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Mechanisms by which resistant starches and non-starch polysaccharide sources affect the metabolism and disposition of the food carcinogen, 2-amino-3-methylimidazo[4,5-*f*]quinoline

P. Kestell, S. Zhu, L.R. Ferguson*

Faculty of Medicine and Health Science, Auckland Cancer Society Research Centre, University of Auckland, Private Bag 92019, Auckland, New Zealand

Abstract

Although both non-starch polysaccharides (NSP) and resistant starches (RS) are included in current definitions of dietary fibre, our previous work has suggested fundamental differences in the way in which these two classes of material affect the disposition and absorption of a dietary carcinogen. The present studies explore whether different effects on carcinogen metabolism could play a role in the contrasting patterns seen previously. Groups of female Wistar rats were pre-fed for 4 weeks one of five types of defined diet (AIN-76). The control diet contained 35% maize starch and no dietary fibre. The RS-containing diets had all the maize starch substituted with either Hi-maize™ or potato starch. In the NSP-containing diets, 10% of the maize starch was substituted with dietary fibre in the form of either lignified plant cell walls (wheat straw) or soluble dietary fibre (apple pectin). Pre-fed rats were gavaged with the food carcinogen, [2-^{14}C] 2-amino-3-methylimidazo[4,5-*f*]quinoline (IQ), and plasma and urinary metabolites characterized using HPLC at various time intervals after administration. After 4 h gavage, plasma from rats on both RS-containing diets contained significantly higher levels of intact IQ and lower levels of the major metabolites, IQ-5-*O*-glucuronide and IQ-5- sulfate, as compared with plasma from the negative control group at this time. In contrast, plasma from animals on the NSP-containing wheat straw diet (and to a lesser extent the apple pectin diet) showed significantly lower levels of intact IQ, and significantly higher levels of the two major metabolites, as compared with those from the control rats. These different metabolite profiles were also reflected in different urinary excretion profiles. Urine from rats pre-fed RS-containing diets revealed significantly slower metabolite excretion as compared with urine from rats that had been given the NSP-containing diets. Western blotting methodologies also profiled differences between the effects of these two types of dietary fibre in the expression of xenobiotic metabolizing enzymes. We conclude that changes in activity and expression of xenobiotic metabolising enzymes could play a role in the contrasting effects of these two types of dietary fibre on carcinogen uptake and disposition.

Keywords: Carcinogen metabolism; 2-Amino-3-methylimidazo[4,5-*f*]quinoline; Resistant starches; Non-starch polysaccharides

1. Introduction

The earliest definitions of dietary fibre and those still used in parts of Europe, considered non-starch polysaccharides (NSP) as the key chemical component [1,2]. However, starches that are resistant to digestion (resistant starches (RS)), non-digestible oligosaccharides (NDOs) and other materials are now permitted onto the market under the label "dietary fibre". In a previous study on mechanisms of cancer protection, we compared the properties of two dietary fibre preparations that were primarily RS with two that were primarily NSP for effects on excretion and disposition of the carcinogen 2-amino-3-methylimidazo[4,5-*f*]quinoline (IQ), in a rodent model [3]. Although both RS-containing diets were less effective than NSP-containing diets in reducing the transit times of faeces in rats, they were highly effective in increasing faecal bulk by as much as 50%. Thus, we might have expected that either type of dietary fibre would increase the excretion of a dietary carcinogen, thereby decreasing the probability of its absorption and carcinogenic

Abbreviations: NSP, non-starch polysaccharides; RS, resistant starches; NDO, non-digestible oligosaccharides; IQ, 2-amino-3-methylimidazo[4,5-*f*]quinoline; CYP, cytochrome P450; GST, glutathione-*S*-transferase; NSP-SDF, non-starch polysaccharides-soluble dietary fibre; NSP-LPCW, non-starch polysaccharides-lignified plant cell walls

* Corresponding author.
E-mail address: l.ferguson@auckland.ac.nz (L.R. Ferguson).

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action. However, we found that any potential advantage of high faecal carcinogen excretion associated with the RS diets was undermined by the fact that these diets also enhanced the systemic absorption of this carcinogen, resulting in higher plasma levels than those observed in rats pre-fed with any of the other diets (1.5–3-fold). In contrast, the NSP sources reduced the systemic absorption of the carcinogen and produced lower plasma levels than those measured in rats from the other dietary groups. Furthermore, the pattern of effect of the two types of dietary fibre on IQ disposition in plasma was distinctly different. In particular, plasma radioactivity profiles of rats fed RS diets had significantly higher concentrations (5–10-fold more than controls) initially after administration, and at a later time point (4–6 h) secondary peaks of radioactivity, indicative of enterohepatic recycling of IQ and its metabolites, were observed. Such a pattern was not seen in plasma profiles of rats pre-fed with diets derived from either of the NSP sources tested. This data could be explained not only by differential effects of the two types of dietary fibre on carcinogen disposition and excretion but also on its metabolism.

Enterohepatic circulation of IQ would produce multiple peaks and a long apparent half-life in the plasma concentration-time profile. As a consequence, the pharmacological or toxicological effect of IQ or its metabolites would be prolonged [4]. Enterohepatic circulation may be significantly influenced by the formation of metabolites occurring in enterocytes and hepatocytes, and during transit through the biliary system, intestinal tract or systemic circulation [4]. The major products of IQ metabolism in rats are IQ-5-*O*-glucuronide and IQ-5-*O*-sulfate, which are formed by conjugation with either glucuronic acid or sulfate after cytochrome P450-mediated ring hydroxylation at the fifth position, most probably by CYP1A1 and1A2 isozymes [5]. The latter have been identified as being responsible for N-hydroxylation and aromatic ring hydroxylation of many heterocyclic aromatic amines [5–10]. In addition, direct conjugation of the exocylic amine group, forming the N2-glucuronide and sulfamate (IQ-*N*-sulfamate) can occur [11–14].

