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 intracolonic 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, intracolonic 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 intracolonically was mainly metabolised to acetate but also to ethanol in the colon.

Conclusions—Significant endogenous intracolonic 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 intracolonically from ingested ethanol by this pathway.

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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.[1-5] Moreover, alcohol associated rectal carcinogenesis[6-8] and cancer of the upper respiratory tract[9 10] have recently been related to acetaldehyde derived from ethanol.

The toxicity of acetaldehyde has been proved with respect to the gonads,[11] heart,[12] and red blood cells.[13] The toxic effects of acetaldehyde have been related to its ability to bind easily to different cellular proteins.[14 15] Furthermore, acetaldehyde may induce generation of free radicals and subsequently lipid peroxidation.[16] It has also been reported that comparatively low concentrations of acetaldehyde may impair cell proliferation and cause abnormalities in cell growth in vitro.[17 18] 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.[19] If ethanol is oxidised to acetaldehyde in the colon also in vivo, this represents a new extrahepatic pathway for the metabolism of ethanol. Furthermore, intracolonically 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 intracolonically 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[20] 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 (N_2) 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 intracolonic, 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) mM 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 intracolonic acetate and ethanol concentrations (Fig 3).
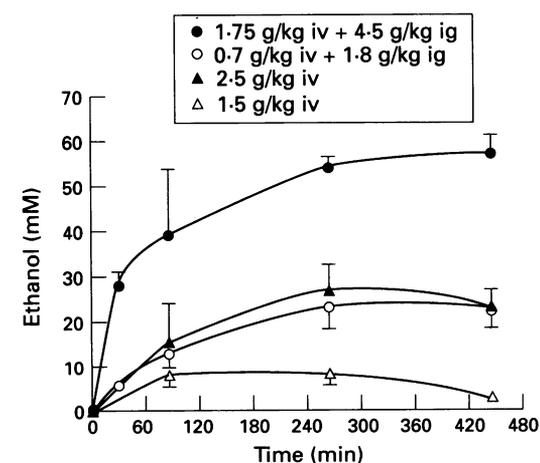

Figure 1: Intracolonic ethanol concentrations (mean (SEM)) in 13 piglets before (0 min) and after the intravenous (iv) administration of 1·5 g (n=3) and 2·5 g (n=3) of ethanol/kg bw, and after the iv administration of 0·7 g (n=3) and 1·75 g (n=4) of ethanol/kg bw followed by the subsequent intragastric (ig) infusion of 1·8 g and 4·75 g of ethanol/kg bw, respectively.
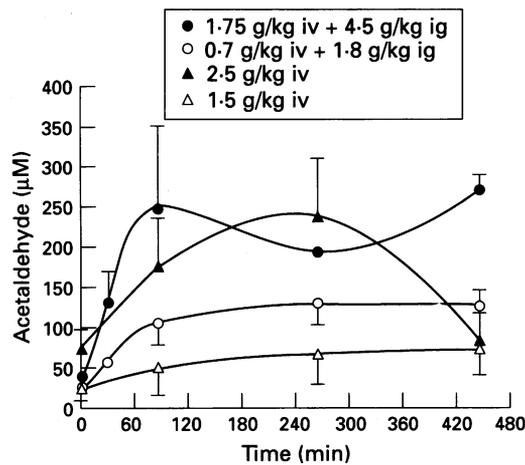
Figure 2: Intracolonic acetaldehyde concentrations (mean (SEM)) in 13 piglets before (0 min) and after the intravenous (iv) administration of 1·5 g (n=3) and 2·5 g (n=3) of ethanol/kg bw, and after the iv administration of 0·7 g (n=3) and 1·75 g (n=4) of ethanol/kg bw followed by the subsequent intragastric (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,[21] stomach,[22] intestine,[23] and bone marrow.[24]

Under anaerobic conditions microbes are capable of producing energy through fermentation.[25] In alcoholic fermentation the end product is ethanol, which is derived from acetaldehyde in a reductive reaction mediated by bacterial alcohol dehydrogenase.[26] Small amounts of ethanol have earlier been found in the contents of the alimentary tract and portal blood of normal rats.[27] An increased production of endogenous ethanol as a result of bacterial overgrowth has been demonstrated in jejunal blind loop contents of rats.[28] In humans significant endogenous ethanol concentrations have been found in jejunal aspirates of patients with tropical sprue[29] and in venous blood of patients after jejunoileal bypass for morbid obesity.[30]

