Studies on the mechanism of cancer protection by wheat bran: effects on the absorption, metabolism and excretion of the food carcinogen 2-amino-3-methylimidazo[4,5-f]quinoline (IQ)

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We examined ways in which dietary supplements of wheat bran may protect against colon cancer. The effects of supplementing the diet of female Wistar rats with 10% wheat bran on the disposition and metabolism of the dietary carcinogen 2-amino-3-methylimidazo[4,5-f]quinoline (IQ) labelled with ¹⁴C was determined. Our data show that the wheat bran had a major effect on both the distribution and metabolism of IQ. At a low dose of IQ (1 mg/kg), we unexpectedly found that up to 2 h after gavage there were higher concentrations of radioactivity in the plasma of rats fed wheat bran compared to the control. At a high dose of IQ (50 mg/kg), there were always lower concentrations of radioactivity in the plasma of rats fed wheat bran compared to the control rats. One of the most marked effects of wheat bran was apparently to significantly retard the metabolism of IQ in the plasma when it was fed at either dose. There were also differences between the rats fed wheat bran and the control in the concentrations and types of IQ metabolites in the urine.

Introduction

Supplementing the diet of both animals and humans with various dietary fibre sources is known to have a potential ability to protect against the development of cancer. Numerous animal studies indicate that supplementing the diet with wheat bran protects against colon cancer. Many of these studies have involved feeding diets supplemented with wheat bran to rats in which colon cancer has been induced by the carcinogen 1,2-dimethylhydrazine (1). There have also been several reports that supplementing the diet with wheat bran protects against breast cancer in animal models (2–4). In human intervention studies, supplementing the diet with wheat bran has been shown to reduce the incidence and size of colonic polyps, an early lesion associated with the development of colon cancer (5,6).

Various mechanisms have been proposed by which wheat bran may protect against cancer. Most of these involve the dietary fibre component of wheat bran. The most commonly cited hypothesis for protection against colorectal cancer proposes that it is the short chain fatty acids, especially butyrate, produced by the fermentation of cell wall polysaccharides in the colon that affect later stages of cancer development (7,8). Other mechanisms involve the dilution of potential dietary carcinogens or procarcinogens by wheat bran supplements reducing transit times in the gastrointestinal tract and by increasing the output of faeces (9). Lignified cell walls (dietary fibre) in wheat bran can also adsorb dietary carcinogens or procarcinogens, at least in vitro (10,11). This could potentially reduce the availability of carcinogens to the colonic mucosa, thereby preventing the carcinogen acting directly on the tissue. Furthermore, the amount of carcinogen absorbed into the bloodstream would be reduced and so decrease the probability of tumours developing at the bases of colonic crypts or at other sites around the body, such as in liver or breast tissues. It would also be expected that more carcinogen would be excreted in the faeces. This hypothesis can be tested in animals by following the fate of a radiolabelled carcinogen.

In this study, we explore whether diet supplemented with wheat bran has significant effects on the absorption, disposition and excretion of the dietary carcinogen 2-amino-3-methylimidazo[4,5-f]quinoline (IQ). This carcinogen has been selected as it is normally present in human diets and is a probable human carcinogen whose routes of detoxification are well characterized (12–14; Figure 1). Furthermore, it adsorbs to wheat bran as well as to other dietary fibres and fibre sources (11,15). Initially, we gave [¹⁴C]IQ by gavage at 50 mg/kg body wt to female Wistar rats whose diet had been supplemented with commercial wheat bran. The total radioactivity was monitored in urine, faeces and plasma and the data related to effects of the wheat bran-supplemented diet on transit time (measured using a particulate marker) and on faecal output. Our results led us to investigate the possibility that wheat bran affects the detoxification of carcinogens. Thus, we further analysed the urine and plasma for IQ and its main metabolites. We used non-fasted rats and orally gavaged the IQ to mimic human feeding as far as possible. Female rats were selected in order to gain information that would be relevant to protection against breast cancer as well as colorectal cancer. These studies were repeated using a lower (1 mg/kg) dose of IQ to bring this also as close as possible to the human diet.

