High intracolonic acetaldehyde values produced by a bacteriocolonic pathway for ethanol oxidation in piglets

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Abstract

Background—Human colonic contents and many colonic microbes produce considerable amounts of acetaldehyde from ethanol in vitro.

Aims—To examine in piglets if acetaldehyde is produced in the colon also in vivo, and if so, what is the fate of intracolonically formed acetaldehyde.

Animals—Seventeen native, non-fasted female piglets (20–25 kg) were used.

Methods—Six piglets received either 1.5 g/kg bw or 2.5 g/kg bw of ethanol intravenously. In seven piglets, 0–7 g or 1–75 g of ethanol/kg bw was administered intravenously, followed by a subsequent intragastric ethanol infusion of 1.8 g/kg bw and 4.5 g/kg bw, respectively. The samples of colonic contents for the assessment of ethanol and acetaldehyde concentrations were obtained up to seven hours. In four additional piglets, the intracolic values of ethanol, acetaldehyde, and acetate were observed for 60 minutes after an intracolonic infusion of acetaldehyde solution.

Results—A raised intracolonic, endogenous acetaldehyde concentration (mean (SEM): 36 (9) μM) was found in all piglets before ethanol infusion. After the infusion of ethanol, intracolic ethanol and acetaldehyde values increased in parallel, reaching the peak values 57 (4) mM of ethanol and 271 (20) μM of acetaldehyde in the group that received the highest dose of ethanol. A positive correlation (r=0.45; p<0.001) was found between intracolonic ethanol and acetaldehyde values. Acetaldehyde administered intracolicly was mainly metabolised to acetate but also to ethanol in the colon.

Conclusions—Significant endogenous intracolic acetaldehyde values can be found in the normal porcine colon. Furthermore, our results suggest the existence of a bacteriocolonic pathway for ethanol oxidation. Increased amounts of acetaldehyde are formed intracolically from ingested ethanol by this pathway.

Keywords: alcohol, ethanol, acetaldehyde, colonic bacteria, ethanol toxicity.

Acetaldehyde is the first product of ethanol oxidation in the body, and is mainly produced by the liver. As a highly reactive and toxic compound, acetaldehyde is generally considered to be an agent responsible for the development of alcoholic liver injury. Moreover, alcohol associated rectal carcinogenesis and cancer of the upper respiratory tract have recently been related to acetaldehyde derived from ethanol.

The toxicity of acetaldehyde has been proved with respect to the gonads and red blood cells. The toxic effects of acetaldehyde have been related to its ability to bind easily to different cellular proteins. Furthermore, acetaldehyde may induce generation of free radicals and subsequently lipid peroxidation. It has also been reported that comparatively low concentrations of acetaldehyde may impair cell proliferation and cause abnormalities in cell growth in vitro. Each of these mechanisms may lead to tissue damage.

We have recently reported that in the presence of ethanol, human colonic contents possess a marked capacity to produce acetaldehyde in vitro. If ethanol is oxidised to acetaldehyde in the colon also in vivo, this represents a new extrabiliary pathway for the metabolism of ethanol. Furthermore, intracolically formed acetaldehyde could exert its toxic effects either on the colonic mucosal cells, and contribute to the development of ethanol associated colonic cancer.

Therefore, the first aim of our study was to examine whether acetaldehyde is produced in the colon from ethanol also in vivo. The second aim was to study the metabolic fate of intracolically administered exogenous acetaldehyde.

Methods

Seventeen 3 month old (20–25 kg), Finnish native, non-fasted female piglets were used. Piglets were preferred to the other laboratory animals because of their numerous anatomical and physiological similarities with humans. In the first part of the study, colonic ethanol concentrations and the intracolonic concentration of acetaldehyde were examined after the administration of different doses of ethanol in 13 piglets. In the second part, the fate of acetaldehyde introduced into the colon was studied in four piglets.

Intracolonic ethanol and acetaldehyde concentrations

All piglets were premedicated with ketamine chloride 20 mg/kg bw followed by inhalation
anaesthesia with enflurane. The animals were normoventilated using a capnometer. To avoid interference with the hepatic enzyme activities, neither barbiturates nor benzodiazepines were used. The left external jugular vein was catheterised. Subsequently, the abdomen was opened, and a tube was placed transmurally into the caecum. Thereafter, three piglets (group I) received 1.5 g of ethanol/kg bw in 500 ml of physiological saline solution intravenously, and three piglets (group II) 2.5 g of ethanol/kg bw, respectively, intravenously over a time period of 30 minutes. Group III (four piglets) was given 0.7 g of ethanol/kg bw intravenously, and this was followed by an intragastric administration of 1.8 g/kg bw of ethanol in 1000 ml of water given via the oesophagus during the next 40 minutes. Three other piglets (group IV) received 1.75 g/kg bw of ethanol intravenously and 4.5 g/kg bw of ethanol intragastrically according to the same protocol. An intravenous infusion of ethanol was used to achieve without delay desired blood ethanol values. Double samples of venous blood and colonic contents for the determination of ethanol and intracolonic acetaldehyde concentrations were taken before starting the ethanol infusion (0 sample), at the end of the intravenous infusion (30 min), at the end of the intragastric infusion (70 min), and after that once every second hour up to seven hours.

