Induction of rat hepatic and intestinal UDP-glucuronosyltransferases by naturally occurring dietary anticarcinogens

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Gastrointestinal tumours are among the most common malignancies in Western society, the majority of which are associated with dietary and lifestyle factors. Many dietary or lifestyle factors have been identified which may have toxic or carcinogenic properties. However, several dietary compounds also able to reduce gastrointestinal cancer rates in both humans and animals have been characterized. Though the exact mechanism leading to the anticarcinogenic action of these compounds is not fully known, it has been demonstrated that this chemopreventive capacity may be due to elevation of the glutathione S-transferase detoxification enzymes. Here we have investigated the effect of several anticarcinogens on the gastrointestinal UDP-glucuronosyltransferase (UGT) enzymes. Diets of male Wistar rats were supplemented with ellagic acid, UDP-glucuronosyltransferase (UGT) enzymes. Diets of male Wistar rats were supplemented with ellagic acid, 31

Introduction

Gastrointestinal tumours are among the most common malignancies in Western society. Epidemiological studies have shown the importance of dietary habits in the risk for gastrointestinal tumours in general and for colon cancer more particularly. Diets rich in vegetables and fruit are associated with a lower risk (1–3). In the face of these epidemiological associations many dietary non-nutritive constituents with anticarcinogenic properties have been identified (4–9). Though the exact mechanism(s) leading to the anticarcinogenic action of these compounds is not fully known, it has been suggested that their chemopreventive capacities may be due to elevation of detoxification enzymes (4,6–8,10,11). Important detoxification or drug-metabolizing enzymes are UDP-glucuronosyltransferases (UGTs) and glutathione S-transferases (GSTs). UGTs catalyse the conjugation of exogenous and endogenous, mainly lipophilic compounds with glucuronic acid, while GSTs catalyse the reaction of glutathione with mainly exogenous electrophiles and endogenous products of oxidative stress. Some overlap in substrate specificity may occur between these detoxification enzymes. Glucuronidation or conjugation with 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 (12). UGTs have been divided into two families, termed UGT1 and UGT2 (13). UGT1 enzymes mainly catalyse glucuronidation of exogenous agents (drugs, pesticides, benzo[a]pyrene, etc.), whilst UGT2 enzymes glucuronidate endogenous agents (steroid hormones and bile acids), however, this classification is not that strict since, for instance, human UGT1A1 is the main enzyme catalysing conjugation of the endogenous substrate bilirubin, whereas non-steroidal anti-inflammatory drugs are conjugated by rat UGT2B1 (14).

In humans, glucuronidation capacity is prominently present in the liver, where most of the various UGT isoforms have been identified at relatively high levels. However, different isoenzymes of UGT1 have also been distinguished in extra-hepatic tissues, including skin, kidney, oesophagus, stomach, small intestine, colon and many other organs (15,16). Since, the gastrointestinal tract is in direct contact with potentially toxic or (pre)carcinogenic agents, ingested in food, medication, drugs, etc., the intestinal mucosa acts as a first line barrier. A striking observation is the significantly lower glucuronidation rate in colon compared with those in liver and small intestine (15,17,18). Tissue-specific expression of the different UGT genes encoding for the various isoenzymes in colon, liver and small intestine could partially contribute to the differences in UGT activity between these tissues (15,18). However, because of the magnitude of the UGT enzyme activity differences between colon and liver or small intestine (15,18), it cannot be ruled out that additional modifiers of UGT activity are responsible for the dramatic differences in functional UGT activities between these tissues.

The majority of malignant tumours in the digestive tract develop in the colon, where UGT enzyme activity is low, and this may contribute to the susceptibility to colon cancer in

Abbreviations: GSTs, glutathione S-transferases; 4-MUB, 4-methylumbelliferone; 4-NP, 4-nitrophenol; UDPGA, UDP-glucuronic acid; UGTs, UDP-glucuronosyltransferases.
humans (13,15,17). Enhancement of the activity of such enzymes could potentially increase the capacity to withstand the burden of toxic agents and (pre)carcinogens we are exposed to daily (7,19,20). Knowledge of the exact protection mechanism(s) of anticarcinogenic compounds present in food may be of importance for the prevention of colon cancer. Previous research demonstrated that several dietary agents increase GST enzyme activity in liver and intestine of male Wistar rats (10). Therefore, we have now investigated the effects of several naturally occurring dietary anticarcinogens on rat hepatic and intestinal UGT enzyme activity. In an earlier study we showed a dose-dependent induction of rat hepatic and intestinal GST enzyme activity after supplementation with the anticarcinogens α-angelicalactone and flavone in varying concentrations, either alone or in combination (21). Now we have also investigated the effects of this dose-dependent and combination treatment on gastrointestinal UGT enzyme activities.