Materials and methods

Materials

Wheat bran was bought from a supermarket as bran flakes (Fleming & Co., Auckland, New Zealand). This had a total dietary fibre content (AOAC) of 35.7% and 10.2% starch determined using a Megazyme starch assay kit (Megazyme International Ireland, Bray, Ireland) (16). [2-¹⁴C]IQ (sp. act. 11 mCi/mmol) and unlabelled IQ were from Toronto Research Chemicals (Ontario, Canada). β-Glucuronidase (bacterial Type VIII) and sulfatase (from abalone entails, Type VIII) were from Sigma Chemical Co. (St Louis, MO). All other chemicals were at least of analytical grade.

Animal maintenance

Female Wistar rats were 6–8 weeks old (average body weight 132 g) when started on the experimental diets. The rats were kept in individual cages (58×24.5×17 cm) with dropped bottom wires and without sawdust and...
Blood sample collection by gavage at either 1 or 50 mg/kg body wt. (0.2 ml). Speciﬁc activities of these solutions were 3 and 150 µCi/mmol. Rats from each subgroup were orally administered 10 µCi IQ. Fresh faeces (30 mg) were shaken with water (10 ml) for 1 h. Aliquots (0.5 ml) of the suspension were digested, solubilized and the radioactivity determined as described above. The remaining suspension was centrifuged (1000 g, 20 min) and similar procedures were followed in which the pellet was shaken with methanol (10 ml). After sampling and centrifugation, the second pellet was shaken with DMSO (10 ml). The sampling and centrifugation procedures were repeated before the ﬁnal pellet was solubilized and its radioactivity determined.

HPLC metabolite proﬁles
Aliquots of plasma or urine (20–100 µl), collected during the 8 h period after [14C]IQ was administered at 50 µg/kg, were analysed directly by HPLC using a NovaPak C18 reverse phase column (4 µm, 3.9×150 mm; Waters Associates, Milford, MA) equipped with a C18 guard column and a Waters 510 solvent delivery system. A linear gradient was used of 0–20% acetonitrile in 0.14 M potassium phosphate buffer (pH 4.7) over 50 min at a ﬂow rate of 1 ml/min. Eluate fractions were collected every 0.5 min to which scintillation ﬂuid (6 ml) was added and the radioactivity determined by liquid scintillation counting.

Urinary and faecal excretion
After dosing, animals were placed in individual metabolic cages that enable the separation of excreta (Metabowls; Jencons Ltd, Poole, UK). The urine and faeces were collected during the 72 h following administration and stored at −70°C.

Determination of radioactivity
Aliquots of plasma or urine (20–100 µl) were added directly to 10 ml water-accepting scintillation ﬂuid (Ready Safe; Beckman Instruments, Fullerton, CA) for scintillation counting with quench correction by the external standardization method. Faeces were assayed similarly after homogenization in water (4:1 v/v) and digestion of aliquots (100 µl) of the resulting homogenates with tissue solubilizer (Protosol; New England Nuclear, Boston, MA) and hydrogen peroxide.

In some experiments, we sequentially extracted the faeces from the two groups of rats given 1 mg/kg IQ. Fresh faeces (30 mg) were shaken with water (10 ml) for 1 h. Aliquots (0.5 ml) of the suspension were digested, solubilized and the radioactivity determined as described above. The remaining suspension was centrifuged (1000 g, 20 min) and similar procedures were followed in which the pellet was shaken with methanol (10 ml). After sampling and centrifugation, the second pellet was shaken with DMSO (10 ml). The sampling and centrifugation procedures were repeated before the final pellet was solubilized and its radioactivity determined.