We have undertaken a series of investigations to determine the mechanisms by which RS and NSP-containing diets affect the metabolism of IQ, and therefore most likely its enterohepatic recycling. In these experiments, the control diet was a defined rat diet (a modified AIN-76^{TM} diet) containing 35% native, normal maize starch. In the RS diets, the native maize starch was completely substituted with one of the following sources of RS: native potato starch or native, high-amylose maize starch (Hi-maize^{TM} starch). The two NSP preparations were provided by wheat straw, an excellent source of non-starch polysaccharides-lignified plant cell walls (NSP-LPCW), and “Apple Pectin”, a non-starch polysaccharides-soluble dietary fibre (NSP-SDF) extracted from plant cell walls, both substituted for starch to a level of 10% in the diet. Initially, profiling of IQ and its most important metabolites in plasma and urine of rats pre-fed with the various diets was performed, using previously described methodology, during the period when the effect of recycling was greatest. Following this, the effects of RS-containing and NSP-containing diets on the expression of hepatic and colonic xenobiotic drug metabolizing enzymes were measured using techniques reported by Helsby et al. [15]. The isozymes of cytochrome P450 and glutathione-*S*-transferase selected for evaluation included not only those associated with IQ metabolism, but also those involved with a wide variety of other potential dietary carcinogens.

2. Experimental

2.1. Materials

A commercial preparation of apple pectin (high methoxyl, type Brown Ribbon P-100) was obtained from Obipektin, Bischofszell, Switzerland, and used as the NSP-SDF preparation. Straw from field grown bread wheat (*Triticum eastivum* cv. Kotuku) was obtained from New Zealand Institute for Crop and Food Research, Christchurch, and cut into <0.5 cm lengths before being added to the diet. This provided a source of NSP-LPCW. The two RS2 preparations used were: native Hi-maize^{TM} starch (a high-amylose maize starch) kindly donated by Ian Brown, Starch Australasia Ltd., Lane Cove, New South Wales 2066, Australia (RS1), and a preparation of food-grade native potato starch from AVEBE Starches International, 9607 PT Foxhol, The Netherlands (RS2). Normal native maize starch was Edmonds Fielder cornflour, marketed by Bluebird Foods Limited, Manukau City, New Zealand. [2-^{14}C]IQ (2-amino-3-methylimidazo[4,5-*f*]quinoline) (specific activity 10 mCi/mmol radiochemical purity >95%) and unlabelled IQ were from Toronto Research Chemicals (Toronto, Ontario, Canada). The purity of unlabelled and radiolabelled IQ was determined by HPLC using conditions described by Kestell et al. (1999) [16] and found to be greater than 95%. All other chemicals were at least of analytical grade.

2.2. Animal maintenance

Six-week-old female Wistar rats, bred within the University of Auckland animal colonies, were provided with experimental diets. All experimental protocols were approved by the University of Auckland Animal Ethics Committee, Permit no. N500. Throughout the experiments, the rats were maintained at a constant environmental temperature of 22 °C, with a 12 h light–dark cycle and free access to drinking water. The rats were kept (three per cage) in cages (58 cm × 24.5 cm × 17 cm) with dropped-bottom wires to stop the rats reaching sawdust, and were randomly allocated into five dietary groups (12 rats/group). Of these 12 rats in each dietary group, three were used for profiling

Table 1
Composition of the rat diets[a]

Component (g/kg)	Control diet	Diet supplemented with dietary fibre preparation	Diet supplemented with RS preparation
Casein	200	200	200
Normal, native maize starch	350	250	–
Sucrose	200	200	200
NSP preparation	–	100	–
RS preparation	–	–	350
Sunflower seed oil	100	100	100
Lard	100	100	100
AIN-76^{TM} vitamin mix	10	10	10
AIN-76^{TM} mineral mix	35	35	35
DL-methionine	3	3	3
Choline bitartrate	2	2	2

[a] The basic diet was a high-fat modification of the AIN-76^{TM} diet [27].

IQ and its metabolites in plasma, whilst three others were utilized for studying their excretion in urine. The livers and colons of the remaining six animals were collected for the determination of the expression of proteins associated with xenobiotic metabolising enzymes. The five diets used were: a basal modified AIN-76^{TM} diet (Bio-Serv Inc, Frenchtown, NJ, USA) containing 35% normal native maize starch, or this diet with 25% normal maize starch, but 10% NSP preparation added; or this diet in which the normal maize starch was replaced with either potato starch or Hi-maize^{TM} starch. Table 1 shows the compositions of these diets, which were prepared dry. Water (100 ml/kg of diet) was added to each diet, which was then molded into 20 g balls.

Diet (80 g per day) was added to each cage, and rats were provided with the diet for 4 weeks before any experimental studies. The amounts of uneaten food were recorded. Each rat was weighed three times per week, and carefully monitored for signs of ill health.

2.3. Formulation and administration of IQ

IQ solutions were prepared by dissolving [2-^{14}C]IQ (1.60 mg), together with unlabelled IQ (0.40 mg), in 0.1 M HCl (1.8 ml) and the pH adjusted to 3 with 5 M NaOH 33.3 μl, 0.5 M NaOH 12.5 μl and 0.01 M NaOH 112.5 μl. Formulated IQ (specific activity 8.3 mCi/mmol) was orally administered to non-fasted rats from each subgroup by gavage at 1 mg/ml/kg body weight and 10 μCi per rat.

