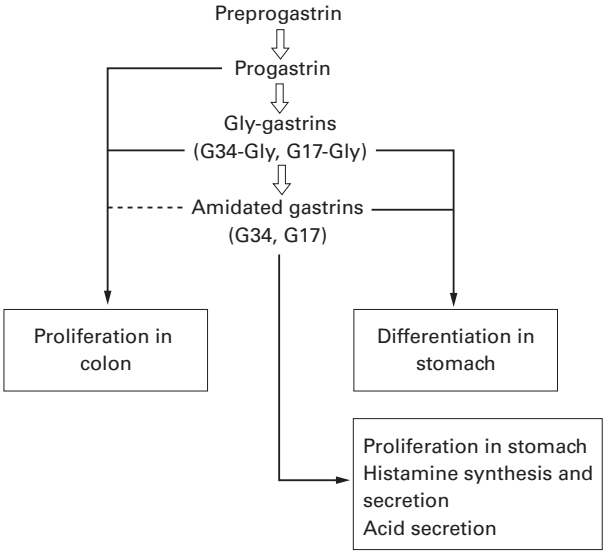

Figure 1 Schematic representation of the pathway of gastrin biosynthesis and the biological properties of the products. Open arrows show the conversion steps by which preprogastrin is converted to amidated gastrins. This sequence of events is completed in G cells but in colorectal cancer cells the main products are progastrin and Gly-gastrin. Progastrin and Gly-gastrins stimulate colon proliferation. Amidated gastrins play a minor role in control of colon proliferation but stimulate acid secretion and gastric proliferation via the gastrin-CCKB receptor (at which progastrin and Gly-gastrin have low affinity).

gastrin biosynthesis (fig 1). In normal G cells, the enzymes responsible for production of carboxy terminal amidated gastrins mostly act in vesicles of the so called regulated pathway of exocytosis[7]; but these vesicles are scarce or absent in polyp and adenoma cells. As a consequence, these cells are poorly equipped both to store hormonal peptides and to complete the full sequence of events giving rise to carboxy amidated gastrins. Instead, peptides mostly corresponding to progastrin and Gly-gastrin are likely to pass directly from the Golgi complex to the cell surface by what is known as the constitutive route of secretion.

What regulates gastrin gene expression in colon polyps and adenomas? Studies by Nakata *et al* have shown that oncogenic Ras stimulates the mitogen activated protein kinase pathway and so increases gastrin gene expression.[8] Recently, Koh *et al* demonstrated that the β-catenin/T cell factor 4 pathway also stimulates gastrin gene expression.[9] Acquisition of mutations that activate the latter pathway is likely to be an early event in the progression to colorectal cancer and could account for the increased gastrin gene expression found by Smith and Watson. Whether or not similar mechanisms account for upregulation of the gastrin-CCKB receptor is not known.

Recently, Singh *et al* reported that mice with elevated plasma progastrin exhibit increased aberrant crypt foci, adenomas, and adenocarcinomas after treatment with azoxymethane.[10 11] Aberrant crypt foci are considered to be a marker for progression to colon cancer. Singh *et al* suggest progastrin is a cocarcinogen, that is, on its own it is not carcinogenic but it increases the pool of transformed cells and so exacerbates the oncogenic progression. Interestingly, mice with elevated plasma concentrations of amidated gastrin did not exhibit increased aberrant crypt foci, adenomas, or carcinomas in response to azoxymethane. The picture emerges then that mutations acquired early in the progression to colorectal cancer lead to increased local production of progastrin and Gly-gastrin that then act as auto- or paracrine growth factors expanding the number of transformed cells. This analysis leaves open the role of the gastrin-CCKB receptor, and further work will certainly be needed on this point. Whether or not these findings can be developed into novel therapeutic strategies will depend on characterisation of the receptor mechanisms mediating the effects of progastrin or Gly-gastrin on colon epithelial proliferation.