Enzymatic and acid hydrolysis of IQ metabolites
To identify the major IQ metabolites, N-(3-methylimidazo[4,5-f]quinoline-2-yl)sulphamic acid (IQ-N-sulphamate), 2-amino-5-(glucosiduronyloxy)-3-D-l-tryptophan, 2-amino-5-(glucosiduronyloxy)-3-D-glucosamine, 2-amino-5-sulphamoyl-D-glucosamine, 2-amino-5-(glucosiduronyloxy)-3-D-glucosamine and 2-amino-5-sulphamoyl-D-glucosamine, were analysed by HPLC using the conditions described above. Brieﬂy, urine was dissolved in 50 mM sodium acetate buffer at pH 5.0 for sulphotase treatment and at pH 6.8 for β-glucuronidase treatment. Sulphotase (50 U) or β-glucuronidase (1000 U) were added and incubated at 37°C for 2 or 48 h, respectively. Samples were also acid hydrolysed using 1 M HCl at 60°C for 2 h. The products were analysed by HPLC using the conditions described above.

Statistical analyses
Increases in the weights of the rats and the rates of faecal output were compared using one way analysis of variance (Tukey test). Otherwise, data were compared using Student’s t-test, with differences being considered signiﬁcant at P < 0.05.

Results
Live weight gain and faecal output
The rats tolerated the two experimental diets well and there was no signiﬁcant difference in the weight of food eaten between the group fed the control diet and the group fed the diet supplemented with wheat bran. The rats in both groups

Table I. Composition of the rat dietsa

<table>
<thead>
<tr>
<th>Component (g/kg)</th>
<th>Control diet (g/kg)</th>
<th>Diet supplemented with wheat bran (g/kg)</th>
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<tbody>
<tr>
<td>Casein</td>
<td>200</td>
<td>200</td>
</tr>
<tr>
<td>Cornstarch</td>
<td>350</td>
<td>210</td>
</tr>
<tr>
<td>Sucrose</td>
<td>200</td>
<td>200</td>
</tr>
<tr>
<td>Wheat branb</td>
<td></td>
<td>140</td>
</tr>
<tr>
<td>Sunflower seed oil</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Lard</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>AIN-76 vitamin mix</td>
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<td>10</td>
</tr>
<tr>
<td>AIN-76 mineral mix</td>
<td>35</td>
<td>35</td>
</tr>
<tr>
<td>dl-Methionine</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Choline bitartrate</td>
<td>2</td>
<td>2</td>
</tr>
</tbody>
</table>

aThe basic diet was a high fat modiﬁcation of the AIN-76 diet (33).

The results of these studies, which were prepared dry. Water (150 ml/kg diet) was then added to each diet to help form bolus. Rats were fed ad libitum and were prefed for 4 weeks before any experimental studies. Each rat was weighted weekly.

Determination of transit time
Groups of six rats were used for these experiments. Each rat was fed a bolus (2.5 g) of the appropriate experimental diet mixed with glass beads (10 mg; 1309±1 µm, diameter range 150–170 µm) at midnight. The rats were fasted (with free access to water) for 24 h before the experiments and the bolus were all completely eaten. One hour later, rats were given free access to food again. Six hours after feeding the bolus, faecal samples were collected in glass scintillation vials every 2 h up to 30 h after feeding and then every 6 h up to 48 h after feeding. Each faecal sample was incubated at room temperature with nitric acid (3–6 ml, 15.7 mol/l) until the faeces dissolved (6 h or overnight). The beads were washed with several changes of 1% Tween 20 (pH 4.72). Aliquots (200 µl) of the dissolved residue were analysed by HPLC as described above except that a 0–10% linear gradient was used over 50 min. Plasma samples were treated with acetonitrile (4:1 v/v) to precipitate proteins and centrifuged (2000 g, 5 min). The supernatant was then removed and evaporated to dryness under vacuum. The residue was dissolved in potassium phosphate buffer before aliquots were analysed as described above for 1 mg/kg IQ urine samples. The recoveries of radioactivity after processing plasma samples were found to be 85–90%.