2.4. Blood and organs collection

For the study of IQ and metabolites in plasma, rats were anaesthetized by halothane and then the blood collected by cardiac puncture into heparinised tubes at 4 h after administration. The plasma was separated by centrifugation (1000 g, 10 min, 4 °C) and then stored at −20 °C until analysis. Those animals assigned to liver and colon collection were culled by CO_2 euthanasia before the organs were removed. These were then snap frozen in liquid nitrogen and stored at −80 °C until they were assayed for enzyme expression.

2.5. Urine collection

After dosing, animals were placed in individual metabolic cages that enable the separation of excreta (Metabowls, Jencons Ltd., Poole, UK). The voided urine was collected in closed systems (reducing water evaporation) at 0–2 and 2–8 h following administration, and stored at −20 °C until analysis.

2.6. Determination of plasma and urinary radioactivity

Aliquots of plasma or urine (100 μl) were added directly to 10 ml of water-accepting Ready Safe scintillation fluid (Beckman Instruments Inc., CA, USA) for scintillation counting, with quench correction by the external standardisation method measured using a 1500 TriCarb Packard Scintillation Counter (Packard Instruments Ltd., Downers Grove, IL, US).

2.7. HPLC metabolite profiles

Urine and plasma samples were collected from rats within each dietary group after [2-^{14}C]IQ gavage, and analysed by HPLC according to previously described conditions that enabled the detection and separation of IQ and its principal metabolites IQ-5-*O*-glucuronide, IQ-*N*-sulfamate and IQ-5-*O*-sulfate [16]. Samples were prepared using protein precipitation or solid phase extraction methods described by Kestell et al. [16] and the resulting extracts injected onto a Novapak C18 5 μ 3.9 mm × 150 mm stainless steel HPLC column (Waters Associates, Milford, MA, USA). Separation of IQ and metabolites was achieved using a mobile phase consisting of acetonitrile and phosphate buffer (0.14 M, pH 4.7), which was pumped at a flow rate of 1 ml/min. A linear gradient was established from 100% of solvent A (phosphate buffer containing 10% acetonitrile) and 0% solvent B (acetonitrile containing 10% phosphate buffer) rising to 10% solvent B over 50 min followed by an increase to 100 % at 55 min which was then held for further 10 min. Solvent delivery was carried out using Waters 510 pumps which were controlled by a Waters Millenium Data Station. Metabolite profiles were established by monitoring the radioactivity in the eluate emerging from the column and were performed in duplicate. Eluate fractions were collected every 0.5 min to which 5 ml of water-accepting Ready Safe scintillation fluid (Beckman Instruments Inc., CA, USA) was added and the radioactivity then measured by scintillation counting as described above.

To ascertain the identity of metabolites, urine and plasma extracts were treated with β-glucuronidase, sulfatase or 1 M hydrochloric acid according to previously

reported procedures [16]. Peaks were assigned according to co-chromatography with authentic IQ, and their susceptibility to chemical and enzymatic hydrolysis.

2.8. Cytosol and microsome preparation

Livers were washed in ice-cold 67 mM phosphate buffer containing 1.15% KCl and kept at −80 °C until subcellular fractions were prepared by differential centrifugation [17]. Protein content was determined by the bicinchoninic acid assay using bovine serum albumin as the standard [18].

2.9. Western immunoblots

Hepatic cytosolic and microsomal samples were diluted to 1 mg/ml with Milli Q water and then further diluted 1:4 with sample buffer. Samples were then boiled at 70 °C for 20 min. SDS/PAGE was performed on 10% acrylamide gels using the BioRad Mini-Protean III apparatus. A sample of 20 μl was added to each lane and run for 40 min at 200 V. Proteins were then transferred at 110 V for 60 min onto polyvinylidene difluoride (PVDF) membrane (Biorad, Auckland, NZ). The PVDF was blocked overnight in 5% non-fat milk protein containing 0.4% FCS. The membrane was then probed with monoclonal antibodies to either CYP1A1/2, CYP3A2, GST Ya (A1) or GST Yc (A2) which were purchased from Gentest Corporation (Woburn, MA, USA). Secondary probing was performed with an Elite ABC Vectastain kit (Vector Labs, Burlingame, CA, USA) and the proteins visualised by enhanced chemiluminescence (ECL) (Amersham Pharmacia Biotech UK Ltd., Amersham, UK) using a film exposure time of 60 s (Kodak Scientific Imaging Film, Rochester, NY, USA). Quantitative analysis of the bands (area/density relationships) was achieved by the MD30 image analysis system (Leading Edge, Adelaide, Australia).

2.10. Statistical analyses

Initial statistical analysis to evaluate differences among the dietary groups was carried out by one-way analysis of variance (ANOVA) followed by Dunnett's multiple comparison test. For data found to be unsuitable by ANOVA treatment, statistical analysis was performed using Student's *t*-test. Data were considered significant if $P < 0.05$.

