Inhibition of retinol oxidation by ethanol in the rat liver and colon

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Abstract

Background—Epidemiological evidence has been presented for an increased risk of development of colon cancer after chronic alcohol abuse. Alcohol is degraded by cytosolic alcohol dehydrogenases that also are capable of retinol oxidation. Inhibition of retinol oxidation to retinoic acid has been shown to occur in parallel with profound impairment of intracellular retinoid signal transduction and loss of cell differentiation control.

Aims—In the present study, the change in cytosolic retinol oxidation and retinoic acid formation by ethanol concentrations that occur in body tissues in humans after social drinking was measured in cells from the liver, and small and large intestine of the rat.

Results—The specific catalytic efficiency $V_{\max}/K_m$ (ml/min/g) of cytosolic retinol oxidation in the large intestine (28.9) was found to be distinctly higher than that in the liver (3.4), while the efficiency in the small intestine was negligible (0.20). In the presence of increasing ethanol concentrations (9, 17, and 34 mM), $V_{\max}/K_m$ for retinol oxidation decreased in a dose dependent manner to 7.8% of the initial value in the large intestine and to 12% in the liver. The $V_{\max}/K_m$ of retinoic acid formation in the liver cytosol decreased to 15%.

Conclusions—Our data demonstrate impairment of hepatic and intestinal cytosolic retinol oxidation and retinoic acid formation by ethanol at concentrations in body tissues after social drinking in humans. The results suggest that the increased risk of developing colorectal neoplasias after alcohol abuse may, at least in part, be caused by impaired retinoid signal transduction.

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result in impaired proliferation control of these cells.^{7,8} To develop its biological function in these cells, retinol has to undergo cytosolic oxidation via retinal to (all *trans*-) retinoic acid,^{9,10} which is an active ligand for retinoic acid receptors (RARs).^{11,12} After isomerisation to 9-*cis*-retinoic acid, it transforms into a ligand for retinoid X receptors (RXR).¹³ Maintenance of the retinoid signalling pathway is vital for normal cell development.¹⁴ RXR, together with 9-*cis*-retinoic acid, can form heterodimers with holo receptors for vitamin D₃, thyroxine, all-*trans*-retinoic acid, and oestrogen.^{15–17} Retinoid signalling interacts closely with that of triiodothyronine¹⁸ and 1,25-dihydroxy vitamin D₃.¹⁹ Both vitamins act synergistically in the induction of differentiation and impairment of proliferation in malignant cell lines²⁰ but retinoic acid isomers can exert their antiproliferative effect only if the corresponding nuclear receptors are present.²¹ This broad ranging involvement of receptors for retinoic acid isomers in signal transduction pathways emphasises the importance of sufficient retinoic acid production for controlled cell growth. The interaction of ethanol with the retinoid signal transduction pathway became clear in experiments where rodents showed increased expression of receptors for triiodothyronine and retinoic acid after alcohol consumption.²²

Previous studies have demonstrated the ameliorating effect of retinoid supplementation on the occurrence of uncontrolled cell growth. In these studies, a reduction in tumour incidence in rats with artificially induced colon carcinomas was demonstrated after retinyl ester supplementation.^{23,24} In addition, a delay in the rate of tumour development after retinoic acid feeding occurred.^{25,26}

Several subclasses of cytosolic alcohol dehydrogenase (ADH) were shown to catalyse the oxidation of retinol to retinal,^{27–29} which is the rate limiting step in retinoic acid synthesis.³⁰ Numerous experimental findings indicate that ADH plays an essential role in retinol oxidation. Some examples of this are impairment of retinoic acid synthesis by specific cytosolic ADH inhibitors,³¹ the close relationship between retinoic acid concentration and ADH distribution in tissues,³² and the existence of a retinoic acid response element in the promoter

Abbreviations used in this paper: RARs, retinoic acid receptors; ADH, alcohol dehydrogenase; TMBP, 4-(1,1,3,3-tetramethylbutyl)-phenol; K_m , Michaelis-Menten constant; v , specific rate of product formation; $V_{\max}$, specific rate of product formation at substrate saturation; K_{ic} , competitive inhibition constant; K_{iu} , uncompetitive inhibition constant; AUC, area under the curve; SDR, short chain dehydrogenases/reductases; CRBP, cellular retinol binding protein; RXR, retinoid X receptors.