G J DOCKRAY

Physiological Laboratory,
University of Liverpool, Crown St,
PO Box 147, Liverpool L69 3BX, UK
g.j.dockray@liverpool.ac.uk

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Alcohol and retinoid metabolism

The complex interactions between the metabolism of retinoids and ethanol have been reported for a long time. Clinically, chronic ethanol consumption leads to vitamin A deficiency but also to enhanced toxicity of vitamin A and beta-carotene when supplemented. Changes in retinol metabolism due to alcohol may have a pathophysiological impact in both alcoholic liver disease and alcohol associated cancer as retinoic acid, the most active form of vitamin A, is an important regulator of normal epithelial cell growth, function, and differentiation. Under normal conditions, ingested retinol is metabolised to retinaldehyde via cytosolic alcohol dehydrogenase (ADH), microsomal retinol dehydrogenase (three types), and several types of cytosolic retinol dehydrogenases, and retinaldehyde is further oxidised to retinoic acid via aldehyde dehydrogenase (ALDH). Retinoic acid binds to retinoic acid receptors (RAR), initiating intracellular signal transduction leading to a cascade of events and finally to a decrease in cell regeneration. The main molecular action of retinoic acid involves either transactivation through direct binding to retinoic acid response elements (RARE) in target gene promoters, thereby transcriptionally activating a series of genes with distinct antiproliferative activity, or transrepression of activator protein (AP-1) and regulation of apoptosis. It is not surprising that this complex interaction between ethanol and metabolism of retinoids occurs as

both substrates share common pathways, namely (a) ADH, (b) ALDH, and (c) cytochrome P4502E1 (CYP2E1).

It has been shown that chronic ethanol consumption decreases hepatic retinol and retinoic acid concentrations due to various mechanisms including, increased mobilisation of retinyl esters to extrahepatic tissues and enhanced hepatic metabolism of retinol and retinoic acid to polar metabolites, predominantly via induced CYP2E1.[1 2] These metabolites include 18-OH-retinoic acid, 4-OXO-retinoic acid, and some unidentified metabolites, possibly with fibrogenic and toxic properties. Decreased hepatic retinoic acid concentrations are associated with functional down-regulation of RAR, enhanced expression of AP-1 gene (c-jun and c-fos), and increased hepatic cell regeneration, all of which return to normal following retinoic acid supplementation.[2 3] In contrast, retinol concentrations in extrahepatic tissues such as the oesophageal and colonic mucosa were found to be increased rather than decreased following chronic ethanol consumption.[4] This was also confirmed in alcoholics with oropharyngeal cancer where normal retinol concentrations were found in normal oral mucosa adjacent to cancerous tissue.[5] It was believed that one mechanism for this observation was increased mobilisation of retinyl esters from the liver to the oral mucosa.

In this issue of *Gut*, Parlesak and colleagues[6] demonstrate another explanation for the lack of loss of retinol in gastrointestinal mucosa of alcoholics (see page 825). These authors showed inhibition of retinol oxidation by ethanol concentrations frequently observed after social drinking in an in vitro study using cytosol from rat liver and intestine. This effect was due to inhibition of ADH. As this inhibition was found not only with low ethanol concentrations (8.6 mM) but also increasingly with higher ethanol concentrations (17 mM, 34 mM), a low K_m ADH (rat ADH3 corresponding to class I ADH) and an ADH with higher K_m (ADH2 corresponding to class III ADH or ADH1 corresponding to class IV ADH, not present in the rat liver and colon, but in the rat rectum[7]) seem to be involved. This inhibition of retinol metabolism by ethanol seems especially relevant in the colon. This may explain the accumulation of retinol in this tissue which may lead to a reduction in retinoic acid levels; however, this needs to be proved. In contrast with the colon, both retinol and retinoic acid concentrations were found to be significantly decreased in rat liver following chronic ethanol ingestion.[2] Thus this finding cannot be explained by inhibition of retinol oxidation by ethanol. Indeed, at least in the liver, a variety of other pathways for oxidation of retinol are involved, as mentioned above.