3. Results

3.1. Plasma HPLC metabolite profiles

Plasma samples were collected from rats within each dietary group 4 h after [2-^{14}C] IQ gavage, analysed by HPLC and representative radio-chromatograms are shown in Fig. 1. It was found that the major proportion of radioactivity in the plasma metabolite profiles of rats fed the control AIN-76 diet could be assigned to six peaks, which accounted for 96% of the total plasma radioactivity. Of these, one peak of retention time 61 min was assigned to parent IQ since it co-chromatographed with the authentic material and accounted for 32% of the total plasma radioactivity. However, the majority of radioactivity associated with these profiles could be assigned to peak M3, which eluted at 29 min. M3 was tentatively identified as IQ-5-*O*-glucuronide (40% of total plasma radioactivity) since it could be eliminated when incubated with β-glucuronidase. In addition, four other peaks (M1, M2, M4 and M5) were detected, each accounting for 5–8% of total plasma radioactivity. Of these, M1 (elution time 43 min) was assigned as being the IQ-5-*O*-sulfate, as it was susceptible to hydrolysis by sulfatase, whilst M2 (elution time 33 min) was characterised as being the IQ-*N*-sulfamate, since it disappeared upon acid hydrolysis of the extract and was unaffected when treated with either β-glucuronidase or sulfatase. The identity of the other two metabolites, M4 (elution time 19.5 min) and M5 (elution time 14.5 min) remained unknown and were not affected by acid or enzymatic hydrolysis.

Analysis of metabolite profiles of plasma collected from rats pre-fed with the RS-containing diets revealed that all of these metabolites were present except M5. Although small radioactive peaks were detected within the same period as M5, it was determined that their retention times were sufficiently different (13.5 and 16 min) to conclude that they were not identical with metabolite M5. The range of metabolites detected in the plasma of rats pre-fed with diets supplemented with both NSP diets was similar to those derived from the RS dietary groups.

In order to quantify the differences between metabolite profiles, the distribution of metabolites and IQ were expressed as a percentage of the total plasma radioactivity in the plasma of rats pre-fed with control AIN-76 as well as the RS and NSP-containing diets (Fig. 2). The profiles thus obtained from the plasma of rats fed diets supplemented with RS appeared to have notably different features from those associated with animals pre-fed the NSP-containing diets. Thus, the proportions of radioactivity associated with IQ in the plasma profiles of rats fed RS diets were 2–2.5-fold greater than those measured in the plasma of rats given the NSP-LPCW diet whilst the amounts of M3, IQ-5-*O*-glucuronide, were 1.5–3-fold lower. These differences were significantly different between the RS1 and NSP-LPCW diets and reflected in the ratios calculated between the proportions corresponding to the major metabolite M3 and parent IQ. Mean values of 0.22 ± 0.04 and 1.89 ± 0.39 were found for profiles associated with the RS1 and NSP-LPCW diets, respectively ($P < 0.05$).

In general, the effect of the both NSP and RS diets on M1, M2 and M4 were minimal and any differences failed to reach statistical significance.

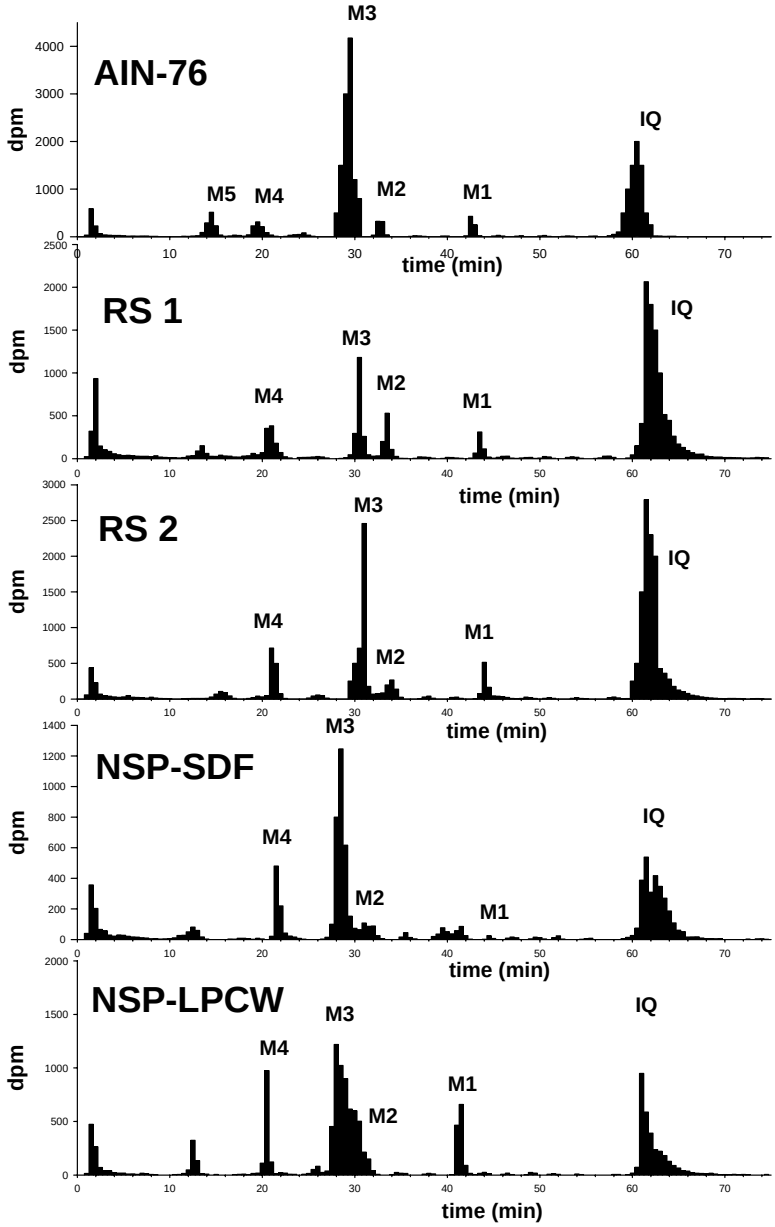

Fig. 1. HPLC metabolite profiles of plasma collected 4 h after oral administration of [2-^{14}C]IQ at 1 mg/kg to non-fasting rats pre-fed either a control AIN-76 diet, or a control diet supplemented with RS derived from RS 1 or RS 2, or NSP prepared from NSP-SDF or NSP-LPCW.