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Epidemiological studies suggest an important role of alcohol abuse in both the development of preneoplastic lesions of the colon and rectum (adenomatous polyps) and induction of colorectal cancer.^{1–5} Alcohol itself is not assumed to act as a direct carcinogen but rather as a promoting agent.⁶ The mechanisms of this promoting effect have not yet been clarified. In common with other factors, alcohol seems to affect mucosal cell proliferation and differentiation.³

Restricted availability of retinol (vitamin A) to intestinal epithelial cells has been shown to

region of ADH genes.³³ Impairment of retinol oxidation by ethanol has been demonstrated in human liver³⁴ and for ADH subclasses capable of retinol oxidation in vitro in rats.³⁵ Furthermore, reduced conversion of retinol to retinoic acid is assumed to be responsible for the development of fetal alcohol syndrome.^{36, 37}

The experimental results mentioned above suggest that impairment of retinol oxidation by ethanol and the associated disturbance in retinoid associated intracellular signal transduction may play an important role in the development of colon neoplasia. To our knowledge, there has been no published information on the enzyme activities of ADH mediated retinol oxidation and its interaction with ethanol in the intestine. Therefore, we investigated the effect of ethanol on overall cytosolic retinol oxidation in the gut and liver under conditions as closely related to physiological circumstances as possible.

Experimental procedures

ANIMALS AND ORGAN PREPARATION

All experiments were performed according to the guidelines of the local ethics committee for animal experiments. Eleven male Wistar rats (average weight 278 (16) g, aged 4–5 months), which were maintained on a standard diet and had access to water ad libitum prior to killing, were decapitated and the liver, small intestine, and large intestine removed immediately. The caecum was discarded. During organ removal, the bodies of the rats were kept on ice. Livers were placed into 15 ml of ice cold KCl solution (1.15%) and kept frozen at -80°C until homogenisation. The lumen of the small and large intestine was purged immediately after killing with 100 ml of ice cold KCl solution (1.15%). Mucosal tissue was removed from serosal tissue by fixing the guts on an ice cooled glass plate and scraping off the mucosa with a sharp edged blade. Mucosal tissue was homogenised with a Potter-Elvehjem homogenisator in 2 ml of ice cold KCl solution (1.15%). Cell debris was removed by centrifugation at 1800 *g* (20 minutes, 4°C) and the mitochondrial/lysosomal pellet was separated after centrifugation at 22 000 *g* (20 minutes, 4°C). A final centrifugation (105 000 *g*, 60 minutes, 4°C) resulted in the separation of the microsomal pellet and supernatant which was used as the “cytosolic fraction” in all experiments.

EXPERIMENTAL DESIGN FOR DETERMINATION OF $V_{\max}$, K_m , K_{ic} , AND K_{iu}

Enzyme constants were measured under conditions established previously with slight modifications.^{29–38} In brief, all incubations for measurement of retinol oxidation by the cytosolic fraction were performed under physiological conditions (37°C , pH 7.4 buffered by *N*-(2-hydroxyethyl)-piperazine-*N'*-(2-ethane sulphonic acid) (HEPES 20 mM; Aldrich, Steinheim), with 2 mM dithiothreitol and 150 mM KCl). Samples containing variable retinol (0, 4, 9, 18, 35, 70, 140, 280 μM) (Sigma, Deisenhofen, Germany) and ethanol (0, 8.6, 17, 34 mM; Braun, Melsungen, Germany) concentrations in the presence of 4.0 mM

NAD^+ and 91 μM butylated hydroxytoluene in a total volume of 450 μl were preincubated for five minutes at 37°C . The reaction was started by addition of 50 μl of the cytosolic fraction.