Materials and methods

Materials

Ellagic acid, furalic acid, quercetin, coumarin, curcumin, flavone, bovine serum albumin, diithiothreitol, 4-methylumbelliferone (4-MUB), UDP-glucuronic acid (UDPGA) and (s)-saccharic acid 1,4-lactone were purchased from Sigma Chemical Co. (St Louis, MO). Ethanol and 4-nitrophenol (4-NP) were from Merck (Darmstadt, Germany). α-Angelicalactone, tannic acid and fumaric acid were obtained from Aldrich Chemie (Zwijndrecht, The Netherlands). Brussels sprouts were collected from local shops.

Study design

Hepatic and intestinal microsomes were prepared from Wistar rats, kept and treated as described in previous studies performed by Nijhoff et al. (10,21). In short, male Wistar rats (Central Laboratory Animal Centre, University of Nijmegen, The Netherlands) were housed individually on wooden shavings in macronol cages maintained at 20–25°C and 30–60% relative humidity. A ventilation rate of 7 air changes/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 laboratory chow (Hope Farms, Woerden, The Netherlands). After acclimatization for 3 days, the animals were fed either the basal diet (control group) or one of the experimental diets.

Diets

Ellagic acid, furalic acid, quercetin, tannic acid, fumaric acid and curcumin were incorporated at the level of 1% (w/w) in the basal diet. α-Angelicalactone and flavone were incorporated at the level of 0.5% (w/w) and coumarin at the level of 0.25% (w/w). Brussels sprouts were incorporated at the level of 20% (w/w) (10). α-Angelicalactone and flavone were incorporated into the diet, either separately or as a combination of both compounds, at 0.01, 0.05, 0.1 and 0.5% w/w, respectively (21). Food and water were available ad libitum. Food cups were replenished with freshly prepared diet every 2–3 days. Food consumption and gain in body weight was recorded daily. After 2 weeks the animals were killed by decapitation.

Tissue preparation

Tissue handling, isolation of liver and intestinal mucosa and preparation of microsomal pellet was performed as described previously (22). In short, liver tissue and intestinal mucosal scrapings were homogenized in buffer A (4 ml buffer A/g tissue or mucosal scraping: buffer A = 0.25 M saccharose, 20 mM Tris–HCl, 1 nM diithiothreitol, pH 7.4), followed by centrifugation at 9000 g (4°C) for 20 min. The resulting supernatant fraction was spun at 150 000 g (4°C) for 50 min, resulting in sedimentation of the microsomes. The microsomal pellet was resuspended in 5 vol buffer A.

Assays

Protein concentration was assayed in duplicate by the method of Lowry et al. (23) using bovine serum albumin as the standard.

UGT activity with 4-NP and 4-MUB as substrates was measured in the microsomes as described previously (24,25). Briefly, liver and intestinal microsomes were resuspended in 50 mM Tris–HCl, pH 7.4, containing 0.25 M sucrose and 1 mM diithiothreitol. Treatment of microsomes with varying concentrations of Triton X-100 failed to activate microsomes and rather inhibited UGT enzyme activity at higher Triton concentrations. Therefore, we did not perform an activation step in the UGT enzyme activity assay.

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 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 0.5 M NaOH, followed by centrifugation for 10 min at 10000 g. Absorbance was measured at 405 nm on a Perkin Elmer Lambda 12 spectrophotometer.

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 of 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 10000 g. 4-MUB was determined fluorometrically with a Shimadzu RF-5000 spectrofluorophotometer (excitation 370 nm, emission 450 nm).

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 glucuronidated 4-NP or 4-MUB. Effects of anticarcinogens on UGT enzyme activity are presented as ratios of treated to control.

