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Role of Bile Acids in Colorectal Carcinogenesis

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Dietary factors are considered important environmental risk determinants for colorectal cancer development. Epidemiological studies have shown that a high fat (or meat) intake is associated positively and a high starch, fibre (non-starch polysaccharide), vegetable and fruit intake negatively with colorectal cancer incidence. One mechanism by which these effects are possibly exerted is through the metabolism of secondary bile acids. Secondary bile acids are formed after enzymatic deconjugation and dehydroxylation of primary bile acids in the large bowel by anaerobic bacteria. It has been shown that these compounds can have tumour-promoting capacities in animal experiments. In epidemiological studies, colonic cancer risk is related to the faecal bile acid concentration. In serum and bile of patients with colonic adenomas, more deoxycholic acid was detected than in healthy controls. Secondary bile acids are toxic to several cell systems at physiological concentrations. The exact mechanism by which these amphiphilic molecules exert their action is not well understood. It might act through membrane damage, intracellular mitochondrial action or genotoxic effects. So far the evidence that bile acids are involved in colonic carcinogenesis is largely circumstantial. It is, however, well accepted that environmental factors, such as dietary habits influence genetic susceptibility. Bile acids could play a promoting role in this process.

Key words: bile acids and salts, cytotoxicity, cell proliferation, colonic carcinogenesis, dietary factors
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INTRODUCTION

The role of dietary factors in colonic carcinogenesis

THE INCIDENCE of colorectal cancer is high in Western countries and is related to dietary habits. Currently it is assumed that dietary factors modulate a genetic susceptibility. In epidemiological observations, the consumption of animal fat is positively related to the incidence of colon cancer [1, 2]. The intake of fibre is possibly negatively related to this incidence; however, many inconsistencies exist [3]. Migrant studies revealed that inhabitants moving from low-incidence to high-incidence areas acquired the colonic cancer risk of the region they moved to [4]. Within a given high-risk population, groups with different life styles have different colon cancer risks. Seventh Day Adventists in the U.S.A. have a lower incidence than the general population, probably because they consume a diet low in fat and high in fibre [5].

Fat

The hypothesis postulates that a high fat diet enhances the formation and degradation of bile acids and neutral sterols exerting a promoting effect in colonic carcinogenesis. Indeed, it has been found that dietary fat increases the output and faecal concentration of bile acids [6]. Epidemiological evidence has shown that populations with a high incidence of colorectal cancer and consuming a high fat and animal protein diet, excrete about twice the amount of secondary bile acids [7]. The concentration of these bile acids is even more increased. However, other studies in the U.S.A., U.K. and New Zealand have failed to demonstrate a correlation between high fat intake and colorectal cancer incidence [8]. Case–control studies have shown conflicting results in this respect [9].

Bile acid metabolism

Bile acids are the major end products of cholesterol metabolism and are synthesised in the liver. The primary bile acids cholic acid (CA) and chenodeoxycholic acid (CDCA) are derived via several intermediate steps from cholesterol and secreted in bile as glycine or taurine conjugates.

They serve as cholesterol solubilising agents by the formation of micelles, and play an important role in the digestion and absorption of lipids in the small intestine. More than 95% of the bile acids passing through the ileum are reabsorbed and return to the liver through the portal vein. An efficient conservation in the so-called enterohepatic circulation is thus achieved. The proportion of bile acids not absorbed in the terminal ileum is 2–5% per cycle, and amounts to an average loss of 20% of the bile acid pool with 6–12 enterohepatic circulations per day. Bile acids that escape absorption in the ileum, are metabolised in the large bowel by the anaerobic bacterial flora. First, deconjugation takes place and the amino acid molecule on the carboxyl group is removed. Secondly, the primary bile acids CA and CDCA are dehydroxylated and converted into the secondary bile acids deoxycholic acid (DCA) and lithocholic acid (LCA), respectively. Further bacterial degradation in the large bowel and alterations in the liver produce the tertiary bile acids. DCA is partly absorbed in the colon and enters the enterohepatic circulation, where it is conjugated in the liver and secreted in