After 20 minutes, samples were placed on ice and the reaction was stopped instantly by addition of 50 μl of an ascorbic acid/ethylene diamine tetraacetic acid/trichloroacetic acid solution (14.2 mM/5.5 mM/6.1 mM, respectively) and 600 μl of ethanol; 20 μl of a 4-(1,1,3,3-tetramethylbutyl)-phenol solution (TMBP, 7.27 mM) in dimethyl sulphoxide were added as an internal standard. Retinoids and TMBP were isolated by vigorous shaking of the solution after addition of 700 μl of toluene with 4.5 mM butylated hydroxytoluene and consequent separation of the organic layer. Toluene was removed in a vacuum centrifuge. The residue was dissolved in 100 μl of mobile phase used for quantification by high performance liquid chromatography.

Retinoids were separated on a reversed phase column (RP 18, particle diameter 5 μm , $\varnothing$ 3 mm $\times$ 125 mm; Chromatographie Service, Langerwehe, Germany) after application of 50 μl of dissolved residue using 130 mM of aqueous ammonium acetate/methanol/acetonitrile 18/21/61 as the mobile phase (flow 1.0 ml/min). Relevant compounds were detected using a diode array detector at 276 nm (TMBP, retention time 2.2 minutes), 340 nm (retinoic acid, 3.0 minutes), 326 nm (retinol, 6.5 minutes), and 382 nm (retinal, 9.5 minutes). Area under the curve (AUC) was used to calculate the concentration of retinoids. AUC was multiplied by the recovery factor calculated from individual recovery of TMBP in each sample. The average of a sixfold measurement of the total TMBP added without extraction was assumed to correspond to 100%. Standardisation was performed using standard solutions of retinol, retinal, and retinoic acid (Sigma) which were treated simultaneously with samples without cytosol. Protein content was measured according to Smith and colleagues³⁹ with bicinchoninic acid/ Cu^{2+} (Sigma) using bovine serum albumin as standard.

During incubation, the substrate (retinol) concentration declined according to losses resulting from enzymatic conversion of retinol to retinal and retinoic acid. As the oxidation rate remained constant during the incubation period, the average substrate concentration in each sample was calculated as the retinol concentration at the end of incubation with cytosol plus half of the concentration of the formed retinoids (retinal+retinoic acid; μM).⁴⁰ To obtain the amounts of retinal and retinoic acid formed by enzymatic conversion only, the amount of these compounds formed by autooxidation was calculated from a blank incubation without cytosol and subtracted from the products formed in the samples with cytosol. The amount of product was assumed to be the sum of the oxidised forms of retinol (retinal+retinoic acid). $V_{\max}$ and K_m were calculated using the Michaelis-Menten equation (equation (1)) obtained after non-linear regression.

$$v(\text{pmol/min/mg}) = \frac{V_{\max} \times c(\text{retinol})}{K_m + c(\text{retinol})}$$

In this equation, v refers to the rate of formation of retinal and retinoic acid, $V_{\max}$ is the rate at substrate saturation, and K_m is the Michaelis-Menten constant of the reaction. The function was approximated using the Levenberg-Marquardt algorithm included in the software Slide Write Plus for Windows V 3.0 (Indigo Informations Systems, Munich, Germany). After determination of $V_{\max}$ and K_m in the absence of ethanol and the apparent $V_{\max}$ (app.) and apparent K_m (app.) in the presence of different ethanol concentrations, inhibition constants for uncompetitive (K_{iu}) and competitive (K_{ic}) inhibition were calculated from equations (2) and (3)⁴⁰:

$$V_{\max}(\text{app})(\text{pmol/min/mg}) = \frac{V_{\max}}{1 + c(\text{ethanol})/K_{iu}}$$

$$K_m(\text{app})(\mu\text{mol}) = \frac{K_m \times (1 + c(\text{ethanol})/K_{ic})}{1 + c(\text{ethanol})/K_{iu}}$$

Statistical differences were verified by ANOVA analysis and the Dunnett post hoc test.