3.2. Excretion of metabolites in urine

HPLC metabolite profiles of urine samples collected from pre-fed rats after oral administration of [2-^{14}C]IQ were determined. All profiles of urine sampled at 0–2 and 2–8 h following gavage of the carcinogen contained two major metabolites, which together accounted for 80–90% of the urinary radioactivity. These compounds were assigned to M3, IQ-5-*O*-glucuronide and M1, IQ-5-*O*-sulfate, based on their retention times and susceptibility to enzymatic hydrolysis. In addition, only a small proportion of urinary radioactivity (2–4%) was found to be associated with the parent IQ. This was also the case for the unknowns M4 and M5 in the control AIN-76 dietary group (each <3%), and M4 and associated unknown peaks in the other groups.

The amount of IQ and each major urinary metabolite excreted within the collection periods was calculated as a percentage of the total radioactive dose administered (Fig. 3). Generally, during the first 2 h after treatment, rats within the NSP dietary groups excreted slightly higher amounts of these compounds than those animals fed diets prepared from RS sources, but statistical significance was only reached for the excretion of M1 (IQ-5-*O*-sulfate) and IQ between the NSP-LPCW and RS1 groups. During the 2–8 h collection period, urinary excretion of IQ, M1 (IQ-5-*O*-sulfate) and M3 (IQ-5-*O*-glucuronide) was found

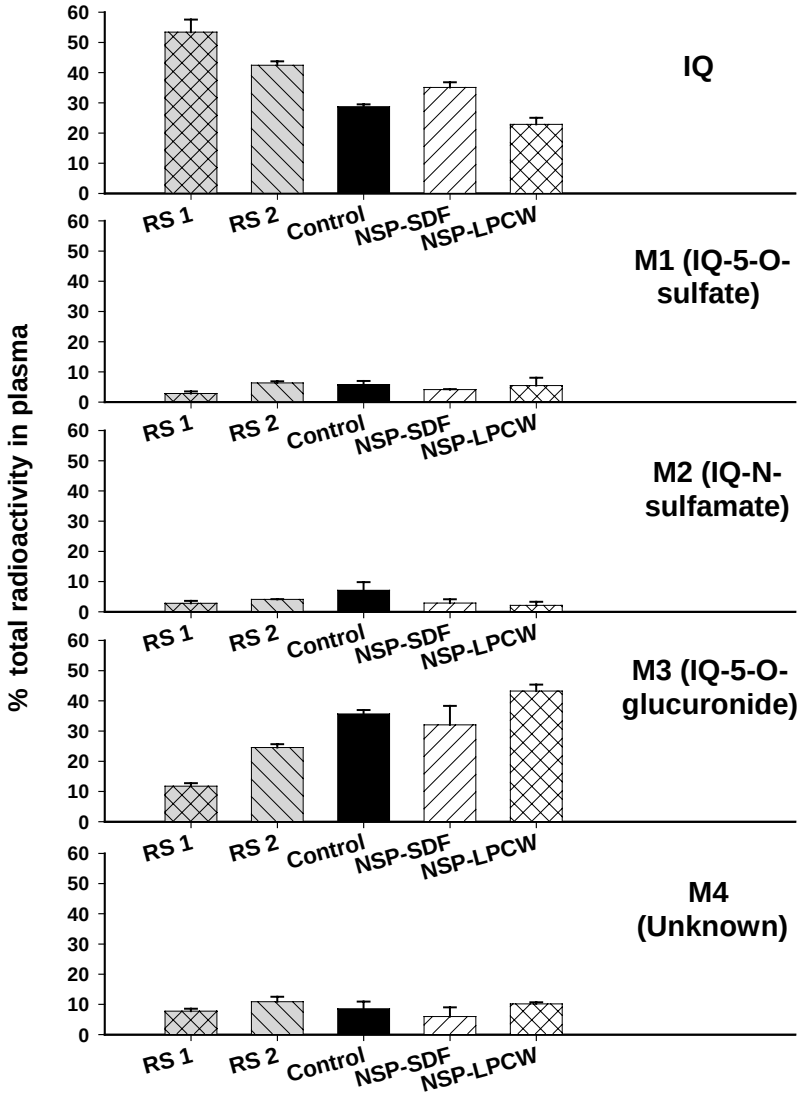

Fig. 2. Percent distribution of IQ and metabolites in plasma as a function of total plasma radioactivity 4 h after rats were gavaged with [2-^{14}C]IQ at 1 mg/kg. Data are from non-fasting animals pre-fed either a control AIN-76 diet, or a control diet supplemented with RS derived from Hi-maize (RS1) or potato (RS2), or NSP prepared from pectin (NSP-SDF) or wheat straw (NSP-LPCW). Results are the mean ± S.E. of data from three animals.

to be significantly different among all groups. In particular, rats pre-fed with the NSP-LPCW diet excreted 1.5–2-fold lower amounts than that seen in animals fed RS diets.

3.3. The effect of RS and NSP diets on the expression of CYP1A1/2, CYP3A2, GST Ya (A1) and GST Yc (A2) proteins in liver and colon

It was found that both RS and NSP-containing diets produced changes in the expression of immunoreactive proteins for CYP1A1/2, 3A2 and GST Yc (A2). Representative Western blots for expression of these hepatic enzymes are illustrated in Fig. 4, and all data are summarized in relation to values for expression of these enzymes in hepatic or colonic tissue from rats pre-fed the control diet on Fig. 5.

