acetaldehyde in vitro at the comparatively low ethanol concentrations known to exist in the colon during normal drinking. Moreover, this acetaldehyde formation takes place at a pH normally found in the colon, although the production is enhanced by increasing pH. Colonic bacteria could thus provide a clinically significant source of acetaldehyde in the large bowel and this acetaldehyde could possibly lead to toxic changes in the intestinal wall. Resulting cellular injury could then lead to compensatory hyperregeneration, which by itself can favour carcinogenesis. Furthermore, acetaldehyde produced in the intestine and reaching the liver by the portal circulation could also potentiate the hepatotoxicity of acetaldehyde originating in the liver, thus making subjects with certain

types of colonic microflora more vulnerable to alcohol induced hepatic injury than others. Acetaldehyde might also have an important role in the pathogenesis of alcohol induced diarrhoea, a well reported, but poorly understood symptom among alcoholics.[10 11 33]

In this study we had no opportunity to obtain bacteriological data from the faecal samples and samples were not stored or incubated strictly anaerobically, which may have reduced the ratio of anaerobic/aerobic organisms. The rather large variation seen in the acetaldehyde forming capacity in this study could, therefore, be explained by different bacterial counts, as well as different bacterial viability in the samples. Further studies are, consequently, already in progress in our laboratory to establish the capacity of various colonic bacterial strains to metabolise ethanol to acetaldehyde in vitro.

As described in this paper, colonic acetaldehyde production is rapidly reduced with lowering of the pH. Dietary fibres decrease the pH of colonic content,[34] and also have a protective role against colonic cancer.[35 36] The mechanism behind this protection could be the reduced colonic acetaldehyde production. Therefore, the possible role of dietary factors in the regulation of colonic pH as well as in production of acetaldehyde also warrants further studies.

In this study, 4-methylpyrazole showed no inhibition on acetaldehyde formation by the colonic content until at a concentration of 100 mM. This finding could reflect poor penetration of 4-methylpyrazole through the bacterial wall or merely, insensitivity of the bacterial alcohol dehydrogenase to the inhibitory effect of 4-methylpyrazole in vitro, as previously speculated in the case of *Helicobacter pylori*.[37] Studies on the effect of aldehyde dehydrogenase inhibitors on acetaldehyde production by colonic contents have already started in our laboratory.

1 Graham S, Dayal H, Rohrer T, Swanson M, Sultz H, Shedd D, *et al.* Dentition, diet, tobacco and alcohol in the epidemiology of oral cancer. *J Natl Cancer Inst* 1977; **59:** 1611–6.
2 McCoy GD. A biochemical approach to the etiology of alcohol related cancers of the head and neck. *Laryngoscope* 1978; **88** (suppl 8): 59–62 (part 2).
3 Wynder EL, Covey LS, Mabuchi K, Muskinski M. Environmental factors in cancer of the larynx. Second look. *Cancer* 1976; **38:** 1591–601.
4 Tuyns A. Epidemiology of alcohol and cancer. *Cancer Res* 1979; **39:** 2840–3.
5 Wynder EL, Mabuchi K. Etiological and environmental factors in esophageal cancer. *JAMA* 1973; **226:** 1546–8.
6 Schottenfeldt D, Gantt RC, Wynder EL. The role of alcohol and tobacco in multiple primary cancers of the upper digestive system, larynx and lung. A prospective study. *Prev Med* 1974; **3:** 277–93.
7 Breslow NE, Enstrom JE. Geographic correlations between cancer mortality rates and alcohol-tobacco consumption in the United States. *J Natl Cancer Inst* 1974; **53:** 631–9.
8 Kono S, Ikeda M. Correlation between cancer mortality and alcoholic beverages in Japan. *Br J Cancer* 1979; **40:** 449–55.
9 Pollack ES, Nomura AMY, Heilbrun LK, Stemmerman GN, Green SB. Prospective study of alcohol consumption and cancer. *N Engl J Med* 1984; **310:** 617–21.
10 Robles EA, Mezey E, Halsted CH, Schuster MM. Effect of ethanol on the motility of the small intestine. *Johns Hopkins Med J* 1974; **135:** 17–24.

11 Berenson MM, Avner DL. Alcohol inhibition of recto-sigmoid motility in humans. *Digestion* 1981; **22:** 210–5.

12 Seitz HK, Simanowski UA, Garzon FT, Rideout JM, Peters TJ, Koch A, *et al.* Possible role of acetaldehyde in ethanol-related rectal cocarcinogenesis in the rat. *Gastroenterology* 1990; **98:** 406–13.

13 Levitt MD, Doizaki W, Levine AS. Hypothesis: activity of the colonic bacteria influences organ injury from ethanol. *Hepatology* **1982; 5:** 598–600.

14 Miyakawa H, Baraona E, Chang JC, Lesser MD, Lieber CS. Oxidation of ethanol to acetaldehyde by broncho-pulmonary washings: role of bacteria. *Alcohol Clin Res Exp* 1986; **5:** 517–20.

