Summary

Colorectal cancer is one of the most common malignancies of the Western world. The American Cancer Society estimates that a total of 130,200 new cases of colorectal cancer will be diagnosed in the United States of America in the year 2000. Five years survival rates vary between 90% in patients diagnosed at an early stage to only 8% in patients with distal metastases at the time of initial diagnosis. The overall five years survival rate in persons diagnosed with symptomatic colorectal cancer is only 50-60%. Because of the extent of the disease and the unfavorable prognosis, much effort is put into increasing our knowledge about the carcinogenic process and developing strategies to prevent colorectal cancer. This thesis describes the effects of chemo- and dietary interventions aimed to influence luminal and epithelial factors associated with colon carcinogenesis. Special attention is paid to differential effects of intervention strategies in various parts of the colorectum. In the second part of this thesis, mechanisms involved in the process of colorectal carcinogenesis are studied, using the presumably carcinogenic sexxoside laxatives as a model.

The development of colorectal cancer is a gradual process, involving both genetic and environmental factors. Modulating this process by intervening in environmental factors such as diet requires the presence of assessable parameters. The most direct approach, using colorectal cancer as primary end-point, is time-consuming and expensive, as it involves the follow-up of numerous patients during many years. Studies with smaller numbers of patients for a shorter period of time can be performed if reliable intermediate endpoints of colorectal cancer risk are available.

One of the factors presumably contributing to colorectal cancer risk is the composition of the diet. A diet high in fat increases the concentrations of fatty acids and secondary bile acids in feces and fecal water, the aqueous fraction of feces. These substances are cytotoxic to colonic epithelial cells and induce hyperproliferation to restore the colonic epithelium from the inflicted damage. Increased proliferative activity of colonic epithelium is generally considered one of the primary steps in the process of colon carcinogenesis. Concentrations of cytotoxic substances in feces and the cytotoxic potential of fecal water as well as the proliferative activity of colonic epithelial cells are being used as intermediate biomarkers of colorectal cancer risk.

Secondary bile acids, deoxycholic acid (DCA) and lithocholic acid (LCA) are formed in the colon by bacterial 7α-dehydroxylation from the primary bile acids cholic acid (CA) and chenodeoxycholic acid (CDCA) respectively. The hydrophobic bile acids DCA and LCA are membrane damaging and therefore cytotoxic to colonic epithelial cells.

Ursodeoxycholic acid (UDCA) is a more hydrophilic bile acid that is normally only present in the colon in small amounts. Several protective properties have been attributed to increased con-
centrations of UDCA in the colon. UDCA inhibits membrane-damaging effects of hydrophobic bile acids and reduces the production of CA, which eventually results in lower concentrations of DCA. We performed a randomized placebo-controlled cross-over study in 15 healthy volunteers to assess the effect of 900 mg/day of UDCA for four weeks on fecal bile acid composition and cytolytic activity of fecal water. Chapter 2 shows that supplementation with UDCA decreased the percentages of CA, CDCA and DCA, whereas the concentrations of these bile acids in fecal water were not affected. Despite high levels of UDCA after UDCA supplementation, cytolytic activity of fecal water was not reduced. This could be due to the increased concentrations of LCA following intervention with UDCA. The results of this study do not support a role for UDCA in the chemoprevention of colorectal cancer in the human situation.

The preferential occurrence of tumors in the distal colon in regions with a high incidence of colorectal cancer can probably be ascribed to dietary factors and their effect on luminal colonic contents. Difficulties in obtaining representative samples from different colonic regions have contributed to the fact that little is actually known about the composition of colonic contents in the proximal and distal colon. In chapter 3 hemicolectomy patients, in whom either the proximal (right hemicolectomy) or distal (left hemicolectomy) colon had been removed, served as a model to evaluate regional differences in bile acid metabolism. In 9 left and 16 right hemicolectomy patients the percentage of DCA in feces was lower than in 17 control subjects with an intact colon. Left hemicolectomy patients had a lower proportion of DCA in duodenal bile than right hemicolectomy patients and those with an intact colon. This is probably caused by the predominant absorption of protonated DCA in the distal colon. Indeed higher concentrations of protonated DCA were present in colonic contents of right hemicolectomy patients. As especially protonated DCA is thought to be cytotoxic, it is not surprising that cytotoxic activity of fecal water was higher in right hemicolectomy patients. This study clearly demonstrates differences in bile acid handling between the proximal and distal colon and may help to understand differences in cancer risk between these colonic regions.