Formulation and administration of IQ
[14C]IQ solutions were prepared by dissolving this, together with unlabelled IQ, in 0.1 M HCl (1.8 ml) and the pH adjusted to 3 with 0.5 M NaOH (0.2 ml). Specific activities of these solutions were 3 and 150 µCi/mmol. Rats from each subgroup were orally administered 10 µCi of formulated [14C]IQ by gavage at either 1 or 50 mg/kg body wt.

Blood sample collection
Blood (0.4 ml) was collected in heparinized tubes from the tips of tails of rats at 0.5, 1, 2, 4, 6, 8 and 24 h after administration and the plasma separated by centrifugation (1000 g, 10 min), which was removed and stored at −70°C.
Cancer protection by wheat bran

Fig. 2. Average transit time in wheat bran-fed compared with control rats. Animals were either fed a control diet (●) or a control diet supplemented with 10% wheat bran (○). Each point represents the mean of six animals.

Table II. Comparison of live weight gain and 24 h faecal dry weight output by the rats fed modified AIN-76 diet (33) containing no dietary fibre or 5% total dietary fibre as provided by wheat bran

<table>
<thead>
<tr>
<th>Group</th>
<th>Control</th>
<th>Wheat bran</th>
</tr>
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<tbody>
<tr>
<td>Weight gain (g/rat/week)</td>
<td>19.5 ± 0.5</td>
<td>21 ± 1.5</td>
</tr>
<tr>
<td>Faecal dry weight output (g/24 h)</td>
<td>0.47 ± 0.05</td>
<td>1.0 ± 0.04</td>
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*The control group of rats differed significantly from the group fed the wheat bran diet (P < 0.05).

Excretion of radioactivity in the urine and faeces

The cumulative amounts of radioactivity excreted in the urine and faeces are given in Figure 3. Urinary and faecal excretion following a single oral dose of [14C]IQ to non-fasted rats pre-fed with the control diet at a dose level of 50 mg/kg accounted for 65.7 ± 11.9 and 27.1 ± 16.7%, respectively, of the administered dose within 72 h. In contrast, rats that were similarly treated but pre-fed a diet supplemented with wheat bran, compared with 26.0 ± 3.8 h for the control group. Independent Student’s t-tests showed that these differences were not statistically significant.

Excretion of radioactivity in the urine and faeces

The cumulative amounts of radioactivity are shown in Figure 2. The mean time for 80% of the glass beads in the faeces was 22.3 ± 2.2 h for the group fed the diet supplemented with wheat bran, compared with 26.0 ± 3.8 h for the control group. Independent Student’s t-tests showed that these differences were not statistically significant.

Transit times

The patterns of fecal output in the two groups are shown in Figure 2. The mean time for 80% of the glass beads to be excreted in the faeces was 22.3 ± 2.2 h for the group fed the diet supplemented with wheat bran, compared with 26.0 ± 3.8 h for the control group. Independent Student’s t-tests showed that these differences were not statistically significant.

Excretion of radioactivity in the urine and faeces

The cumulative amounts of radioactivity excreted in the urine and faeces are given in Figure 3. Urinary and faecal excretion following a single oral dose of [14C]IQ to non-fasted rats pre-fed with the control diet at a dose level of 50 mg/kg accounted for 65.7 ± 11.9 and 27.1 ± 16.7%, respectively, of the administered dose within 72 h. In contrast, rats that were similarly treated but pre-fed a diet supplemented with wheat bran accounted for 50.4 ± 11.9 and 39.5 ± 23.2% of the total administered dose in the urine and faeces, respectively, within the same period after gavage. Although the excretion of radioactivity in this subgroup of rats was different from the control group, the mean cumulative amounts at each time point were not statistically different between the two groups. Similar results were obtained after rats were given [14C]IQ at 1 mg/kg.

