Abstract. Background: Dietary compounds or nonsteroidal anti-inflammatory drugs (NSAIDs) may reduce cancer rates. Elevation of phase II detoxification enzymes might be one of the mechanisms leading to cancer prevention. We investigated the effects of dietary anticarcinogens and NSAIDs on rat gastrointestinal UDP-glucuronosyltransferases (UGT). Materials and Methods: Diets of Wistar rats were supplemented with oltipraz, α-tocopherol, β-carotene, phenethylisothiocyanate (PEITC), sulforaphane analogue compound-30, indole-3-carbinol, D-limonene, relafen, indomethacin, ibuprofen, piroxicam, acetyl salicylic acid or sulindac. Hepatic and intestinal UGT enzyme activities were quantified by using 4-nitrophenol and 4-methylumbelliferone as substrates. Results: Compound-30, D-limonene, indomethacin, ibuprofen or sulindac enhanced proximal small intestinal UGT activities. Only compound-30 was able to induce mid- and distal small intestinal UGT activities. Large intestinal UGT activities were increased by ibuprofen and sulindac, whereas oltipraz, PEITC and D-limonene gave enhanced hepatic UGT activities. Conclusion: Mainly rat proximal small intestinal and hepatic UGT enzyme activities were induced by dietary anticarcinogens or NSAIDs. Enhanced UGT activities might lead to a more efficient detoxification of carcinogenic compounds and thus could contribute to the prevention of gastrointestinal cancer.

There is considerable interest in identifying dietary or synthetic compounds with anticarcinogenic properties. From epidemiological studies there is growing evidence that diets containing abundant vegetables and fruit may reduce the risk of cancers, especially cancers of the gastrointestinal tract (1, 2). The human diet may contain a large number of both (pre)carcinogens as well as a variety of compounds with potential anticarcinogenic properties (3, 4).

The carotenoids, α-tocopherol and β-carotene, protected rodents against development of chemically-induced tumours in the gastrointestinal tract (5-7), whereas epidemiological data on their anticarcinogenic capacity are somewhat contradictory. A case-control study showed that α-tocopherol levels in blood were inversely correlated with cancer risk (8). Subsequently, β-carotene intake was also associated with a decreased risk for developing colorectal adenomas in a case-control study (9). In addition, strong evidence was provided for a protective role of α-tocopherol or β-carotene against oesophageal, but not gastric cancer risk (10). However, in a prospective cohort study, no significant association between dietary α-tocopherol or β-carotene and risk for colorectal cancer was found (11). Phenethylisothiocyanate (PEITC) and indole-3-carbinol, breakdown products of glucosinolate precursors present in cruciferous vegetables, possess anticarcinogenic properties. PEITC was shown to inhibit chemically-induced carcinogenesis in the oesophagus and colon of rats (12, 13), while indole-3-carbinol was able to inhibit chemically-induced tumours in the forestomach (14), colon (15, 16) and liver (17) in rodents. In contrast, indole-3-carbinol treatment for 25 weeks strongly induced glutathione S-transferase Pi (GST-Pi) foci in the liver of rats (16). The monoterpane D-limonene inhibited chemically-induced gastric (18), colonic (19) or hepatic cancer (20). Compound-30 is a structural analogue of sulforaphane (21) and a component of broccoli. Sulforaphane was demonstrated to have anticarcinogenic properties in the colon of rats (13). Oltipraz is a substituted dithiolthione, which was used in humans as an antischistosomal drug. Dithiolthiones occur in cruciferous vegetables. Oltipraz was found to inhibit
chemically-induced carcinogenesis in the stomach (22), colon (23) and liver (24) of rats. The first clinical trial with oltipraz to investigate the effects on aflatoxin biomarkers was conducted in 1995 in 234 residents of Qidong, who were at high risk for exposure to aflatoxin and development of hepatocellular carcinoma (25). Intermittent, high-dose oltipraz was shown to inhibit phase I activation of aflatoxins, while sustained low-dose oltipraz increased phase II conjugation of aflatoxin (26).

Nonsteroidal anti-inflammatory drugs (NSAIDs) are among the most prescribed drugs worldwide and have anti-inflammatory, analgesic and antipyretic activities. In addition to their therapeutic use, there is strong evidence that NSAIDs may have anticarcinogenic effects in humans. Epidemiological, animal and clinical studies suggest that NSAIDs may reduce the risk of development of and mortality from gastrointestinal cancer (27).