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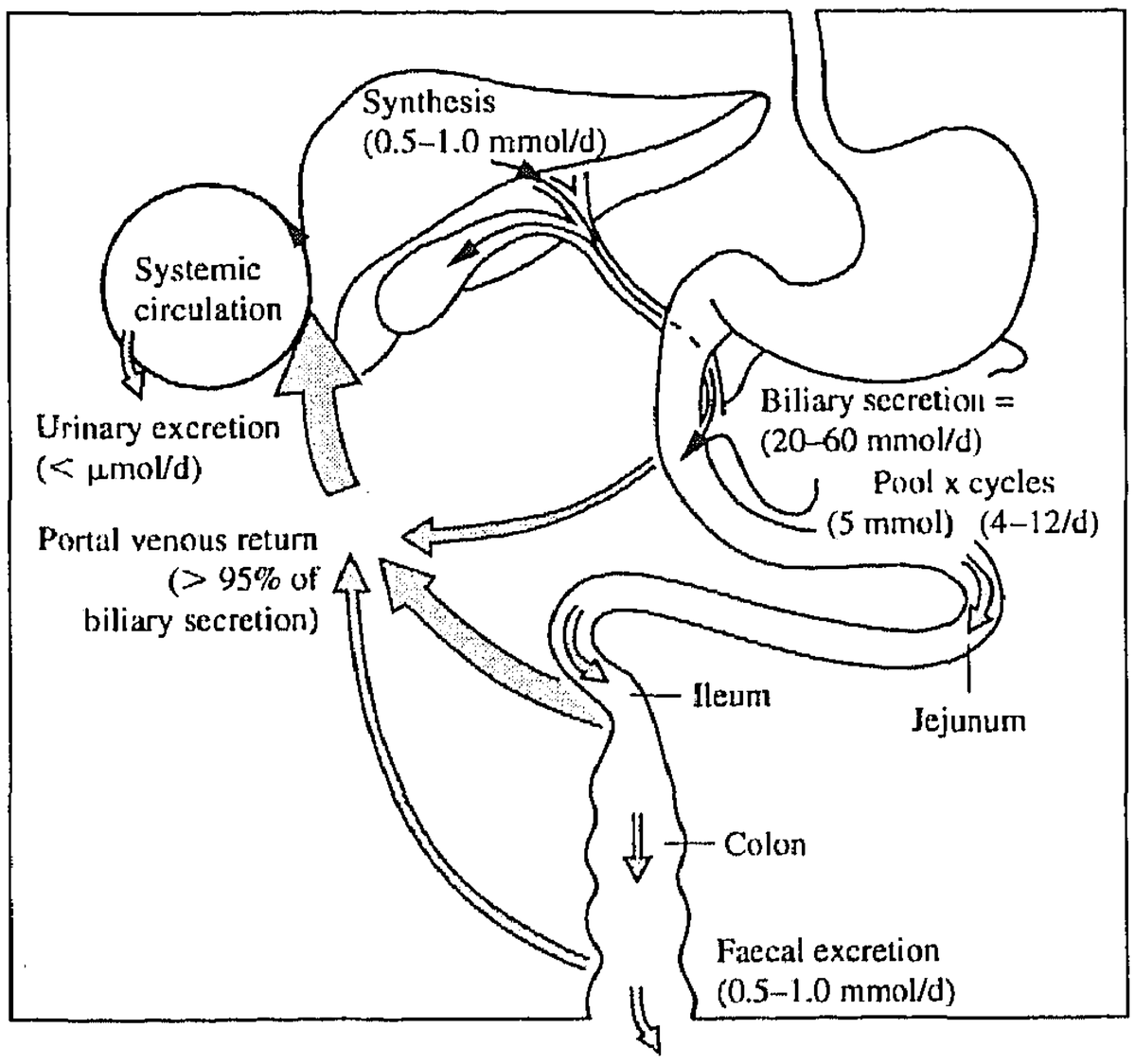