Results

In the liver and large intestine, product (retinal+retinoic acid) formation followed classic Michaelis-Menten kinetics, both with and without ethanol (fig 1). In relation to protein content, the rate of retinol oxidation at substrate saturation ($V_{\max}$) catalysed by the cytosolic fraction from the large intestine was about twice as high as that of the liver (table 1). For retinol oxidation catalysed by hepatic ADH, the Michaelis-Menten constant was more than fivefold higher than that measured for the colon mucosa (table 1).

The catalytic efficiency of the cytosol obtained from the mucosa of the small intestine in retinol oxidation was less than 1% of that measured in the large intestine. Within the concentration range investigated, retinol oxidation in the small intestine showed a linear increase depending on the substrate concentration and no saturation. Therefore, $V_{\max}$ and K_m could not be calculated separately but the slope of the linear regression ($r^2=0.987$) was generally accepted to correspond to the catalytic efficiency $V_{\max}/K_m$ ⁴⁰ (table 1). Because of the low enzyme activity in the small intestine, no attempt was made to measure inhibition of retinol oxidation by ethanol in this organ.

In hepatic cytosol, retinoic acid formation from retinol was measured (table 2). Because of the lower average protein concentration of the cytosolic fraction of the large intestine (3.55 (0.30) mg/ml) compared with the cytosolic fraction from the liver (41.2 (2.2) mg/ml), retinoic acid levels were below the quantification limit in incubations performed with preparations from the colon. The protein concentration in the small intestine was about twice as high (7.63 (1.02) mg/ml) but because

of the inability of the cytosol of these cells to cause retinol oxidation, no retinoic acid formation was found if retinol was offered as substrate.

Retinol oxidation in the hepatic cytosol was inhibited by ethanol in a dose dependent manner (table 1, fig 1). Even at low alcohol concentrations (0.05%=8.6 mM), the calculated $V_{\max}(\text{app.})$ decreased to approximately 40–43% of the initial value in both the liver and large intestine. $V_{\max}(\text{app.})$ was also decreased to approximately 25–31% at a higher but physiologically relevant ethanol concentration (0.2%=34 mM). In parallel, $K_m(\text{app.})$ of retinol oxidation increased in a dose dependent manner indicating mixed inhibition (competitive and uncompetitive) of retinol oxidation by ethanol (table 1). Inhibition constants were 2.7 mM for competitive (K_{ic}) and 5.8 mM for uncompetitive (K_{iu}) inhibition.

The overall formation of retinoic acid in the presence of hepatic cytosol was also shown to be impaired by ethanol (fig 3). To determine if this reduction in retinoic acid formation was caused only by inferior substrate availability for enzymes catalysing the oxidation of retinal, the rate of retinoic acid synthesis was plotted against the average concentration of the

Figure 1 Effect of different ethanol concentrations (without (w/o) alcohol, and 8.6 mM, 17 mM, and 34 mM ethanol) on cytosolic (c) retinol dehydrogenase activity in the liver (A) and large intestine (B). In both organs, retinol oxidation is inhibited by increasing ethanol concentration in a dose dependent manner.

Table 1 *Michelis-Menten constants, maximum rates of total retinol oxidation and their coefficients in the cytosol obtained from the liver, and small and large intestine of rats (n=11) at different ethanol concentrations*

Organ	Ethanol concentration (%) (mM)	V_{max} (pmol/min/mg)	K_m (μ M)	V_{max}/K_m (ml/min/g)
Liver	0	382 (4.9)	113 (3.2)	3.4
	0.05 (8.6)	158 (5.8)	185 (12.8)	0.9
	0.1 (17)	120 (4.3)	201 (13.5)	0.6
	0.2 (34)	99 (8.4)	236 (35.0)	0.4
Large intestine	0	604 (14)	21 (1.8)	28.9
	0.05 (8.6)	259 (17)	43 (8.6)	6.0
	0.1 (17)	225 (20)	66 (15)	3.4
	0.2 (34)	186 (25)	70 (24)	2.3
Small intestine	0	—	—	0.20