Relafen inhibited the development of aberrant crypt foci (ACF) in azoxymethane-treated rats and suppressed development of intestinal tumours in adenomatous polyposis coli (APC) Min mice (28). Indomethacin inhibited chemically-induced carcinogenesis in the forestomach (29), colon (15) and liver (30). Ibuprofen also inhibited colon carcinogenesis (15, 27). Furthermore, ibuprofen reduced tumour multiplicity and incidence in the forestomach (31). Piroxicam inhibited carcinogen-induced ACF in the colon of rats (27, 32). In APC Min mice, piroxicam reduced tumour number and multiplicity (33). Chemically-induced carcinogenesis was inhibited by acetyl salicylic acid in the colon (27, 32) and liver (34). Recently, a clinical trial with aspirin reported significant reduction in the incidence of colorectal adenomas in patients with previous colorectal cancer (35). Sulindac suppressed the development of colonic preneoplastic lesions induced by azoxymethane (27, 36). In addition, sulindac reduced the relative risk of development of oesophageal cancer (37) and reduced tumour multiplicity and incidence in the forestomach (31). In patients treated with sulindac a reduced polyp number in the rectal segment was observed (38). However, this was not confirmed by Giardiello et al. (39).

The exact mechanisms of action of the above-mentioned inhibitors of carcinogenesis have not been clearly defined yet. Although the chemopreventive potential of these dietary and synthetic compounds may be due to multiple mechanisms, one mode of action may be enhancement of phase II detoxification enzymes, such as UDP-glucuronosyltransferases (UGTs, 40) and glutathione S-transferases (GSTs, 41). UGTs conjugate a wide variety of compounds to UDP-glucuronic acid (UDPGA), while GSTs catalyse the conjugation with glutathione. Conjugation with glucuronic acid or glutathione in general results in less biologically active molecules and enhances the water-solubility of the conjugated products, which facilitates excretion from the body via bile or urine (42). In the digestive tract, the colon is the site where the majority of malignant tumours do develop, whereas the detoxification capacity in the colon may be critically low (43). Therefore, enhancement of the activity of such enzymes could potentially increase the capacity to withstand the burden of toxic agents and (pre) carcinogens that we are exposed to daily (3, 4). Knowledge of the exact protection mechanism(s) of dietary anticarcinogens and NSAIDs may be of importance for the prevention of gastrointestinal cancer. Previous research demonstrated that several dietary agents and NSAIDs increased GST enzyme activities in the liver and intestine of male Wistar rats (44-46). Therefore, we here investigated the effects of naturally occurring or synthetic anticarcinogens and NSAIDs on rat hepatic and intestinal UGT enzyme activities.

Materials and Methods

Materials. Oltipraz was from Rhone Poulenc Rorer (France). Bovine serum albumin, dithiothreitol, 4-methylumbelliferone, UDPGA and D-saccharic acid 1,4-lactone, α-tocopherol, β-carotene, phenethylisothiocyanate, indole-3-carbionil, relafen, indomethacin, ibuprofen, piroxicam, acetyl salicylic acid and sulindac were purchased from the Sigma Chemical Company (USA). D-limonene was obtained from Aldrich Chemie (Germany). 4-Nitrophenol was from Merck (Germany). Sulforaphane analogue compound-30 was synthesised as described before (21). All dietary anticarcinogens and NSAIDs used were of the highest grade purity commercially available.

Study design. Hepatic and intestinal microsomes were prepared from Wistar rats, kept and treated as described in previous studies performed by Van Lieshout et al. (44-46). In short, male Wistar rats (Central Laboratory Animal Centre, University of Nijmegen, the Netherlands) were housed in pairs on wooden shavings in macrolon cages, maintained at 20-25°C and 30-60% relative humidity. A ventilation rate of seven air cycles/h and a 12 h light/dark cycle were used. The rats were randomly assigned to one of the dietary treatment groups. All groups were fed powdered RMH-TM lab chow (Hope Farms, Woerden, The Netherlands). After acclimatisation for seven days, the animals were fed either the basal diet (control group) or one of the experimental diets.