Figure 1. The enterohepatic circulation of bile acids in man.

bile; LCA is almost insoluble and very little is reabsorbed. Both secondary bile acids are excreted in the stool and make up to 95% of the total amount of excreted bile acids. In the stool, the major part of the bile acids are bound to dietary and bacterial residues. In the circulating bile acid pool, CA and CDCA each comprise about 30–40%, DCA about 20–30% and LCA less than 5% of the total amount [10]. Figure 1 shows the enterohepatic circulation of bile acids.

EXPERIMENTAL EVIDENCE

The hypothesis that the relationship between diet and colorectal cancer is established through bile acid metabolism has led to many studies on cell systems, to experimental studies in animals (mostly rodents) and metabolic and interventional studies in humans.

Epidemiology

Colon cancer incidence is positively related to dietary fat intake and negatively to fibre and probably even more to total starch intake [11]. A high fat consumption leads to a higher bile acid excretion. Several epidemiological studies have shown that the concentration of faecal bile acids is positively related to colonic cancer incidence [12]. Case–control experiments, however, have shown conflicting results: in some a higher faecal bile acid concentration was found in patients with adenomas or cancer, others found no difference between cases and controls [13].

Part of the discrepancy can be attributed to confounding factors, such as age and dietary consumption, which were not controlled for in these investigations. Faecal bile acid concentration proves to be age-dependent and inversely related to dietary fibre intake [14]. In some studies bile acid kinetics or biliary and serum bile acids were measured. The absorption of DCA from the large bowel is also age-dependent [15, 16], higher in adenoma patients than in age-matched controls, and coincides with a more anaerobic environment [17, 18]. One study showed a higher biliary CDCA fraction in adenoma and carcinoma patients [19]. Recently it was demonstrated that adenoma patients have a higher serum DCA concentration than healthy controls [20, 21].

Animal experiments

Secondary bile acids can act as tumour promoters in animal experiments, which are ideally performed in rodents. Because spontaneous colon cancer rarely occurs in rodents, initiating carcinogens such as azoxymethane have to be used [13]. Studies have been performed both by dietary manipulation (fat and fibre) and by direct application of bile acids to the colonic mucosa. Feeding high fat diets resulted in a higher tumour yield and an increased faecal bile acid concentration. Fibre addition has an opposite effect, although results were conflicting in this respect [13]. After diversion of bile ducts or small bowel resection more tumours can occur [22]. Direct installation of bile acids in the large bowel can be tumour promoting [23]. In one study, infusion of DCA led to damage of the mucosa thereby provoking an increased cell renewal. This was accomplished by increased cell proliferation which might be the key mechanism in the effect of bile acids in colonic carcinogenesis [24]. From this and other experiments, the concept has emerged that the concentration of soluble bile acids rather than the total faecal bile acid concentration determines possible cytotoxic effects of these molecules. The former is reflected by the concentration in the aqueous phase of the stool.

Genotoxicity and mutagenicity

Secondary bile acids can have co-mutagenic effects as has been shown in the Ames test [25]. It has also been demonstrated that LCA can transform hamster embryo cells in culture. LCA can break DNA strands in cultured L1210 cells and enhance the activity of repair mechanisms after DNA strand breakage caused by 2-aminoanthracene [26, 27].

Both LCA and DCA stimulated the incorporation of tritiated thymidine in mouse liver and biliary tract epithelium, suggestive of enhanced cell proliferation [28]. Among components contributing to faecal mutagenicity are reactive glyceryl ethers, known as fecapentaenes. Their biosynthesis might be stimulated by bile salts [29]. In general conjugated bile salts have less or no genotoxic effects and unconjugated dihydroxy and monohydroxy bile salts are more genotoxic. However, it must be kept in mind that the most abundant monohydroxy bile acid in the human colonic lumen, LCA, is very poorly soluble in water.