Fate of intracolonically administered acetaldehyde
Four piglets were anaesthetised as described above. The abdomen was opened and two separate tubes were placed transmurally into the caecum. Thereafter, 500 ml of 5 mM acetaldehyde water solution was introduced intracolonically over a time period of 10 minutes. The colonic contents for the assessment of acetaldehyde, acetate, and ethanol concentrations were taken before starting acetaldehyde infusion (0 sample) and thereafter at five minutes intervals up to one hour. During both experiments the animals were carefully supervised and all respiratory and circulatory parameters were monitored.

For the determination of acetaldehyde, samples were processed according to the method described by Stowell and kept in ice until analysed. The ethanol samples were diluted 1:10 in ice cold water and put into vials. Acetaldehyde and ethanol concentrations in the samples were determined by head space gas chromatography at 65°C. Conditions for analysis were as follows: column 60/80 Carbopack B/5% Carbowax 20 M, 2 m × 1/8" (Supelco, Bellefonte, PA); oven temperature 85°C; dosing line and detector temperature 200°C; carrier gas (N2) flow rate 20 ml/min.

The study was conducted in accordance with the principles of the Declaration of Helsinki and approved by the Committee of Animal Experimentation of County Council.

Statistical analysis
All results are expressed as mean (SEM). The possible correlations were tested by using the linear regression analysis.

Results
Intracolonic ethanol and acetaldehyde concentrations
An increased intracolonolic, endogenous acetaldehyde concentration (36 (9) μM, range 8–118 (μM)) was found before ethanol administration. The mean intracolonic endogenous ethanol value was 0.4 (0–2) mM. During the intravenous infusion of ethanol, intracolonic ethanol increased slowly reaching the levels of 9 (3) mM, 16 (9) mM, 6 (1) mM, and 28 (12) mM of ethanol in the groups I, II, III, and IV, respectively. After the subsequent intragastric infusion in the groups III and IV, a further increase of ethanol up to 23 (5) mM and 57 (4) mM, respectively, was recorded in the colon (Fig 1). A highly significant positive correlation (r=0.85; p<0.001) was found between blood and intracolonic ethanol values.

A considerable increase in the intracolonic acetaldehyde concentration after the administration of ethanol could be recorded in all groups. Intracolonic acetaldehyde concentrations (Fig 2) increased in parallel with rising intracolonic ethanol values, reaching the peak level 57 (4) μM of ethanol and 271 (20) μM of acetaldehyde in the group that received the highest dose of ethanol. A significant positive correlation (r=0.45; p<0.001) between the intracolonic ethanol and acetaldehyde concentrations was found.

Fate of intracolonically administered acetaldehyde
The base endogenous acetaldehyde concentration in the colon was 24 (5) μM, ranging from 19 to 33 μM. The major part of acetaldehyde administered intracolonically was effectively metabolised in the colon. This associated with a significant increase in the intracolonnic acetate and ethanol concentrations (Fig 3).

Figure 1: Intracolonic ethanol concentrations (mean (SEM)) in 13 piglets before (0 min) and after the intravenous (iv) administration of 1.5 g (n=3) and 2.5 g (n=3) of ethanol/kg bw, and after the iv administration of 0.7 g (n=3) and 1.75 g (n=6) of ethanol/kg bw followed by the subsequent intragastric (ig) infusion of 1.8 g and 4.75 g of ethanol/kg bw, respectively.
concentrations in the administration of 1.5 g (n=3) and 2.5 g (n=3) of ethanol/kg bw, and after the iv administration of 0.75 g (n=3) and 1.75 g (n=4) of ethanol/kg bw followed by the subsequent intragastric (ig) infusion of 1.8 g and 4.75 g of ethanol/kg bw, respectively.

Figure 2: Intracolonic acetaldehyde concentrations (mean (SEM)) in 13 piglets before (0 min) and after the intravenous (iv) administration of 1.5 g (n=3) and 2.5 g (n=3) of ethanol/kg bw, and after the iv administration of 0.75 g (n=3) and 1.75 g (n=4) of ethanol/kg bw followed by the subsequent intragastric (ig) infusion of 1.8 g and 4.75 g of ethanol/kg bw, respectively.

Discussion

Acetaldehyde, the first metabolite of ethanol oxidation, is chemically a potent and reactive compound. It is generally agreed that acetaldehyde is mainly formed from ethanol by liver alcohol dehydrogenase. To a smaller extent, however, ethanol is also oxidised to acetaldehyde in the other tissues such as kidneys, stomach, intestine, and bone marrow.