Diets. The diets were prepared by supplementation with either one of the dietary compounds: 0.03% (w/w) oltipraz, 0.02% (w/w) α-tocopherol, 0.02% (w/w) β-carotene, 0.045% (w/w) phenethylisothiocyanate, 0.145% (w/w) sulforaphane analogue compound-30, 0.025% (w/w) indole-3-carbionil and 1.0% (w/w) D-limonene, or with one of the following NSAIDs: 0.02% (w/w) relafen, 0.0025% (w/w) indomethacin, 0.04% (w/w) ibuprofen, 0.04% (w/w) piroxicam, 0.04% (w/w) acetyl salicylic acid and 0.032% (w/w) sulindac. Food and water were available ad libitum. Food cups were replenished every 2-3 days. Food consumption and gain in body weight was recorded daily. After two weeks the animals were killed by decapitation.
Tissue preparation. Tissue handling, the isolation of liver and intestinal mucosa and preparation of microsomal pellet was performed as described previously (44). In short, liver tissue and intestinal mucosa were homogenised in buffer A (4 ml buffer A/g tissue (liver) or mucosal scraping (intestine); buffer A: 0.25 M saccharose, 20 mM Tris-HCl, 1 mM dithiothreitol, pH 7.4), followed by centrifugation at 9000 g (4°C) for 30 min. The resulting supernatant fraction was spun at 150,000 g (4°C) for 60 min, resulting in the sedimentation of the microsomes. The microsomal pellet was resuspended in 5 volumes of buffer A.

Assays. Protein concentration was assayed in duplicate by the method of Lowry et al. (47) using bovine serum albumin as the standard. UGT activity with 4-methylumbelliferone (4-MUB) or 4-nitrophenol (4-NP) as substrates was measured in the microsomes as described previously (48). Briefly, liver and intestinal microsomes were resuspended in 50 mM Tris-HCl, pH 7.4, containing 0.25 M sucrose and 1 mM dithiothreitol.

Conjugation of 4-MUB was performed in the presence of 4 mM UDPGA, 10 mM MgCl₂, 0.025-0.25 mg microsomal protein, 1 mM saccharic acid-1,4-lactone and 0.1 mM 4-MUB in a final volume of 0.1 ml Tris-HCl, pH 7.4. 4-MUB was dissolved in ethanol (50 mM) and diluted with assay medium just before use. After incubation at 37°C for 0.5-20 min, 1 ml 0.5 M glycine/NaOH, pH 10.35, was added to the reaction mixture to terminate the reaction. Subsequently, samples were centrifuged for 10 min at 10,000 g. 4-MUB was determined fluorometrically with a Shimadzu RF-5000 spectrofluorophotometer (excitation 370 nm, emission 450 nm).

Conjugation of 4-NP was measured in a final volume of 0.1 ml Tris-HCl, pH 7.4, containing 3.5 mM UDPGA, 10 mM MgCl₂, 0.05-0.5 mg of microsomal protein, 1 mM saccharic acid 1,4-lactone and 1 mM 4-NP. 4-NP was dissolved in 0.01 M NaOH. After incubation for 1-20 min at 37°C, the reaction was terminated by adding 2 ml of 0.3 M NaOH, followed by centrifugation for 10 min at 10,000 g. Absorbance was measured at 405 nm on a Perkin Elmer Lambda 12 spectrophotometer. All samples were measured in duplicate. In all assays a control sample without UDPGA was run simultaneously. The absorbance difference between the control sample and the sample incubated in the presence of UDPGA represents the amount of 4-MUB or 4-NP that was conjugated. The effects of anticarcinogens on UGT enzyme activity are presented as ratios treated/control.

### Table I. Effects of dietary anticarcinogens or NSAIDs on rat intestinal- and hepatic 4-methylumbelliferone UGT enzyme activities.