Right hemicolectomy patients probably have an increased risk of developing metachronous tumors in the remaining distal colon. This might be due to the higher cytotoxic activity of fecal water as shown in chapter 3. Calcium and resistant starch are promising agents for dietary intervention in patients with increased risk of colorectal cancer. Calcium probably exerts its protective effect by binding to secondary bile acids and other cytotoxic substances, thus rendering them harmless for the colonic epithelium. The protective effect of resistant starch is probably brought about by the fermentation of resistant starch to short chain fatty acids (SCFA). These SCFA's can either exert a direct protective effect on colonic epithelium or indirectly influence the carcinogenic process by diminishing the production of secondary bile acids
Summary, conclusions and future perspectives

Chapter 11

due to a reduction of fecal pH. Chapter 4 describes the results of two randomized placebo-controlled cross-over intervention trials in 2 x 15 right hemicolectomy patients with 1.0 g calcium and 19 g resistant starch. After two months of supplementation calcium reduced fecal water alkaline phosphatase activity, a putative marker for intestinal epitheliolysis, but did not alter cytotoxicity of fecal water. Proliferative activity in the luminal part of colonic crypts was decreased. A reduction in luminal proliferative activity, even without affecting total proliferation, is generally assumed to be beneficial. Resistant starch did not affect either cytotoxic activity of fecal water or proliferative activity of colonic epithelial cells but reduced alkaline phosphatase activity. This study thus suggests that only calcium supplementation but not resistant starch may have a protective effect on colorectal cancer risk in right hemicolectomy patients.

As described in chapter 5, the effects of calcium and resistant starch were further evaluated in patients with a history of benign colorectal adenomas, who are known to have an increased rate of epithelial cell proliferation and an enhanced risk of developing colorectal carcinoma. After two months of supplementation with either 1.0 g calcium plus placebo, 19 g resistant starch plus placebo or two placebos, site specific effects on cell proliferative activity were studied in biopsies of coecum, transverse colon, sigmoid colon and rectum obtained from 86 patients with a history of adenomatous polyps. Proliferative activity, expressed as labeling index (LI), was similar in the three groups in all areas of the colorectum.

Proli erative activity of the colonic epithelium is only one side of the carcinogenic process. In the early development of tumors the balance between cell proliferation and cell death is de ranged. When DNA is damaged by e.g. chemical substances, cells enroll in a genetically controlled programmed cell death, apoptosis, which is started by increased production of the p53 protein. P53 in conjunction with p21/WAF, an inhibitor of cyclin-dependent kinases, which are necessary for entry into the S-phase of the cell cycle, stops cell division at the G1-S phase. At this point either DNA is repaired and cell division is resumed, or cells undergo p53-mediated apoptosis, a process which is further regulated by members of the bcl-2 family, containing both inhibitors and promoters of apoptosis. Failure of cells to undergo apoptotic cell death despite irreparable DNA-damage can lead to uncontrolled proliferation of genetically altered cells and ultimately the formation of a neoplasm. Bcl-2, an inhibitor of p53 mediated apoptosis, is indeed frequently abnormally activated at an early stage of colorectal carcinogenesis. The second part of this thesis focuses on mechanisms involved in carcinogenesis and the role of proliferation, apoptosis and mediators of the apop-
totic pathway, using sennoside laxatives as a model to study the effects of a cytotoxic and possibly carcinogenic substance on colonic epithelium.

Sennoside laxatives are drugs of natural origin commonly used for short-term treatment of constipation and for diagnostic procedures that require bowel cleansing. Short-term administration of these laxatives is generally considered safe without any acute or long-term danger of enhanced colorectal cancer risk. However, chronic use of these laxatives induces pseudomelanosis coli, a condition characterized by a brownish pigmentation of the colonic mucosa, and this has been associated with an enhanced risk of colorectal cancer. Chapter 6 reviews sennoside and other anthranoid laxatives and their potential carcinogenic effects as evident from in vitro, animal and human studies.

A single high dose of sennoside laxatives is often used either as a single agent or together with other laxatives to ensure an optimal bowel preparation for colonoscopies or other diagnostic procedures. Chapter 7 investigates whether sennoside laxatives have any additional value on bowel lavage and whether they induce any adverse effects on colonic mucosal histology. A total of 171 patients were randomized for bowel preparation with either 1 ml/kg bodyweight of a senna-containing (2 mg/ml) syrup and 3-5 l lavage solution or lavage solution alone. Sennoside laxatives did not improve bowel preparation but an increase of mononuclear infiltrate was observed in the lamina propria of the colonic mucosa, which could interfere with diagnostic interpretation of colonic biopsies. Therefore bowel preparation without sennosides is preferable.