There were, however, significant differences in the amounts of radioactivity that were extracted by different solvents. Thus, for the faeces from the control rats given 1 mg/kg IQ, 64.4 ± 0.3% of the radioactivity was extracted with water, a further 4.4 ± 0.1% was extracted with methanol and 7.6 ± 0.1% with DMSO. Of the radioactivity, 23.5 ± 1.4% remained in the faecal residue. In contrast, water extracted only 15.6 ± 0.5% of the radioactivity from the faeces of the comparable group fed wheat bran. A further 6.7 ± 0.1% was extracted with methanol and 9.8 ± 0.3% with DMSO. However, in this group of rats, 67.9 ± 4.6% of the radioactivity remained in the residue at the end, suggesting that it may be covalently bound to the wheat bran.

Significant differences were also observed in the amounts of urinary radioactivity which were excreted. Thus, rats pre-fed with the control diet supplemented with wheat bran excreted 44.6 ± 2.9% of the administered dose over 72 h following gavage. This was considerably less than the radioactivity excreted by rats pre-fed the control diet. With these rats the urinary excretion accounted for 67.3 ± 7.9% of the administered dose.

Radioactivity in plasma

Plasma concentration–time profiles of total radioactivity after oral administration of [14C]IQ at 1 and 50 mg/kg to groups of non-fasting rats which were either pre-fed with a control diet or a control diet supplemented with wheat bran are shown in Figure 4. Mean total radioactivity concentrations of [14C] in plasma after [14C]IQ was given at 50 mg/kg were lower in the group of rats fed the diet supplemented with wheat bran than those measured in the plasma of the control group. However, this difference failed to reach statistical significance. More complex profiles were obtained in the subgroup given
1 mg/kg $[^{14}\text{C}]$IQ. Up to 2 h after giving the $[^{14}\text{C}]$IQ, the mean total radioactivity concentrations in the plasma were higher in the group fed the diet supplemented with wheat bran than those observed in the control group. This difference was most striking 0.5 h after gavage, when it was 2.5-fold and was statistically different. In contrast, from 4 h, the concentration of radioactivity in the plasma was higher in the control group than in the group fed the diet supplemented with wheat bran.

**HPLC urinary profiles of IQ and IQ metabolites**

Urine collected during the 0–8 h period after rats were orally administered $[^{14}\text{C}]$IQ by gavage at a dose of 50 mg/kg was analysed by HPLC for the presence of IQ and IQ metabolites. These were identified by their retention times and by the behaviour of spiked samples. Representative chromatograms are shown in Figure 5 and the distribution of metabolites and IQ as a function of dose are given in Figure 6. Small, but significant, amounts of unmetabolized IQ (retention time 34 min), accounting for <2.4% of the total dose, were recovered in the urine of rats pre-fed with either diet. The major metabolite M3 in the urine collected from rats pre-fed a control diet was identified as IQ-N-sulphamate (retention time 24 min) (12.7 ± 1.1% of the administered dose), since it was extremely labile in HCl and appeared to be unaffected by enzyme hydrolysis using β-glucuronidase or sulphatase (Figure 5A). However, two minor metabolites, M2 and M4, which had retention times of 22 and 27 min and accounted for 4.1 ± 0.8 and 2.8 ± 0.5% of the dose, respectively, were susceptible to hydrolysis by these enzymes. These compounds were identified as IQ-5-O-glucuronide (M2, retention time 22 min) and IQ-5-O-sulphate (M4, retention time 27 min). Another minor metabolite M1 (retention time 17 min), representing 2.8 ± 0.6% of the dose, was not identified since its chromatographic properties were unchanged on chemical and enzymatic hydrolysis. In contrast, the major metabolite in the urine of rats fed the diet supplemented with wheat bran was IQ-5-O-glucuronide (M2), which amounted to 13.3 ± 2.6% of the dose. However, only a small proportion of the administered dose was found to be excreted as IQ-N-sulphamate (4.1 ± 1.4% of the dose), IQ-5-O-sulphate (4.8 ± 0.4% of the dose) and M1 (1.5 ± 0.2% of the dose). Thus, significantly different amounts of IQ-N-sulphamate (M3) and IQ-5-O-glucuronide (M2) were found to be excreted from rats in the group fed the diet supplemented with wheat bran at either dose of $[^{14}\text{C}]$IQ ($P < 0.05$).