Either potato or Hi-maize RS diets led to small increases in the expression of CYP1A/2 in both the liver and the colon, but only the latter data reached statistical significance ($P < 0.01$). In contrast, the NSP-SDF-diet decreased the expression of both these proteins, and again the data are statistically significant only for the colon. The pattern produced by the NSP-LPCW-diet was different again. This dietary regime increased the levels of CYP1A1/2 in the liver by 2–3-fold in the liver but only by around 30% in the colon (both $P < 0.001$).

Both RS-containing diets significantly increased CYP3A2 by 2–3-fold ($P < 0.001$) in the liver but decreased levels in the colon. Statistical significance ($P < 0.001$) was only reached for colonic tissue derived from rats pre-fed the RS potato starch diet. The NSP-SDF-diet decreased the expression of both these proteins, and again the data

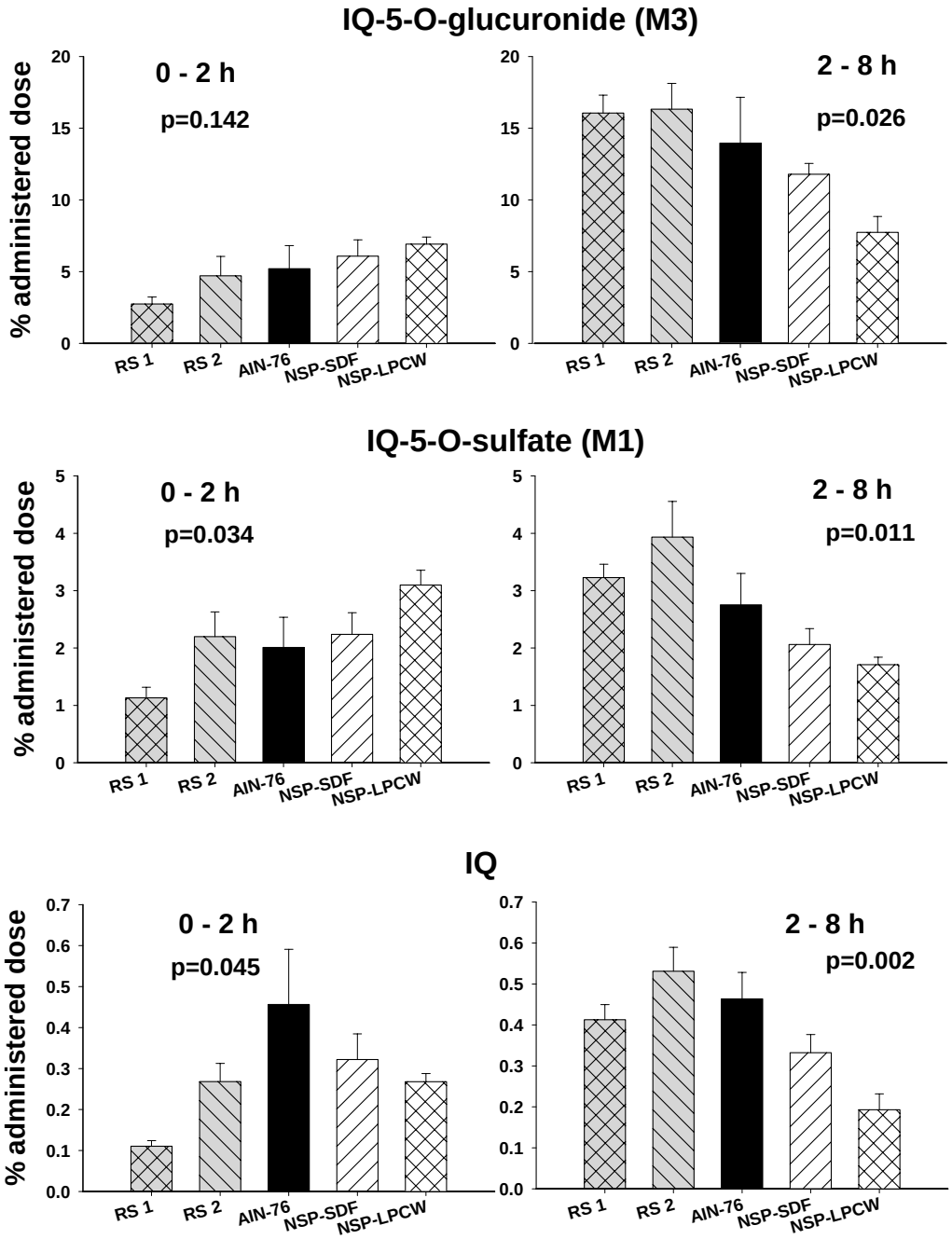

Fig. 3. Urinary excretion of IQ and major metabolites M1 and M3 during 0–2 and 2–8 h periods following oral administration of [2-^{14}C]IQ at 1 mg/kg to non-fasting rats pre-fed a control AIN-76 diet, as compared with rats pre-fed either RS-containing or NSP-containing diets. Each point represents the mean ± S.E. of data from three animals.

are statistically significant ($P < 0.001$) only for the colon. However, the NSP-LPCW diet significantly increased the hepatic CYP3A2 approximately 2-fold and the colonic enzyme between 2–3-fold (both $P < 0.001$).

Neither the RS nor NSP-containing diets affected the expression of hepatic or colonic GST Ya (A1). The most consistent changes were observed with GST Yc (A2) protein whereby all RS and NSP-diets decreased expression in the colon and liver but the effect only reached high statistical significance ($P < 0.001$) in the latter.