Table 2 *Michelis-Menten constants, maximum rates of retinoic acid formation and their coefficients in the cytosol obtained from the liver of rats (n=11) at different ethanol concentrations, calculated using retinol and retinal as substrates*

Substrate	Ethanol concentration (%) (mM)	V_{max} (pmol/min/mg)	K_m (μ M)	V_{max}/K_m (ml/min/g)
Retinol	0	28.1 (2.1)	18.8 (5.1)	1.49
	0.05 (8.6)	11.2 (0.3)	34.4 (2.6)	0.33
	0.1 (17)	9.8 (0.5)	43.4 (7.1)	0.23
	0.2 (34)	7.4 (0.6)	33.8 (8.7)	0.22
Retinal	0	31.5 (3.3)	2.20 (0.7)	14.3
	0.05 (8.6)	12.7 (0.5)	1.19 (0.1)	10.7
	0.1 (17)	11.2 (0.9)	1.12 (0.2)	10.0
	0.2 (34)	8.1 (1.0)	0.67 (0.2)	12.2

intermediary formed retinal in fig 4. As can be seen, ethanol also significantly reduced the conversion of retinal to retinoic acid.

Discussion

Numerous studies have demonstrated the anti-proliferative effect of vitamin A and its derivatives (reviewed by Nagy and colleagues⁴¹). This effect is associated with regulation of gene expression,¹² and cytokine specific transcription factors such as activators of transcription (STAT) and interferon regulatory factors were suggested to mediate modulation of cellular proliferation and differentiation (reviewed by Matikainen and colleagues⁴²). To activate this signalling pathway, retinoic acid has to be formed from retinol to enable activation of RAR and RXR dependent gene transcriptions.⁴³ Several subclasses of ADH such as classes I and IV in the rat²⁷ and classes I, II, and IV in humans^{28, 29} were demonstrated to catalyse oxidation of both retinol and ethanol. Despite the fact that microsomal short chain dehydrogenases/reductases (SDR) are also capable of retinol oxidation if retinol is bound to cellular retinol binding protein (CRBP),⁴⁴ striking evidence has been presented that ADH activity plays a crucial role in retinol oxidation, the first step in retinoic acid synthesis. Feeding of either a specific ADH inhibitor (4-methylpyrazole) or ethanol to rats resulted in a dramatic decrease in retinoic acid synthesis *in vivo*,^{31, 36} while SDRs were not inhibited by these compounds.⁴⁵ As reviewed by Duester,⁴³ the key role of ADH in retinol oxidation is supported by further findings such as the cosubstrate (NAD⁺/NADP⁺) ratio,⁴⁶ which favours retinol oxidation by ADHs and retinal reduction by SDRs. The closely related distribution of both ADH and retinoic acid in mammal tis-

sue³² and existence of a retinoic acid response element in the promoter region of the ADH3 gene³³ are further arguments for an important role of ADH in retinol oxidation. Ethanol has been shown to impair retinoid metabolism in the mouse embryo³⁰ and to suppress retinol oxidation in human liver,³⁴ cattle retina,³⁴ and in some isolated isozymes in the rat.³⁵

From the total concentration of retinol in the cytosol (~5 μ M), only about 2.5 nM is estimated to be present in the unbound state, while the major part is assumed to be bound to CRBP I.^{47, 48} For an unbiased assessment of inhibition of retinol oxidation by ethanol, this low concentration of free retinol has to be taken into account. As the physiological concentration of free retinol in the cytosol is well below the K_m measured in this study (table 1), product formation shows a linear correlation to substrate concentration. The proportionality factor of this correlation, which corresponds to the initial slope of the Michealis-Menten function, is the catalytic efficiency V_{max}/K_m .⁴⁰ V_{max}/K_m has been demonstrated to be the best indicator of the cytosol catalysed oxidation of free retinol.²⁸