Statistical analysis

The Wilcoxon rank sum test was used to assess 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 the anticarcinogens on 4-NP UGT activity in the five different parts of the gastrointestinal tract investigated (proximal, mid and distal small intestine, large intestine and liver) are shown in Table I. All compounds tested, except fumaric acid, increased 4-NP UGT activity at one or more sites. The most striking statistically significant enhancement was seen in small intestine by curcumin (5.4×, 6.7× and 7.2× in the proximal, mid and distal part, respectively), in large intestine by curcumin (3.1×) and in liver by flavone (7.6×). α-Angelicalactone and coumarin gave an enhancement in 4-NP UGT enzyme activity at all five sites investigated. The effects of the anticarcinogens on 4-MUB UGT activity are the same as observed for 4-NP UGT activity. However, quercetin was able to induce 4-MUB UGT activity in the proximal small intestine (2.7×) and large intestine (2.2×), α-angelicalactone had no effect on mid small intestinal activity and tannic acid did not have any effect. On the other hand flavone induced distal small intestinal 4-MUB UGT activity (2.2×), whereas fumaric acid and curcumin increased hepatic activity (2.2× and 2.6×, respectively).

The effects on 4-NP UGT activity in animals fed diets with α-angelicalactone, flavone or their combination are summarized in Table II. In general, 4-NP UGT activity was increased most in rats that were fed the highest dose of α-angelicalactone or flavone or the combination of both compounds. In large intestine and liver treatment with the highest concentration of α-angelicalactone resulted in enhancement of 4-NP UGT activity. Treatment with the highest dose of flavone or the combination of both agents increased 4-NP UGT activity at all sites investigated. Furthermore, treatment with flavone resulted in a larger increase in 4-NP UGT activity as compared with α-angelicalactone treatment. With increasing concentrations of α-angelicalactone, flavone or the combination of both agents, hepatic 4-NP UGT activity was gradually increased. Even the lowest concentration (0.01% w/w) of flavone and the
Table I. Effects of naturally occurring anticarcinogens on intestinal and hepatic 4-NP UGT enzyme activity

<table>
<thead>
<tr>
<th>Treatment group</th>
<th>n</th>
<th>4-NP UGT activity (ratio treated/control)</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Small intestine</td>
<td>Mid</td>
<td>Distal</td>
<td>Proximal</td>
</tr>
<tr>
<td>Ellagic acid</td>
<td>5</td>
<td>1.1 (0.5–11.0)</td>
<td>1.4 (0.6–7.0)</td>
<td>2.0 (1.5–9.0)</td>
<td>1.4 (0.6–3.7)</td>
</tr>
<tr>
<td>Ferulic acid</td>
<td>6</td>
<td>2.2 (0.4–10.3)</td>
<td>2.0 (0.7–4.1)</td>
<td>3.3 (0.7–4.2)</td>
<td>1.2 (0.5–1.8)</td>
</tr>
<tr>
<td>Brussels sprouts</td>
<td>3</td>
<td>0.4c (0.2–0.4)</td>
<td>0.9 (0.8–1.1)</td>
<td>1.1 (0.5–1.5)</td>
<td>1.0 (0.7–1.4)</td>
</tr>
<tr>
<td>Quercetin</td>
<td>6</td>
<td>4.0 (0.4–13.3)</td>
<td>2.8 (0.9–6.6)</td>
<td>4.0 (1.4–8.3)</td>
<td>3.8 (0.6–5.2)</td>
</tr>
<tr>
<td>α-Angelicalactone</td>
<td>6</td>
<td>2.8b (1.2–6.7)</td>
<td>2.1b (1.5–3.0)</td>
<td>3.9b (3.1–4.8)</td>
<td>2.3b (1.7–3.0)</td>
</tr>
<tr>
<td>Tannic acid</td>
<td>3</td>
<td>1.6 (0.7–4.9)</td>
<td>2.1 (1.2–3.1)</td>
<td>3.4 (1.6–3.6)</td>
<td>1.4 (0.6–2.8)</td>
</tr>
<tr>
<td>Coumarin</td>
<td>6</td>
<td>3.4c (1.8–6.2)</td>
<td>3.0 (1.0–7.0)</td>
<td>4.3c (2.2–5.5)</td>
<td>3.0b (1.8–7.7)</td>
</tr>
<tr>
<td>Fumaric acid</td>
<td>3</td>
<td>3.5 (0.6–4.7)</td>
<td>1.6 (1.4–2.5)</td>
<td>1.4 (0.8–1.9)</td>
<td>1.4 (1.4–1.5)</td>
</tr>
<tr>
<td>Curcumin</td>
<td>6</td>
<td>5.4c (2.4–25.2)</td>
<td>6.7b (2.0–8.9)</td>
<td>7.2b (3.8–8.3)</td>
<td>3.1b (1.3–6.1)</td>
</tr>
<tr>
<td>Flavone</td>
<td>6</td>
<td>1.2 (0.8–1.4)</td>
<td>1.5 (0.8–1.8)</td>
<td>1.5 (0.8–3.4)</td>
<td>1.9b (1.4–3.0)</td>
</tr>
</tbody>
</table>