**HPLC plasma profiles of IQ and IQ metabolites**

Plasma collected from rats pre-fed with either diet and given $[^{14}\text{C}]$IQ by gavage at 50 and 1 mg/kg was analysed by HPLC.
Representative chromatograms of samples taken 0.5 and 4 h after administration are shown in Figure 7A and B, respectively. The distribution of metabolites and IQ, expressed as a percentage of the total plasma radioactivity, is shown in Figures 8 and 9. All profiles contained five radioactive peaks that were tentatively assigned as for the peaks in the chromatograms of urine by co-chromatography with authentic IQ and peaks associated with the urinary metabolites. In the subgroup of rats given IQ at the dose of 50 mg/kg, HPLC radiochromatograms of all plasma samples collected at 0.5 h were virtually identical and not affected by pre-feeding of either diet. The major proportion of radioactivity (68–70%) in these samples eluted at 48 min and corresponded to unmetabolized IQ. In addition, small amounts of radioactivity co-eluted with the unknown urinary metabolite M1 and IQ-N-sulphamate (M3) (10 and 8% of the total plasma radioactivity, respectively), together with traces of IQ-5-O-glucuronide and IQ-5-O-sulphate (<1%). Similar profiles were obtained with plasma collected from wheat bran pre-fed rats sampled within this subgroup at 4 h after being given [14C]IQ. However, analysis of equivalent samples taken from [14C]IQ-treated rats in this subgroup but pre-fed with the control diet showed significantly different profiles at 4 h. Here, the major proportion of plasma radioactivity was associated with metabolites, particularly IQ-5-O-glucuronide (M2) and IQ-N-sulphamate (M3), which accounted for 26 and 24% of the total plasma radioactivity, respectively. Only 12% of the total plasma radioactivity corresponded to the parent IQ. Analysis of plasma from the subgroup of rats given 1 mg/kg IQ and sampled 0.5 h after treatment showed that the major radioactive peak in the profiles derived from rats pre-fed a wheat bran-supplemented diet was associated with IQ (44%), whereas in those profiles that were from rats pre-fed control diet, the major radioactive peak was IQ-5-O-glucuronide (M2) (40%). This metabolite was also the major radioactive component (41%) in plasma sampled from the rats fed wheat bran 4 h after [14C]IQ treatment at this dose, whereas in the plasma of rats fed the control diet, the major radioactive peaks were M1 and IQ-5-O-glucuronide (M2) (31% and 27%, respectively). Small, but significant, quantities of plasma radioactivity (8%) assigned to IQ-5-O-sulphamate (M4) were present in all profiles. Only trace amounts of radioactivity were present as IQ-N-sulphamate (M3) (<1%) in all samples.

Discussion

Reductions in transit times, increased faecal bulk and adsorption of carcinogens or procarcinogens by dietary fibres have all been suggested as being important in wheat bran protecting against colorectal cancer. These all result in carcinogens having less opportunity to interact with colonic cells (7,17). In the present animal study, the diet supplemented with wheat bran increased faecal output and reduced gastrointestinal transit time as previously found (18). Furthermore, we have evidence that wheat bran can adsorb IQ, at least in vitro (10,15). The present data on the excretion of radioactivity in the faeces of rats fed the diet supplemented with wheat bran appears to confirm these physiological effects. The diet supplemented with wheat bran increased the amounts of radioactivity (as a
measure of IQ plus metabolites) excreted in the faeces, especially in the first 24 h after administration. There was evidence that this radioactivity was more strongly associated with the faecal pellet in the wheat bran-fed group, possibly indicating covalent linkage of an IQ metabolite to the wheat bran. There were also concomitant reductions in the amounts of radioactivity excreted in the urine. However, these physiological changes failed to predict the complex concentration versus time profiles of plasma radioactivity or the differences in the plasma and urinary IQ metabolite profiles. These suggest to us that although these physiological changes may play some role in the effects of wheat bran on carcinogenesis, such changes may be less important than previously believed (7,17).