To gain insight into the suggested cancer promoting effect of chronic sennoside ingestion, the effect of a single high dose sennoside on apoptosis, crypt length, proliferative activity, p53 and bcl-2 expression was evaluated in chapter 8. Biopsies were obtained from 4 regions of the colorectum i.e., coecum, transverse colon, sigmoid colon and rectum, from 15 patients 18 hours after sennoside administration and 15 control patients. Sennoside laxatives reduced crypt length and increased proliferative activity. Bcl-2 expression was increased in both groups, when crypts were shorter and/or proliferative activity was increased. No effect on apoptosis or p53 expression could be observed.

We hypothesized that sennosides rapidly induce apoptosis of colonic epithelial cells, which results in the observed shorter crypts. Probably as a compensatory mechanism to restore cellularity, proliferative activity is subsequently increased and apoptosis inhibited. To test this hypothesis, another study, as described in chapter 9, was performed in 15 subjects receiving sennosides and 15 control patients. This time, sigmoid biopsies were obtained 6 hours after sennoside ingestion and evaluated for apoptosis, p53, p21/WAF and bcl-2 expression and for proliferative activity. Archival material from 27 patients with pseudomelanosis coli, representing long-term sennoside use, was collected. Histologically a distinction was made between moderate and severe forms of pseudomelanosis coli. This material was also analyzed for the aforemen-
tioned parameters. Six hours after sennoside administration apoptosis was induced, resulting in shorter crypts. Expression of p53 and p21/WAF was increased as well, whereas no effect on bcl-2 or proliferative activity was observed. In biopsies of patients with a severe form of pseudomelanosis coli the degree of apoptosis, intensity of p53 staining and p21/WAF expression were also enhanced. Again no alteration of bcl-2 expression or proliferative activity could be demonstrated. Remarkably, crypt length was increased in biopsies of severe pseudomelanosis coli patients. From this study it can be concluded that within 6 hours sennosides induce apoptosis probably by a p53, p21/WAF mediated pathway, which leads to crypt shortening. Despite a higher degree of apoptosis in patients with severe pseudomelanosis coli, an increasing number of cells seems to become refractory to apoptotic cell death, causing crypt lengthening without rise in proliferative activity or bcl-2 expression and thus possibly enhancing the risk of colorectal cancer.

Chapter 10 investigates the in vitro effects of different anthranoid laxatives, including rhein, the active metabolite of senna, aloe emodin and danthron. Induction of apoptosis, interaction with DNA and cytotoxicity of these substances were evaluated using different carcinoma cell lines. Interference with anthranoid-induced cytotoxicity by mechanisms involved in multidrug resistance, such as the drug efflux pumps Pgp and MRP1 and the topoisomerase enzymes, were studied as well. Pgp and MRP1, drug efflux pumps are present in normal colonic epithelium and probably protect colonic epithelial cells against damage induced by xenobiotics by pumping these substances out of the cells. All anthranoid laxatives tested by means of the MTT cytotoxicity assay were cytotoxic to different carcinoma cell lines. The MRP1 drug efflux pump was involved in protection against rhein-induced cytotoxicity as rhein was more toxic to the GLC4 cell-line than to its MRP1-overexpressing subline GLC4/ADR. Pgp and topoisomerases were not involved in reduction of rhein cytotoxicity. Rhein did not intercalate in DNA but induced apoptosis in all cell lines tested. No resistance mechanism was observed for aloe emodin and danthron. From this study it can be concluded that rhein induces apoptosis and that the drug efflux pump MRP1 protects against rhein-induced cytotoxicity in an in vitro setting.

Conclusions and future perspectives

In this thesis colorectal cancer is approached from three different sides: regional differences of luminal and epithelial factors involved in colorectal carcinogenesis, modulation of these factors by means of chemical and dietary intervention and mechanisms of the carcinogenic process itself.

Regional differences of luminal and epithelial factors involved in colorectal carcinogenesis

In populations with a high risk of colorectal cancer tumors predominantly develop in the distal colon. This is generally ascribed to dietary factors and composition of luminal colonic con-