4-NP UGT activity was measured in duplicate as described in Materials and methods. n, number of rats used in each group. Results are given as ratios of treated to control (median, range). The Wilcoxon rank sum test was used for statistical evaluation.

αP < 0.01.
βP < 0.005.
γP < 0.05.

Table II. Effects of dietary α-angelicalactone and flavone, individually and in combination, on rat intestinal and hepatic 4-NP UGT enzyme activity

<table>
<thead>
<tr>
<th>Treatment group</th>
<th>Per cent w/w</th>
<th>4-NP UGT activity (ratio treated/control)</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Small intestine</td>
<td>Mid</td>
<td>Distal</td>
<td>Proximal</td>
</tr>
<tr>
<td>α-Angelicalactone</td>
<td>0.01</td>
<td>1.0 (0.6–1.1)</td>
<td>1.0 (0.7–1.2)</td>
<td>0.8 (0.6–0.9)</td>
<td></td>
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<tr>
<td></td>
<td>0.05</td>
<td>0.9 (0.7–1.4)</td>
<td>1.0 (0.9–1.3)</td>
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<tr>
<td></td>
<td>0.1</td>
<td>1.0 (0.9–1.4)</td>
<td>0.9 (0.7–1.2)</td>
<td>1.4a (1.3–1.8)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.5</td>
<td>1.0 (0.8–1.6)</td>
<td>1.1a (1.1–1.3)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Flavone</td>
<td>0.01</td>
<td>0.8 (0.6–1.1)</td>
<td>1.1 (0.9–1.3)</td>
<td>1.6b (0.9–2.2)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.05</td>
<td>1.2 (0.8–1.4)</td>
<td>1.0 (0.7–1.3)</td>
<td>1.7b (1.3–2.7)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.1</td>
<td>1.2 (1.0–1.4)</td>
<td>1.2 (0.9–1.3)</td>
<td>3.3b (2.4–4.8)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.5</td>
<td>1.2b (1.1–1.3)</td>
<td>1.4b (1.0–1.7)</td>
<td></td>
<td>11.0c (6.4–14.2)</td>
</tr>
<tr>
<td>Combination</td>
<td>0.01/0.01</td>
<td>1.1 (0.9–1.3)</td>
<td>0.9 (0.7–1.3)</td>
<td>1.6b (1.4–2.1)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.05/0.05</td>
<td>1.2 (1.1–1.3)</td>
<td>0.9 (0.7–1.3)</td>
<td>3.1b (2.1–5.7)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.1/0.1</td>
<td>0.8 (0.7–1.4)</td>
<td>1.2 (0.9–1.7)</td>
<td>7.2b (5.8–8.8)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.5/0.5</td>
<td>1.7b (1.3–2.0)</td>
<td>2.2a (1.5–3.0)</td>
<td></td>
<td>10.7b (2.9–15.1)</td>
</tr>
</tbody>
</table>

4-NP UGT activity was measured in duplicate as described in Materials and methods. Number of animals: control group, n = 9; treatment groups, n = 6. Results are given as ratios of treated to control (median, range). The Wilcoxon rank sum test was used for statistical evaluation.

αP < 0.05.
βP < 0.005.
γSynergistic induction of 4-NP UGT activity, P < 0.05.