Cytotoxicity of bile acids

Damaging effects of various bile acids on the colonic mucosa have been described at the concentrations present in the aqueous phase of stool [24]. Bile acids can disrupt the integrity of the cell membrane of colonic mucosal cells [24, 30–32]. The increased cell loss will stimulate a compensatory cell renewal by increased mucosal proliferation. Thus, dietary manipulation resulting in a rise in colonic bile acid concentration can cause increased mucosal proliferation [33, 34]. In addition to the attractive hypothesis that hyperproliferation is induced by the cytotoxic potential of bile acids, there is also evidence of a direct stimulatory effect of several bile acids on proliferation. Bile salts (DCA) can release prostaglandin E_2 (PGE_2) from colonic tissues. The proliferative activity of colonic epithelial cells is among other things suppressed by PGE_2. Bile salts can enhance the release of arachidonate from colonocytes and subsequently the synthesis of PGE_2. This could be another explanation of the link between cell proliferation and bile acids [35]. A third effect could be on a family of enzymes within the cell membrane known as protein kinases. Protein kinase C appears to play a critical role in tumour promotion and in the action of growth factors [36]. Bile acids might have a direct stimulatory effect on subclasses of these enzymes [37].

Recently, a putative mechanism of hepatocyte necrosis was published. Toxic bile salts (in the liver GCDC) impair mitochondrial function, leading to an inhibition of oxidative phosphorylation and enhanced formation of toxic oxygen species by the mitochondrial respiratory chain. This results in oxidative stress and ATP depletion causing an increase in Ca^{2+} concentration with stimulation of hydrolases. This could lead to hydrolysis of lipid membranes and structural proteins causing cell death by necrosis [38].

We do not know whether this mechanism could be operative in colonocytes, since the type of cell and amount of cytotoxic bile salts differs considerably from the liver, but it remains an attractive hypothesis for cytotoxicity. Indirect evidence that this mechanism might also be applicable to cells other than hepatocytes, comes from experiments in which cytotoxicity of bile salts is determined in colon cancer cell lines (HT-29, CACO-2). In these cell lines cytotoxicity can be measured by the MTT assay, in which the mitochondrial function of the cell is tested. In viable cells MTT (3-(4,5-dimethylthizol-2-yl)-2,5-diphenyl tetrazolium bromide) is converted by the mitochondria to formazan, a blue dye, which can be detected in a fluorometer. With this assay, we and others have shown that the unconjugated dihydroxy bile acids DCA and CDCA are cytotoxic in a range that can be found in faecal water [32, 39]. Conjugated dihydroxy bile acids and cholic acid are not cytotoxic in this assay. So, as stated previously, the bile acid-induced increase in mucosal proliferation may be the key step in the association between bile acids and colon carcinogenesis. It has been demonstrated that a hyperproliferative colonic mucosa is more susceptible to carcinogens than a quiescent mucosa [40–42].

When proliferation is increased, the fraction of cells in S-phase (target cells) is relatively high, possibly resulting in an increased potency of intraluminal mutagenic substances. Large bowel neoplasms are associated with changes in proliferative characteristics, and in patients with colonic adenomas and cancer, an overall increased colonic mucosal proliferation has been demonstrated [43]. Furthermore, the proliferative compartment expands from the basal part of the crypts to the luminal surface. Similar changes in proliferative acitivity can be seen in patients with familial adenomatous polyposis, who are at high risk of developing colonic cancer. So, within the adenoma–carcinoma sequence, hyperproliferation might be a relatively early event leading towards an increased susceptibility to colonic cancer. Bile acids possibly play an important intermediate role in this process. It has to be kept in mind, however, that many other factors contribute to this cascade of events. Most intriguing, of course, are the successive genetic events that occur in the adenoma–carcinoma sequence [44]. In Figure 2, a hypothesis of colonic carcinogenesis is shown with special reference to the role of bile acids.