4. Discussion

This study revealed that the metabolism and excretion of the dietary carcinogen IQ and the expression of hepatic and colonic xenobiotic metabolising enzymes could be changed

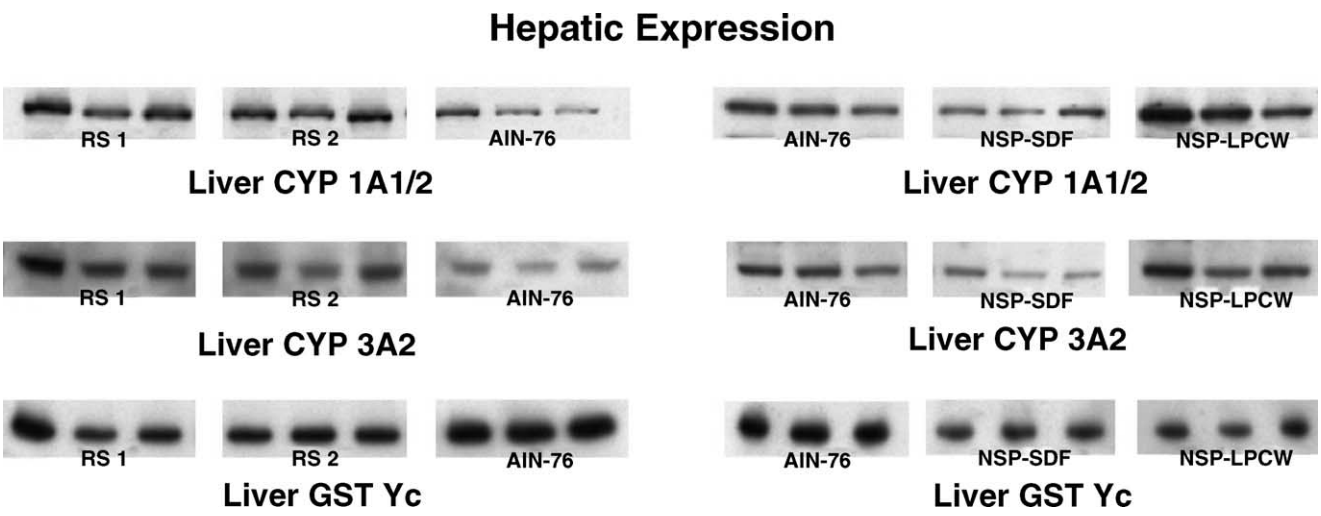

Fig. 4. The expression of hepatic xenobiotic metabolising enzymes in rats pre-fed with either a control AIN-76 diet, or a control diet supplemented with RS derived from RS1 or RS2 or NSP prepared from NSP-SDF or NSP-LPCW as detected by immunoblot (Western) analysis of CYP1A1/2, CYP3A2 and GST Yc (A2). Representative samples are presented from rats within each of the dietary groups, which contained six animals.

according to the type of dietary fibre that was incorporated into the diet. We suggest that the differential effects are a consequence of the RS and NSP diets eliciting a unique set of physiological changes that combine to modulate the absorption, metabolism and excretion of this carcinogen. A schematic overview of these mechanisms is given in Fig. 6.

Our previous studies have shown that an NSP-LPCW diet has a profound reducing effect on IQ absorption from the rat gut, which leads to a lower plasma IQ level and a higher amount of IQ excreted in faeces. [3]. We propose that reduced faecal transit times (60% lower than the control diet [19]) and the substantial carcinogen binding capacity of NSP-LPCW [20–23] are chiefly responsible for the pharmacological modulation of IQ. The binding capacity of NSP-LPCW is so strong that only an aprotic solvent such as dimethyl sulfoxide can remove IQ and its metabolites from wheat straw residues in the faeces [3]. When combined together they would substantially reduce contact time with the gut wall and directly impair IQ absorption. Urinary excretion data from the present study is consistent with this hypothesis as these animals excreted the lowest amounts in total of IQ, M1 (IQ-5-*O*-sulfate) and M3 (IQ-5-*O*-glucuronide) in comparison to rats from the other dietary groups.

The reduced transit time and strong carcinogen binding capacity of NSP-LPCW are also likely to be important factors in preventing reabsorption of parent IQ, which is excreted either in the bile [12] or in the small intestine by processes that could be mediated by transport proteins such as MRP2 [24]. This is supported by data from our previous study [3] in which there was a little evidence of enterohepatic recycling and from the current study in which the majority of the radioactivity in plasma HPLC metabolite profiles derived from rats pre-fed with the NSP-LPCW diets was associated with IQ metabolites and not parent IQ. The contributions of up-regulation of hepatic and colonic xenobiotic metabolising enzymes, CYP1A and 3A might be of some significance to this effect too. In particular, the apparent 2–3-fold increase in the expression of CYP1A in the liver