In the present study, V_{max}/K_m of cytosol mediated retinol oxidation in rat colon and liver was found to decrease markedly even in the presence of low concentrations of ethanol (table 1), paralleling findings in cytosol from human liver³⁴ and isolated hepatic ADH isozymes.^{28, 38, 49} A high requirement for retinoic acid seems likely in the case of controlled frequent cell divisions associated with repeated fusions of all-*trans*-retinoic acid and 9-*cis*-retinoic acid with the corresponding receptors RAR and RXR during signal transduction.⁵⁰ In accordance with this assumption is the higher V_{max}/K_m value of retinol dehydrogenases measured in the cytoplasm of colonic cells compared with hepatic cells (table 1). At this point, the vulnerability of cells with high proliferation rates such as cells from the colon mucosa becomes clear as not only vitamin A deficiency⁵¹ but also consumption of alcohol in larger quantities⁶ result in increased proliferation of cells in the large intestine. In contrast, increased concentrations of retinoic acid can reduce proliferation of malignant cells.⁵²

Despite the high proliferation rate of cells in the small intestine, the V_{max}/K_m of retinol oxidation in the cytosol of these cells is less than 1% of that measured in cells from the large intestine. A possible explanation may be the availability of retinal generated from carotenoids by dioxygenase, which is further reduced by a specific retinal reductase.^{53, 54} The fact that the small intestine is the primary site of β -carotene cleavage and formation of retinal^{55, 56} implies that these cells can use this retinal for retinoic acid synthesis and the need for retinol oxidation is reduced.⁵⁷ If so, this would imply that the unfavourable competition of retinol dehydrogenase and retinal reductase in the cytosol forming a "futile cycle" would be avoided. In line with this hypothesis, malignoma associated with alcohol abuse are found frequently in the colon, but rarely in the small intestine.^{1, 2, 4, 6}

Figure 2 After reverse phase separation, the internal standard (4-(1,1,3,3-tetramethylbutyl)-phenol solution; TMBP 2.26/2.20 minutes at 276 nm) and retinol (6.25/6.04 minutes at 326 nm) were quantified in the first channel of the diode array detector (upper chromatogram) while retinoic acid (2.87/2.78 minutes at 340 nm) and retinal (7.76/7.50 minutes at 382 nm) were measured in the second one (lower chromatogram). The HPLC chromatograms resulted from incubation (20 minutes, 37° C) of a buffered retinol solution (140 mM) with 4 mM NAD⁺ in the presence of 10% (v/v) cytosolic fraction obtained from rat mucosa cells without ethanol (A) and with 0.2% ethanol (B). Ethanol significantly inhibited the amount of formed retinal while the concentration of retinoic acid remained at levels which also occurred in control assays without cytosol. For calculation of enzyme kinetics, differences between concentrations found in the experiments with cytosol and blank incubations were applied.

The risk of developing hepatocellular carcinoma after alcohol abuse is linked to the occurrence of pronounced fibrosis or cirrhosis.⁵⁸ Therefore, the postulated effect of ethanol on impaired retinoid signalling pathways does not seem to increase the risk of hepatocellular carcinoma per se. The different impact of alcohol abuse on the development of carcinoma of the colon and liver may be explained by the distinctly higher proliferation rate of colonic cells compared with hepatocytes.⁵⁹ In the case of cell death occurring in pathological stages before and during cirrhosis, hepatic cell proliferation is increased due to the attempt of the body to maintain a constant ratio between hepatic functional mass and body mass.⁵⁸ The resulting increase in proliferation rate may result in increased susceptibility of these cells

Figure 3 Retinoic acid formation in hepatic cytosol of the rat at different ethanol concentrations (without (w/o) alcohol, and 8.6 mM, 17 mM, and 34 mM ethanol). The ethanol associated inhibition of enzyme activity, which is related to the concentration of the applied retinol concentration, is in parallel with overall retinol oxidation.