<table>
<thead>
<tr>
<th>Treatment group</th>
<th>n</th>
<th>4-Methylumbelliferone UGT activity (ratio treated/control)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>proximal</td>
</tr>
<tr>
<td>Oltipraz</td>
<td>8</td>
<td>1.3 (0.8-1.8)</td>
</tr>
<tr>
<td>α-Tocopherol</td>
<td>8</td>
<td>1.1 (0.8-2.0)</td>
</tr>
<tr>
<td>β-Carotene</td>
<td>8</td>
<td>0.89 (0.8-1.0)</td>
</tr>
<tr>
<td>PEITC</td>
<td>8</td>
<td>0.86 (0.8-1.1)</td>
</tr>
<tr>
<td>Compound-30</td>
<td>8</td>
<td>1.4 b (1.0-2.1)</td>
</tr>
<tr>
<td>Indole-3-carbinol</td>
<td>8</td>
<td>1.1</td>
</tr>
<tr>
<td>D-Limonene</td>
<td>8</td>
<td>1.2 a (0.7-1.8)</td>
</tr>
<tr>
<td>Relafen</td>
<td>8</td>
<td>1.0</td>
</tr>
<tr>
<td>Indomethacin</td>
<td>8</td>
<td>1.1 a (0.5-1.3)</td>
</tr>
<tr>
<td>Ibuprofen</td>
<td>8</td>
<td>1.2 a (1.0-1.6)</td>
</tr>
<tr>
<td>Piroxicam</td>
<td>8</td>
<td>1.0</td>
</tr>
<tr>
<td>Acetyl salicylic acid</td>
<td>8</td>
<td>1.1 (0.8-1.4)</td>
</tr>
<tr>
<td>Sulindac</td>
<td>8</td>
<td>1.2 a (0.7-1.6)</td>
</tr>
</tbody>
</table>

4-MUB UGT activity was measured in duplicate as described in Materials and Methods. n = number of rats used in each group. Results are given as median ratios treated/control (range). In the control group (n = 8) median 4-MUB UGT activities (range) were 0.25 (0.17-0.29), 0.29 (0.21-0.41), 0.31 (0.20-0.49), 0.24 (0.10-0.33) and 10 (6-15) nmol/min.mg protein for proximal-, mid- and distal small intestine, large intestine and liver, respectively. The Wilcoxon rank-sum test was used for statistical evaluation; a p<0.05, b p<0.01, c p<0.005.
Statistical analysis. Wilcoxon rank-sum test was used to assess the statistical significance of differences between control and treatment groups. Correlation analyses between enzyme activities were performed using Spearman rank correlation. $P < 0.05$ was considered to be significant.

Results

The effects of feeding the dietary agents and NSAIDs on intestinal and hepatic 4-MUB UGT enzyme activities are summarised in Table I. Compound-30 was able to induce 4-MUB UGT activity in all studied parts of the small intestine (1.4x, 1.5x and 1.4x, respectively). Proximal small intestinal 4-MUB UGT activity was also enhanced by D-limonene (1.2x), indomethacin, ibuprofen and sulindac (1.1x, 1.2x, and 1.2x, respectively). Large intestinal 4-MUB UGT activity was only increased by ibuprofen (1.4x). Furthermore, hepatic 4-MUB UGT activity was enhanced after treatment with oltipraz (2.4x) PEITC (2.1x) and D-limonene (3.2x). No statistically significant changes in 4-MUB UGT activity were found with $\alpha$-tocopherol, $\beta$-carotene, indole-3-carbinol, relafen and acetyl salicylic acid in all organs investigated. The effects of the different anticarcinogens on intestinal and hepatic 4-NP UGT enzyme activities are the same as observed for 4-MUB UGT activity, except for acetyl salicylic acid which also induced 4-NP UGT activity in the proximal small intestine (1.3x). However, sulindac was not able to enhance 4-NP UGT activity in the proximal small intestine, though it increased large intestinal 4-NP UGT enzyme activity (1.7x).

Discussion

It is well known that environmental factors affect the development of human cancers. The human diet contains a large number of both (pre) carcinogens as well as a variety of compounds that may inhibit mutagenesis and/or carcinogenesis as tested in laboratory models (3, 4). Anticarcinogens are very diverse in chemical structure and their protective mechanisms are generally unclear. Although prevention of cancer may be due to multiple mechanisms, one mode of action of anticarcinogens may be to enhance the carcinogen detoxification systems, such as UGTs and GSTs (40, 41). These detoxification systems can minimise carcinogenicity by conjugation reactions, which add functional groups to the carcinogen, thereby lowering their biological activity and increasing their excretion.