Under anaerobic conditions microbes are capable of producing energy through fermentation. In alcoholic fermentation the end product is ethanol, which is derived from acetaldehyde in a reductive reaction mediated by bacterial alcohol dehydrogenase. Small amounts of ethanol have earlier been found in the contents of the alimentary tract and portal blood of normal rats. An increased production of endogenous ethanol as a result of bacterial overgrowth has been demonstrated in jejunal blind loop contents of rats. In humans significant endogenous ethanol concentrations have been found in jejunal aspirates of patients with tropical sprue and in venous blood of patients after jejunoileal bypass for morbid obesity.

This study also shows significant concentrations of endogenous ethanol in the colon of piglets. An intravenous ethanol administration resulted in intracolonic ethanol concentrations that paralleled those of the blood within about an hour. After the oral dose of ethanol intracolonic ethanol concentration still remained and equal to that of the blood. Accordingly, ethanol was distributed to the water phase of the colon via blood circulation, which is in accordance with the earlier finding in humans demonstrating that ethanol is effectively absorbed from the upper gastrointestinal tract and that ileal and colonic ethanol concentrations are equal to those in the blood. In contrast, with the conversion of acetaldehyde to ethanol during fermentation, the reaction catalysed by bacterial alcohol dehydrogenase can also run into the opposite direction with acetaldehyde as an end product.

In this study, this reversed reaction, mediated most probably by several colonic aerobic bacteria, resulted in an appreciable intracolonic accumulation of endogenous acetaldehyde. The large variation in the endogenous acetaldehyde values is presumably due to the individual differences in the colonic flora — that is, in the capacity of the bacteria to oxidise ethanol to acetaldehyde.

In 1982, Levitt et al reported a significant production of higher alcohols and unidentified metabolites by human faecal homogenates incubated with ethanol. Acetaldehyde, however, was not measured. Furthermore, after intragastric ethanol administration, rats with a jejunal self filling diverticulum, a condition accompanied by bacterial overgrowth, presented a considerably increased concentration of acetaldehyde in colonic lumen and portal vein when compared with controls with self emptying diverticulum. We have recently shown that human colonic contents and isolated colonic microbes are capable of producing significant amounts of acetaldehyde when incubated with ethanol in vitro.

Bacterial alcohol dehydrogenase mediated oxidation of ethanol to acetaldehyde could form a new pathway for ethanol oxidation if acetaldehyde is effectively enough removed so that substrate inhibition cannot prevent the reaction. As shown before, the capacity of intracolonic contents to oxidise further ethanol derived acetaldehyde in vitro is limited. However, as shown in this study acetaldehyde is effectively removed from the colon in vivo. At least in part it is oxidised to acetate in the colon, presumably by the aldehyde dehydrogenase present in the colonic mucosal cells or by some as yet unknown pathway prevailing in colonic bacteria. It is also possible that some of intracolically formed acetaldehyde is absorbed to the portal blood.

The development of hepatic injury resembling that seen in alcoholic subjects is a common complication among patients with jejunoileal bypass, a state favouring bacterial overgrowth in the gut. Bode et al have demonstrated that alcohol feeding to rats subjected to jejunoileal bypass leads to severe liver injury presenting the features of alcohol induced liver disease in humans. Most recently it has been shown that liver injury after longterm exposure of rats to ethanol can be prevented by antibiotics killing Gram-negative, aerobic colonic bacteria.

Furthermore, ciprofloxacin has been shown to reverse the inhibitory effects of short-term ethanol exposure on hepatic regeneration in the rat. Finally, we have recently shown that acetaldehyde given to the rats in drinking water seemed to be 30 times more hepatotoxic than intrahepatic acetaldehyde formed from ethanol oxidised in the liver. All these findings strongly advocate the role of intestinal bacteria in the oxidation of ethanol to acetaldehyde and the potential hepatotoxicity of intracolically formed acetaldehyde.

In addition to alcohol induced liver injury, longterm ethanol consumption has been found to stimulate rectal cell proliferation in the rat. A considerable increase in the activity of
mucosal alcohol dehydrogenase, an enzyme converting ethanol to acetaldehyde, in the distal colon. Mice 
were found to have higher levels of acetaldehyde in the colon than in the liver, which was 
consistent with previous studies showing that the colon is the major site of ethanol metabolism 
in the body.

In conclusion, the colon plays a critical role in ethanol metabolism, and interventions that 
reduce colon-derived acetaldehyde may be effective in reducing the risk of cancer and other 
ethanol-related diseases.
Intracolonic ethanol oxidation - a new link to empty calories and blood acetaldehyde. Hepatology 1994; 20: 313A.


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