In humans it has been shown that retinol is a physiological substrate for ADH3 (class I ADH) in the gastrointestinal mucosa. ADH3 has a low K_m for ethanol (1–2 mM) and is the only class I ADH gene that contains an RARE in the promoter region. It has been suggested that retinoic acid activation of ADH constitutes a positive feedback loop regulating retinoic acid synthesis.[8] Ethanol was found to be a competitive inhibitor of retinol for class I ADH, but also for classes II and IV ADH.[9 10] Indeed, it has been shown that class IV ADH (only present in the mucosa of the upper gastrointestinal tract) has a low K_m for all-*trans*-retinol of 15–60 μM and has the highest catalytic efficiency of 3800–4500 mmol/min.[10 11] In vitro studies using class IV ADH enzyme preparations have shown strong inhibition of metabolism of all-*trans*-retinol and 9-*cis*-retinol by ethanol with a K_i of 6–10 mM.[10] Oxidation of retinol to retinaldehyde is probably the rate limiting step in the generation of retinoic acid. However, recently it was shown in the rat oesophagus that in addition to ethanol, acetaldehyde also inhibits generation of retinoic acid, possibly by inhibition at the retinaldehyde level.[12]

The data of Parlesak *et al* extended these in vitro experiments with isolated ADH by using rat cytosol from liver and colonic mucosa. An important next step would be to measure retinoic acid in colonic mucosa following chronic ethanol consumption and, as there are differences in ADH patterns between rats and humans, to also measure retinoic acid in the mucosa of alcoholics.

It is interesting that class IV in contrast with class I ADH is not expressed in human colorectal mucosa. However, it was found recently that in a number of biopsies from colorectal polyps of alcoholics, class IV ADH was expressed.[13] One explanation for such de novo expression of class IV ADH could be retinoic acid deficiency in a critical premalignant condition to guarantee increased generation of retinoic acid.

Chronic alcohol consumption is associated with an increased risk of both hepatic and colorectal cancer. Whereas for the liver, cirrhosis is possibly the most important precondition for cancer development, mechanisms for colorectal carcinogenesis are complex and less clear. One important morphological feature of chronic ethanol consumption in rats and humans is colorectal cellular hyperproliferation associated with extension of the proliferative compartment of the crypt towards the lumen, a condition associated with increased cancer risk.[14] This early event in colorectal carcinogenesis was thought to be related to acetaldehyde induced cell injury as acetaldehyde (produced during mucosal and bacterial ethanol metabolism) and crypt cell production rate showed a significant positive correlation.[14] However, this important alteration in cell cycle behaviour, which was also observed in the upper alimentary tract, could also be due to retinoic acid deficiency, and acetaldehyde may contribute by preventing its generation, as discussed above.

In summary, the paper by Parlesak *et al* shows the importance of ethanol in the inhibition of retinol metabolism in the liver and colon. However, it raises more questions than it answers. One major question arising from these data is whether decreased retinoic acid concentrations can be found in extrahepatic tissues, especially in the colorectal mucosa, following chronic ethanol ingestion of rodents, and even more important in alcoholic. If so, a new mechanism for alcohol associated carcinogenesis has to be considered.

H K SEITZ

Department of Medicine, Salem Medical Centre and Laboratory of Alcohol Research, Liver Disease and Nutrition, Heidelberg, Germany

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Web extra

A new website has been developed to help gastroenterologists calculate CDAI (Crohn's Disease Activity Index) and PCDAI (Paediatric Crohn's Disease Activity Index).

The user registers his/her patient under a name/code and registers the sex, age, and weight of the patient, and is then asked to fill in raw data. The website will then calculate CDAI (and for patients below 19 years, PCDAI). The site enables the user to store indices over time, and the disease activity of patients will also be presented as a diagram. The data are stored on the Örebro Medical Centre Hospital for one year. You can visit the website at:

http://www.orebroll.se/rso/barn/crohn/Home_Eng.asp

Users must first register; this is done by entering a user name and password, which the user chooses (user names and passwords are not distributed by Dr Ludvigsson).

For further information: **Dr Jonas Ludvigsson,**
Scientific Coordinator,
Department of Paediatrics,
Örebro Medical Centre Hospital,
701 85 Örebro,
Sweden

jonasludvigsson@yahoo.com
www.geocities.com/jonasludvigsson