In the present study, we found that rats fed the diet supplemented with wheat bran and given IQ at a dose of 1 mg/kg had more complex profiles of plasma concentrations of total radioactivity versus time than equivalent rats given IQ at a dose of 50 mg/kg. During the 2 h period after gavage, it appeared that the diet supplemented with wheat bran significantly increased, rather than decreased, the plasma concentrations of total radioactivity associated with IQ and its metabolites. These results are difficult to interpret, but they may result from the effects of wheat bran on gastrointestinal motility and gastric emptying. Previous studies, mostly with soluble dietary fibres, have shown that dietary fibres have substantial effects on gastrointestinal motility and gastric emptying (20). However, the data available in the literature for the effects of wheat bran are inconsistent. For example, in pigs wheat bran had little or no effect on gastric emptying (21), but in man it reduced the emptying rates of gastric liquid and solid phases (22). If wheat bran enhanced both gastrointestinal tract motility and gastric emptying, then the contents of the stomach, including the IQ, would pass more rapidly into the intestine, where they may be absorbed. This could result in higher plasma concentrations at earlier times. However, if this is the explanation for the early time effects versus time profiles of plasma radioactivity or the differences in the plasma and urinary IQ metabolite profiles. These suggest in the present experiments, it is not easy to explain why the same effect did not also occur at the higher dose of IQ; a later peak in the control rats might also be expected.

Our results also indicate that wheat bran affects the plasma radioactivity profiles in another way, particularly at times longer than 2 h after gavage with the lower dose of IQ, where enterohepatic cycling of IQ may have important effects. Like many other xenobiotics, IQ is absorbed quickly by the small intestine and metabolized principally by the liver, where the resulting metabolites undergo biliary excretion. These can then be hydrolysed back to the parent compound by the gut microflora and reabsorbed into the bloodstream, giving rise to a secondary peak of plasma radioactivity. Sjödin and Jegerstad (23) showed that after giving rats radioactive IQ at a dose of 3–4 mg/kg, 70% of the radioactivity appeared in the bile fluid within 24 h. They concluded that most of the IQ entered the bloodstream by the process of enterohepatic circulation. In contrast, Inamasu et al. (13) gave rats IQ by gavage at a dose $[14C]IQ$ at 1 mg/kg and are expressed as mean per cent of total plasma radioactivity ± SE for three rats. *P < 0.05 (unpaired Student’s t-test). M1, unknown; M2, IQ-5-O-glucuronide; M3, IQ-N-sulphamate; M4, IQ-5-O-sulphate. (A) 0.5 h after gavage; (B) 4 h after gavage. ■, control diet; ♦, wheat bran diet.

**Fig. 8.** Per cent distribution of IQ and major metabolites in plasma as a function of total plasma radioactivity. Data are from rats treated with $[14C]IQ$ at 50 mg/kg and are expressed as mean per cent of total plasma radioactivity ± SE for three rats. *P < 0.05 (unpaired Student’s t-test). M1, unknown; M2, IQ-5-O-glucuronide; M3, IQ-N-sulphamate; M4, IQ-5-O-sulphate. (A) 0.5 h after gavage; (B) 4 h after gavage. ■, control diet; ♦, wheat bran diet.

**Fig. 9.** Per cent distribution of IQ and major metabolites in plasma as a function of total plasma radioactivity. Data are from rats treated with $[14C]IQ$ at 1 mg/kg and are expressed as mean per cent of total plasma radioactivity ± SE for three rats. *P < 0.05 (unpaired Student’s t-test). M1, unknown; M2, IQ-5-O-glucuronide; M3, IQ-N-sulphamate; M4, IQ-5-O-sulphate. (A) 0.5 h after gavage; (B) 4 h after gavage. ■, control diet; ♦, wheat bran diet.
of either 20 or 40 mg/kg and concluded that only ~15% appeared in the bile. Although we did not investigate the extent of biliary excretion and enterohepatic cycling of IQ and its metabolites, it is likely to have been significant, as illustrated by the plasma radioactivity profile of rats fed the control diet after dosing with IQ at 1 mg/kg. Thus, in comparison, the reduction of the second peak of plasma radioactivity in rats fed the diet supplemented with wheat bran may have been caused by an effect on enterohepatic cycling of IQ and its metabolites.