This study also shows significant concentrations of endogenous ethanol in the colon in 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 constant 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[31] and colonic[32] 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.[33] [34] In
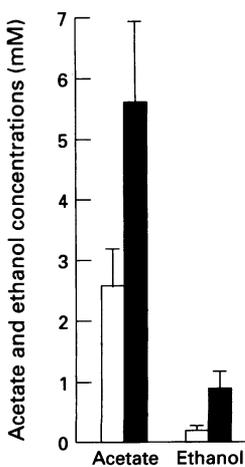

Figure 3: Intracolonic acetate and ethanol concentrations (mean (SEM)) immediately after (□) and 60 min after (■) the intracolonic administration of 500 ml of 5 mM acetaldehyde solution in four piglets.

this study, this reversed reaction, mediated most probably by several colonic aerobic bacteria,[35] 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.[35]

In 1982, Levitt *et al*[32] 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.[28] We have recently shown that human colonic contents[19] and isolated colonic microbes[35] are capable of producing significant amounts of acetaldehyde when incubated with ethanol in vitro.
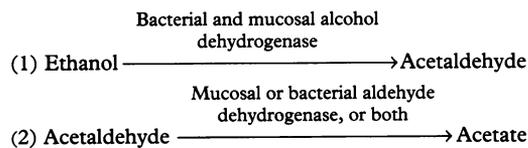
Bacterial alcohol dehydrogenase mediated oxidation of ethanol to acetaldehyde could form a new pathway for ethanol oxidation if acetaldehyde is effectively enough removed so that substrate inhibition cannot prevent the reaction. As shown before,[19] the capacity of intracolonic contents to oxidise further 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[36] or by some so far unknown pathway prevailing in colonic bacteria. It is also possible that some of intracolonically formed acetaldehyde is absorbed to the portal blood.[37]

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.[30] [38] Bode *et al*[39] 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.[40] Furthermore, ciprofloxacin has been shown to reverse the inhibitory effects of short-term ethanol exposure on hepatic regeneration in the rat.[41] 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.[42] All these findings strongly advocate the role of intestinal bacteria in the oxidation of ethanol to acetaldehyde and the potential hepatotoxicity of intracolonically formed acetaldehyde.

In addition to alcohol induced liver injury, longterm ethanol consumption has been found to stimulate rectal cell proliferation in the rat.[43] A considerable increase in the activity of

mucosal alcohol dehydrogenase, an enzyme converting ethanol to acetaldehyde, in the distal colorectum was found in longterm ethanol fed rats compared with their pair fed controls.[44] Accordingly, an association has been suggested between acetaldehyde, probably generated through bacterial ethanol oxidation, and the pathogenesis of the chemically induced rectal carcinogenesis in rats.[45] Similarly, acetaldehyde derived from ethanol has been suggested to contribute to the development of colorectal cancer in heavy drinkers.[45]

In conclusion, our results show that the colon is an important organ for acetaldehyde production. This is the first study, to our knowledge, in which significant intracolonic endogenous acetaldehyde concentrations have been described. Moreover, our results show that ingested ethanol is effectively oxidised to acetaldehyde in the colon and that increased intracolonic acetaldehyde concentrations are found during normal ethanol metabolism. Intracolonic acetaldehyde can be metabolised further inside the colon to acetate. We consider that these findings strongly advocate the existence of a bacteriocolonic pathway for ethanol oxidation, capable of eliminating ingested ethanol as follows:

<center>Bacterial and mucosal alcohol
dehydrogenase</center>

(1) Ethanol $\xrightarrow{\hspace{3cm}}$ Acetaldehyde

<center>Mucosal or bacterial aldehyde
dehydrogenase, or both</center>

(2) Acetaldehyde $\xrightarrow{\hspace{3cm}}$ Acetate

In addition, intracolonically produced acetaldehyde and acetate may be absorbed from the colon to the portal blood, and, subsequently, metabolised further in the other tissues.

The bacteriocolonic pathway offers a new explanation for the disappearance of a part of ethanol calories.[46] In addition, high intracolonic acetaldehyde values may contribute to the development of colorectal cancer in heavy drinkers.[6-8] The quantitative contribution of the bacteriocolonic ethanol oxidising pathway for the total ethanol oxidising capacity of the body as well as the rate limiting steps of the pathway remain to be established in future studies. To approach these questions, studies with piglets treated with different non-absorbable antibiotics have already started in our laboratory.

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