15 Pikkarainen PH, Salaspuro MP, Lieber CS. A method for the determination of 'free' acetaldehyde in plasma. *Alcoholism* 1979; **3:** 259–61.

16 Lundquist F. The metabolism of alcohol. In: Israel Y, Mardones J, eds. *Biological basis of alcoholism.* New York: John Wiley, 1971: 1–52.

17 Peters TJ. Ethanol metabolism. *Br Med Bull* 1982; **38:** 17–20.

18 Lamboeuf Y, de Saint Blanquat G, Derache R. Mucosal alcohol dehydrogenase- and aldehyde dehydrogenase-mediated ethanol oxidation in the digestive tract of the rat. *Biochem Pharmacol* 1981; **30:** 542–5.

19 Julkunen RJK, DiPadova C, Lieber CS. First pass metabolism of ethanol: a gastrointestinal barrier against the systemic toxicity of ethanol. *Life Sci* 1985; **6:** 567–73.

20 Lieber CS, Baraona E, Matsuda Y, Salaspuro M, Hasumura Y, Matsuzaki S. Hepatotoxicity of acetaldehyde. In: Begleiter H, ed. *Biological effects of alcohol.* New York: Plenum, 1980: 397–411.

21 Salaspuro M, Lindros K. Metabolism and toxicity of acetaldehyde. In: Seitz HK, Kommerell H, eds. *Alcohol related diseases in gastroenterology.* Berlin: Springer-Verlag, 1985: 106–23.

22 Barry RE, Williams AJK, McGivan JD. The detection of acetaldehyde/liver plasma membrane protein adduct formed in vivo by alcohol feeding. *Liver* 1987; **7:** 364–8.

23 Lin RC, Smith SR, Lumeng L. Detection of protein-acetaldehyde in the liver of rats fed alcohol chronically. *J Clin Invest* 1988; **81:** 615–9.

24 Sillanaukee P, Seppä K, Koivula T. Effect of acetaldehyde on hemoglobin: HbA_{1ach} as a potential marker of heavy drinking. *Alcohol* 1991; **8:** 377–81.

25 Sillanaukee P, Koivula T. Detection of a new acetaldehyde-induced hemoglobin fraction HbA_{1ach} by cation exchange liquid chromatography. *Alcohol Alcohol* 1990; **6:** 842–6.

26 Sorrel M, Tuma D. Hypothesis: alcoholic liver injury and the covalent binding of acetaldehyde. *Alcoholism* 1985; **9:** 306–9.

27 Espina N, Lima V, Lieber CS, Garro AJ. In vitro and in vivo inhibitory effect of ethanol and acetaldehyde on O^6-methylguanine transferase. *Carcinogenesis* 1988; **9:** 761–6.

28 Niemelä O, Klajner F, Orrego H. Antibodies against acetaldehyde modified protein epitopes in human alcoholics. *Hepatology* 1987; **7:** 1210–4.

29 Laskin CA, Vidins E, Blendis L, Solonoka CA. Autoantibodies in alcoholic liver disease. *Am J Med* 1990; **89:** 129–33.

30 Seitz HK, Czygan P, Waldherr R, Veith S, Raedsch R, Kässmodel H, Kommerell B. Enhancement of 1,2-dimethylhydrazine-induced rectal carcinogenesis following chronic ethanol consumption in the rat. *Gastroenterology* 1984; **86:** 886–91.

31 Baraona E, Julkunen RJK, Tannenbaum L, Lieber CS. Role of intestinal bacterial overgrowth in ethanol production and metabolism in rats. *Gastroenterology* 1986; **90:** 103–10.

32 Halsted CH, Robles EA, Mezey E. Distribution of ethanol in the human gastrointestinal tract. *Am J Clin Nutr* 1973; **26:** 831–4.

33 Pirola RC, Davis AE. Effects of intravenous alcohol on the motility of the duodenum and of the sphincter of Oddi. *Australas Ann Med* 1970; **19:** 24–9.

34 Pye G, Crompton J, Evans DF, Clarke AG, Hardcastle JD. Effect of dietary fibre supplementation on colonic pH in healthy volunteers. *Gut* 1987; **28:** A1366–7.

35 Dales LG, Friedman GD, Ury HK, Grossman S, Williams SR. A case-control study of relationship of diet and other traits to colorectal cancer. *Am J Epidemiol* 1978; **109:** 132–44.

36 Heilbrun LK, Nomura A, Hankin JH, Stemmermann GN. Diet and colorectal cancer with special reference to fiber intake. *Int J Cancer* 1989; **44:** 1–6.

37 Kaihovaara P, Salmela KS, Roine RP, Kosunen TU, Salaspuro M. Purification and characterization of Helicobacter pylori alcohol dehydrogenase. *Alcoholism* (in press).