Discussion

It is well known that environmental factors affect the development of human cancers. The human diet may contain a large number of (pre)carcinogens (19,20). However, apart from carcinogens, our diet may also contain a wide variety of compounds which inhibit mutagenesis and/or carcinogenesis, as tested in laboratory models (19,20–28). These anticarcinogens are very diverse in chemical structure and their protective mechanisms are generally unclear. However, there are strong indications that anticarcinogens are effective by virtue of enhancing detoxification systems (4,8,10). In a recent study in humans by Hoensch et al. (11) high intake of fruits or vegetables was associated with high upper gastrointestinal levels of isoenzymes of the GST system. Detoxification systems such as UGTs and GSTs can minimize carcinogenicity by conjugation reactions, which add functional groups to the carcinogen, thereby lowering their biological activity and increasing their excretion. Although in a few studies induction of hepatic UGT enzyme activity has been suggested as a protective mechanism of some dietary anticarcinogens (29,30), little is known about the effects of anticarcinogens.
on UGT enzyme activity in the small intestine or colon, the latter being the organ where most gastrointestinal tumours are formed.

Flavone has been shown to induce hepatic 4-NP UGT activity (30,31), which is in comparison with the results observed here. In our study, quercetin significantly increased UGT enzyme activity in liver and proximal and distal small intestine. In contrast, Siess et al., Brouard et al. and Canivenc-Lavier et al. (30–32) found no effect on hepatic 4-NP UGT activity. This difference may be caused by variations in dietary treatment, since Siess et al. and Canivenc-Lavier et al. used a lower dose of quercetin (0.3 versus 1.0% w/w in our study). However, Brouard et al. also used a dose of 1% w/w quercetin for 2 weeks, suggesting that other factors influence the level of UGT activity. For example, starvation may affect UGT activity, since rats were fasted 18 h before collecting tissues in the study of Brouard et al. (31), whereas in our study animals had free access to food until decapitation. Since starvation is known to rapidly decrease phase II enzyme activity (33), an initial increase in UGT enzyme activity may have disappeared during this starvation period.

After treatment with quercetin, increased UGT activity in proximal small intestine was only seen with 4-MUB as substrate. In distal small intestine this treatment induced both 4-NP and 4-MUB UGT activity. In contrast, Brouard et al. (31) found no induction of intestinal 4-NP UGT activity after feeding quercetin to rats. This difference may be explained as follows: Brouard et al. studied the overall 4-NP UGT activity of the intestine, while we measured the UGT activity in three different parts of the intestine, revealing an increase in 4-NP UGT activity in the distal part of the intestine only. Furthermore, as mentioned above, due to a starvation period in the study of Brouard et al., eventual effects may have disappeared. Like Brouard et al. (31), we found no effect of flavone on intestinal 4-NP UGT activity, whereas dietary ellagic acid was shown by Ahn et al. (29) to increase hepatic 4-NP UGT activity, which was confirmed by us. However, we used a higher dose of ellagic acid, which resulted in a more elevated UGT activity.

For all other compounds studied no effects on UGT enzyme activity have been reported before. All agents had an enhancing effect on UGT activity at one or more sites investigated, except for Brussels sprouts, which had an inhibitory effect on proximal small intestinal UGT activity. Nevertheless, Brussels sprouts were able to enhance hepatic UGT activity. This discrepancy might be explained by the fact that certain metabolites of glucosinolates present in Brussels sprouts may be responsible for induction of hepatic UGT activity, whereas these metabolites may not yet have been formed in the intestine. Colonic 4-NP UGT enzyme activity was significantly increased by α-angelicalactone, coumarin, curcumin and flavone, whereas the same agents as well as quercetin significantly induced colonic 4-MUB UGT activity. In general, the same anticarcinogens enhanced both small intestinal as well as large intestinal UGT enzyme activities.

A study on the dose dependency effects of dietary flavone on hepatic 4-NP UGT activity was performed by Siess et al. (34). Increased 4-NP UGT activity was found after treatment with flavone at 0.002, 0.005, 0.02, 0.05 and 0.2% w/w. In accordance with these results, we found that both 4-NP and 4-MUB hepatic UGT activity was significantly induced by 0.01, 0.05, 0.1 and 0.5% w/w flavone. In the intestinal tissues studied here, only the highest dose of dietary flavone resulted in enhancement of UGT activity. For α-angelicalactone, for which no data on dose–response effects have been reported before, a dose dependency for both 4-NP and 4-MUB UGT activity was only seen in the liver. In conclusion, increasing concentrations of α-angelicalactone or flavone did show a gradual increase in hepatic UGT activity.