In human organs at high risk for cancer development, low UGT levels were measured (43). At present little information on the effects of dietary and synthetic anticarcinogens on the UGT activity of the digestive tract is available. Recent data, mostly obtained from animal studies, have indicated that naturally occurring dietary anticarcinogens may be able to elevate UGT activity (49, 50). Oltipraz has been shown to elevate hepatic 4-NP UGT enzyme activity (23, 49, 51), which is similar to the results observed here. Rao et al. (23) also described a significant induction of colonic 4-NP UGT activity after oltipraz treatment in F344 rats, whereas we found no effect on colonic UGT activities in Wistar rats. Furthermore, oltipraz...
treatment did not significantly change UGT activities in rat proximal small intestine (51) and in other parts of the small intestine investigated here. In agreement with the results reported by Astorg et al. (52), we found no effect of β-carotene on hepatic 4-NP UGT enzyme activity. For PEITC, we found hepatic 4-NP UGT enzyme activity to be significantly increased, whereas a somewhat lower enhancement was also observed by Guo et al. (50), which may be explained by monitoring 48 h after treatment. Except for one recent report on changes in UGT1A1 mRNA and protein levels in HepG2 and HT29 cells (53), induction of UGT enzyme activity by sulforaphane or one of its analogues has not been reported before. Here we demonstrated that the sulforaphane analogue compound-30 significantly enhanced UGT enzyme activities in the small intestine, but not in the large intestine and liver. In the past, the effect of indole-3-carbinol on hepatic UGT enzyme activity was only examined using 1-naphthol as substrate, and no effect could be observed (54). This is in accordance with our results, since we found no effect of indole-3-carbinol on either hepatic or intestinal UGT enzyme activity with both 4-NP and 4-MUB as substrates. Like Elegbede et al. (55) we investigated the effects of D-limonene treatment on hepatic UGT enzyme activity. We found that 1% D-limonene significantly elevated 4-NP and 4-MUB UGT activities, whereas Elegbede et al. only observed an inducing effect with 5% and not 1% D-limonene on α-naphthol UGT activity. Such variations in results may either be caused by the use of different substrates (4-nitrophenol vs. α-naphthol) or by the different strains of rats. With regard to the effects of NSAIDs, Falzon et al. (56) described that 8.5 mg/kg indomethacin given intraperitoneally for 3 days significantly decreased hepatic 4-NP UGT activity by 22%. Furthermore, pallor of the liver and severe intestinal lesions were observed. We found no macroscopic signs of toxicity of indomethacin in all organs studied. In addition, we observed no inhibitory effects of indomethacin on 4-NP or 4-MUB UGT enzyme activities. No earlier reports on the effects of α-tocopherol, relafen, ibuprofen, piroxicam, acetyl salicylic acid and sulindac on hepatic or intestinal UGT enzyme activity were found.

Until recently, the tissue distribution of only the UGT1A family had been examined in the liver and gastrointestinal tract of rats (57). However, Shelby et al. (58) reported on the mRNA expression of the different members of the UGT1 and UGT2 families in the rat gastrointestinal tract. In the intestine many UGT1 and only a few UGT2B mRNAs were expressed, in contrast with the liver where many UGT2B mRNAs were predominantly expressed. The glucuronidation of NSAIDs may be mainly catalysed by the isoenzymes UGT1A6 (intestine) and UGT2B (liver, 59). In this study we measured UGT enzyme activity with 4-nitrophenol and 4-methylumbelliferone as substrates: both substrates mainly react with the isoenzymes of the UGT1 family (40, 42). This might explain why we did not find any effect of NSAIDs on the UGT enzyme activity in the liver.

Finally, we examined the associations between 4-MUB and 4-NP UGT, and GST enzyme activities, as measured in our earlier studies (44-46), and the data are presented in Table II. In general, strong correlations were observed between 4-MUB and 4-NP UGT enzyme activities in the liver and, too a much lesser extent, in the small and large intestine. Bock et al. (60) also found a strong association between 4-MUB and 4-NP UGT activities in the liver. This may be due to the fact that glucuronidation of both substrates may be mainly catalysed by the same UGT isofoms, and that by far the highest activities are present in liver (40, 42). In rats UGT1A1, 1A6 and 1A7 are highly expressed in the liver and intestine (57) and therefore the total UGT enzyme activity in these organs may be mainly covered by these UGT isofoms. Furthermore, significant associations between GST and UGT enzyme activities are also observed, however the correlation coefficients are low. The associations found may be explained by the fact that both GST and UGT genes are regulated by the same transcription factor Nrf2 (61).

In the present study, we demonstrated that dietary anticarcinogens or NSAIDs are capable of inducing UGT enzyme activities in the rat gastrointestinal tract, particularly in the proximal small intestine or liver. This may be of direct significance in the protection against cancer in the particular organ. However, organs such as the colon could also benefit from a more efficient detoxification in the proximal part of the digestive tract, since lower levels of carcinogens may reach the colon.

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