Figure 4 Retinoic acid formation in the hepatic cytosol of the rat shown at different ethanol concentrations (without (w/o) alcohol, and 8.6 mM, 17 mM, and 34 mM ethanol) is dependent on the average intermediary formed retinal concentration. The reduction in retinoic acid formation from retinal by ethanol indicates ethanol induced reduction of retinol oxidation in addition to impairment of retinol oxidation.

to procarcinogenic effects such as impairment of retinol oxidation by ethanol.

The production of retinoic acid from retinol in the presence of hepatic cytosol from rodents has been demonstrated previously,^{30 60 61} but to the best of our knowledge, until now no information was available on retinoic acid synthesis being dependent on retinol concentration, as shown in this study. Ethanol associated inhibition of retinoic acid production from retinol in hepatic cytosol showed a similar pattern as that of all retinol oxidation products (table 2). The catalytic efficiency of $V_{\max}/K_m$ decreased with increasing ethanol concentration only if the enzyme constants were calculated with retinol as substrate (fig 4), and not if the average intermediary formed retinal was assumed to be the substrate for retinoic acid formation. In the event of increasing ethanol concentration,

$V_{\max}/K_m$ seems to change slightly for this (these) enzyme(s) (table 2), indicating that oxidation of retinol to retinal is the rate limiting step of retinoic acid formation. Furthermore, $V_{\max}$, K_m , and $V_{\max}/K_m$ of retinol oxidation change with increasing ethanol concentration. This indicates a mixed (competitive and uncompetitive) type of inhibition⁴⁰ while the $V_{\max}/K_m$ of retinoic acid formation from retinal does not show this trend. This represents pure uncompetitive inhibition by ethanol and/or its metabolite acetaldehyde. This observation may be limited to the complex system with the simultaneous presence of both ADHs and cytosolic acetaldehyde dehydrogenases, arising from the dynamic interactions of these enzymes. Our investigation indicated a pronounced decrease in cytosolic retinoic acid formation from retinol by alcohol at tissue levels resulting from social drinking. Consumption of only 1.4 drinks (17 g) results in a cytosolic ethanol concentration of 8.6 mM (male, 70 kg body weight), as can be calculated from the homogeneous distribution of ethanol in body water.^{62, 63} This concentration corresponds to the lowest ethanol concentration used in our experiments which suppressed the catalytic efficiency of retinol oxidation by 73% in hepatic cytosol and by 79% in the cytosol of colon mucosa cells.

Different K_m values for retinol oxidation and different inhibition constants for ethanol in the cytosol of rat liver and the small and large intestine measured in this study indicate different subsets and/or concentration ratios of isozymes in these organs. ADH class IV has been shown to have the highest catalytic efficiency for retinol oxidation of the four classes found in humans.²⁸ Expression of the corresponding gene, however, could not be detected in the liver or colon of rodents, either at the protein⁶⁴ or mRNA levels.⁶⁵ In rats, class I and II ADHs expressed in the intestine of rodents^{64, 66} are therefore the most likely candidates for enzymes catalysing cytosolic retinol oxidation. The activity of the latter is impaired by ethanol in this organ but the exact mechanism requires further investigations.

In conclusion, apart from mechanisms such as glucuronisation,^{67, 68} transformation to polar metabolites,^{43, 69} and binding to CRBP,⁴⁹ the catalytic efficiency $V_{\max}/K_m$ of retinol oxidation seems to be another essential control mechanism of intracellular retinoic acid formation. Our data support the hypothesis that alcohol in the concentration range which results from consumption of 1–2 drinks by an adult human impairs markedly acute intracellular retinoic acid formation by inhibition of cytosolic retinol oxidation in the large intestine and liver. This impairment may be a possible explanation for the increased risk for the development of colonic neoplasias in chronic alcohol abusers. Because of the different subsets and kinetic properties of ADHs among species, this assumption can only be verified using specimens from human colon mucosa.

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