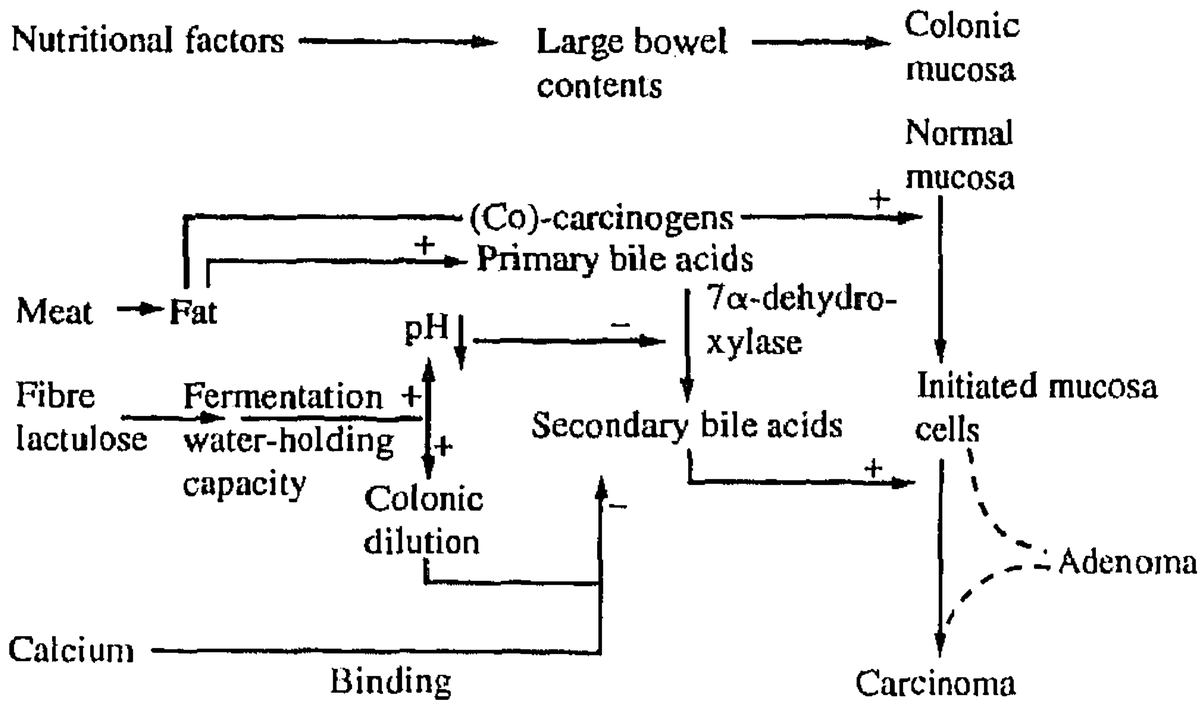

Figure 2. Hypothesis for the role of bile acids in colonic carcinogenesis.

CONCLUSIONS

Bile acids (salts) are amphiphilic molecules synthesised in the liver from cholesterol. They play an important role in the solubility of cholesterol in bile and in the digestive process in the small bowel through formation of micelles. An effective enterohepatic circulation keeps most of these bile acids within the body. During every cycle approximately 5% of the primary bile acids are lost into the large bowel. Here extensive degradation by the anaerobic flora occurs. The main events are deconjugation and dehydroxylation leading to the formation of unconjugated secondary bile acids. These last compounds have been incriminated in colonic carcinogenesis. Thus far, evidence is largely circumstantial and is derived from epidemiological and experimental studies in both animals and humans. Bile acids are probably cytotoxic to colonocytes and lead to a compensatory cell proliferation. The mechanism of cytotoxicity is not well understood, but can be attributed to membrane as well as intracellular effects. The increase in colonic cell proliferation is probably one of the key steps in the risk of development of colorectal cancer. Further studies are necessary to elucidate the mechanisms which are involved in the interaction of luminal events with the cascade of genetic changes that occur within the colonic mucosa during colonic carcinogenesis.

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