In our study, in the rats fed the diet supplemented with wheat bran, most of the plasma radioactivity was present as unmetabolized IQ at all times and for either dose of IQ. This contrasted with the rats fed the control diet, in which only after 0.5 h and particularly after treatment at the higher dose of IQ was most of the plasma radioactivity present as metabolized IQ. Thus the diet supplemented with wheat bran in some way interfered with the metabolism of IQ. In the urine, IQ-N-sulphamate was the major metabolite in rats fed the control diet, whereas IQ-5-O-glucuronide was the major metabolite in rats fed the diet supplemented with wheat bran. It is well documented that the metabolic pathway leading to the formation of the N-sulphamate is dose dependent for both IQ and MeIQx. Moreover, it has been postulated that when the major cytochrome P450 metabolic pathway that forms the 5-hydroxy metabolite becomes saturated, further metabolism of the parent compound occurs by sulphation of the N1 amino group to form the sulphamate (12,13,24). Thus, the significantly lower concentrations of IQ-N-sulphamate in the urine of rats fed the diet supplemented with wheat bran might be expected if the main effect of wheat bran was to reduce absorption of IQ into the bloodstream. Xu et al. (25) found similar effects on the urinary excretion of IQ metabolites in rats treated with indole-3-carbilon. Our urinary metabolite data from rats given IQ at 1 mg/kg are also consistent with wheat bran reducing the systemic concentrations of IQ. However, data from the total plasma radioactivity and also plasma metabolites were not entirely consistent with this hypothesis.

It is also possible that wheat bran could be having a direct effect on metabolizing enzymes (phase I and II enzymes) in the liver or the small intestine (26–28). Only minor differences were found in the activities of hepatic metabolizing enzymes in mice fed a diet supplemented with 30% wheat bran (19). However, small intestinal enzyme activities were not measured in these studies. Another site of IQ metabolism is the intestinal microflora. Wheat bran may affect microbial enzymes directly or by altering the numbers and types of bacteria present. O’Neill and co-workers (29–31) provided evidence that dietary components, such as fat and dietary fibre, could change the gut microflora and this in turn could change the activities of gut microfloral enzymes.

Overall, this study showed that wheat bran significantly affects the absorption, disposition and excretion of the dietary carcinogen IQ. The results cannot simply be explained by physiological effects, such as by an increase in faecal bulking and by a reduction in transit time. Furthermore, in the upper gastrointestinal tract of humans, wheat bran concentrations may be too low for much hydrophobic partitioning of carcinogens or procarcinogens onto the bran dietary fibre. It is not unusual in the human diet for the major intake of food mutagens to be more than 4 h away from the major bran intake.

The wheat bran caused changes in the IQ metabolite profile in both urine and plasma, which have not been reported previously. These changes are consistent with the wheat bran impeding the metabolism of IQ. Furthermore, the changes in the IQ metabolite profile of the urine were dose dependent. We believe that these effects could have quite profound implications for human carcinogenesis, if they occur at the low concentrations of IQ found in the human diet. Recent estimates indicate that the intake of heterocyclic amines by humans is ~1 µg/day (32), which is 10⁷ times less than the lower amount of IQ we fed to the rats. Experiments using these low amounts of IQ would require very sensitive detection techniques, such as accelerator mass spectrometry.

Acknowledgements

We thank Marlene Goedhart, Kirsten Wabnetz and Bradley Anderson for technical assistance. We acknowledge the financial support of the Cancer Society of New Zealand and its Hawkes Bay and Auckland divisions.

References


Received March 2, 1999; revised and accepted August 16, 1999