Since humans may be exposed to mixtures of anticarcinogens in their diets, it may be worthwhile to study combinations of such compounds in laboratory animals in order to learn more on possible additional effects. In general, combination treatment with α-angelicalactone and flavone showed only additive effects. However, in small intestine the 4-MUB UGT activity was significantly enhanced in the 0.1 and 0.5% w/w combination groups (1.6- and 1.8-fold, respectively), whereas in the corresponding flavone or α-angelicalactone groups no significant induction was noticed. In large intestine 4-MUB UGT activity was significantly induced in the 0.1% w/w combination group, whereas treatment with flavone or α-angelicalactone alone did not result in a significant increase. In addition, a synergistic effect on hepatic UGT activity was seen after the 0.1% w/w combination treatment. Thus, exposure to mixtures of anticarcinogens can have additive or even synergistic effects on the activities of biotransformation enzymes.

We observed higher UGT activities, as measured with 4-NP, in rat large intestine as compared with small intestinal values. This is quite remarkable since in human studies phase II biotransformation enzymes were shown to be much more active in small intestine as compared with colon (17,35). Contradictory data about rat large intestinal versus small intestinal UGT activities have been reported. Hänninen et al. (36), using mucosal scrapings, found that 4-NP UGT activity decreased along the small intestine, but except for the first part of the small intestine, large intestinal activity was found to be higher. However, Koster et al. (37), measuring 1-naphthol or morphine glucuronidation capacity in intestinal cells isolated by vibration, measured much lower activities in cecum and colon as compared with the small intestine.

It has often been reported that the specific activities of GSTs and UGTs in rats gradually decrease down the small intestine, when mucosal scrapings or isolated cells were analyzed (36–39). However, in studies with in situ perfused segments of the rat intestine no significant difference in the total glucuronidation capacity of proximal, intermediate and distal small intestine could be found (40,41). In the study described here, the 4-NP or 4-MUB UGT enzyme activity also remained constant in different parts of the rat small intestine. The apparent discrepancy in distribution of UGTs along rat small intestine may thus be explained by either the methods used for studying the intestinal tissue, as well as by the different UGT substrates used for measurement of enzyme activity. The latter is further supported by an earlier study by us (42), in which the distribution of UGT enzyme activities along the human small intestine showed various patterns, depending on the substrates used. The activity for bilirubin UGT declined, whereas the activity for 4-NP and 4-MUB UGT seemed to increase and remain constant, respectively. This may indicate that the different UGT isoenzymes may have different expression patterns along the small intestine.

Finally, we examined the associations between 4-NP and 4-MUB UGT enzyme activities and GST activities, as measured in our earlier studies (10,21). Like Bock et al. (43), a strong significant correlation was observed between 4-NP and 4-MUB UGT enzyme activity in all organs investigated. The
correlation coefficient varied from 0.24 ($P < 0.05$) to 0.91 ($P < 0.0001$) for mid small intestine and liver, respectively. This may be due to the fact that glucuronidation of both substrates may be mainly catalyzed by the same isoforms of UGT (12,16). In rats, UGT1A1, 1A6 and 1A7 are highly expressed in liver and intestine (44) and therefore total UGT enzyme activity in these organs may be mainly covered by these UGT isoenzymes. In liver there is also a strong association between GST and UGT enzyme activities (GST, 4-NP glucuronidation) in liver and intestine. An explanation could be the different expression patterns of GST and UGT isoenzymes in rat intestine and liver, in combination with the different substrate specificities of the GST or UGT isoenzymes (35,38). Since expression of UGT or GST isoenzymes may differ considerably from one organ to another and since UGT or GST isoenzymes may have different substrate specificities, an association between GST and UGT enzyme activities as found in one organ (liver) does not necessarily mean that such an association also exists in another organ.

In conclusion, most naturally occurring dietary anticarcinogens tested, and in particular α-angelicalactone, coumarin and curcumin, were able to increase UGT enzyme activity in liver and, to a lesser extent, in small and large intestine. Furthermore, dose-dependent effects of α-angelicalactone, flavone or a combination of both compounds on the UGT detoxification system of the liver and, to a lesser extent, the intestine were demonstrated. Such high detoxification capacity may account, at least in part, for the observed chemopreventive action of these compounds. However, one should realise that the dose of anticarcinogens applied here is unlikely to be reached in the human diet, but since the human diet may contain many such compounds and since effects on UGTs may be additive or even synergistic, enhancement of UGT activity by these minor compounds could still play a significant role in the prevention of gastrointestinal tumours in humans.

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References


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