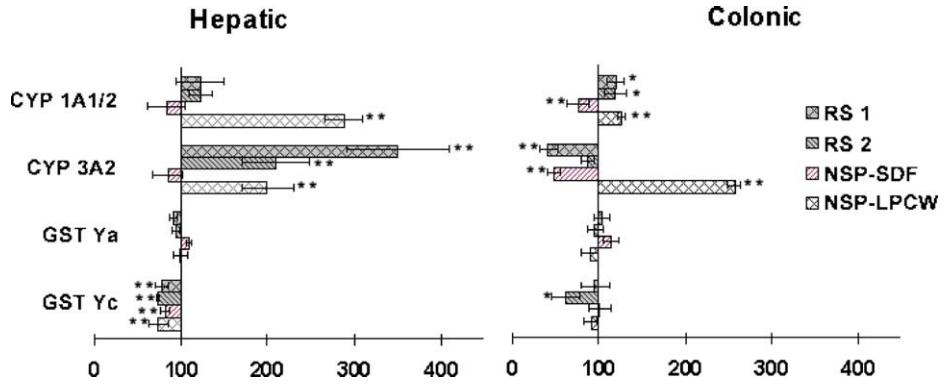

Fig. 5. The change in expression of CYP1A1/2, CYP3A2, GST Ya (A1) and GST Yc (A2) as a percentage of control (mean ± S.E.M., $n = 6$) in the liver and colon of rats pre-fed with different RS and NSP diets. * Significantly different than control animals, $P < 0.01$. ** Significantly different than control animals, $P < 0.001$.

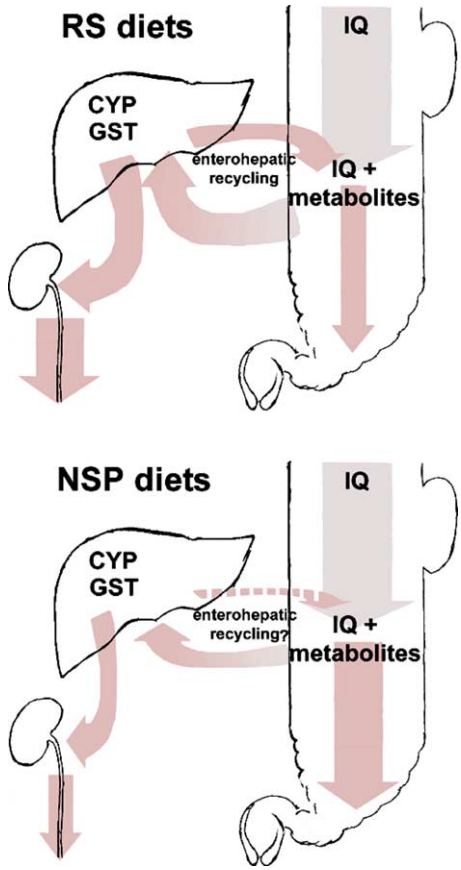

Fig. 6. Schematic overview of mechanisms by which RS and NSP diets affect the absorption, metabolism and excretion of the dietary carcinogen IQ. In rats fed RS diets, the majority of IQ is absorbed into the body, metabolised in the liver by up-regulated xenobiotic metabolising enzymes and eliminated in urine. Further systemic exposure of IQ and its metabolites occurs through enterohepatic recycling. In rats fed NSP diets, the majority of IQ is not absorbed into the body but eliminated in the faeces where it is tightly bound to this matrix. As a consequence, enterohepatic recycling does not occur to the same extent as that seen with rats fed RS diets.

and colon of rats within the NSP-LPCW dietary group might lead to an increase in IQ hydroxylation at the fifth position of the quinoline ring [5]. The resulting metabolite from this metabolic pathway is the precursor of IQ-5-*O*-glucuronide.

The NSP-SDF diet, just as the NSP-LPCW diet, is effective in decreasing transit times and increasing faecal bulk, which could account in part for the lower plasma IQ and its metabolite levels and the higher amount of them excreted in the faeces [19,3]. An alternative explanation of the reduced absorption might be due to the unique property of pectin that allows substantial amounts of undigested starch going through to the caecum, thereby increasing the viscosity of digesta [19]. This could provide a physical barrier to IQ absorption and might impact on enterohepatic recycling by preventing reabsorption. Thus, the effects of this diet on plasma HPLC metabolite profiles and urinary excretion of IQ, M3 (IQ-5-*O*-glucuronide) and M1 (IQ-5-*O*-sulfate) were similar to those produced by the NSP-LPCW diet, but less pronounced. Any subtle differences might be accounted for by the changes in the expression of hepatic and colonic xenobiotic metabolising enzymes. The NSP-SDF diet had the opposite effect to the NSP-LPCW diet in down-regulating CYP 1A1/2 and CYP 3A2 enzymes. This could result in reduction of the extent to which IQ becomes metabolically activated via N-hydroxylation, leading to an increase in the urinary excretion of the parent molecule.

The effects of the RS-containing diets are different again. We previously found that they were less effective than either of the NSP-containing diets in decreasing transit time, and also had a lower carcinogen binding capacity [20–23]. Rather than preventing the systemic absorption of IQ, they actually appeared to enhance it [3]. This was confirmed in the present studies by the presence of IQ-*N*-sulfamate in the plasma of rats pre-fed with RS diets, particularly the Hi-maize diet, in which the majority of the radioactivity was associated with parent IQ. Previous studies have established that the presence of IQ-*N*-sulfamate in plasma and urine is considered a sensitive indicator of systemic exposure to IQ at high levels [11,12,25]. However, data from the current study was somewhat contradictory in relation to this as M2, IQ-*N*-sulfamate, levels were not significantly different in the plasma of rats among the various dietary groups.

The present study also reveals that there are significant differences between RS-containing and NSP-containing diets in modulation of xenobiotic metabolism. Coupled with the lower carcinogen-binding binding capacity of RS materials, these differences may lead to greater amounts of carcinogen being available for absorption. It is proposed that these diets have additional factors that facilitate IQ absorption such as changes in gut pH, ammonia concentration and short-chain fatty acid content. The effects of RS diets on biochemical and physiological parameters have been reported by Silvi et al. [26] and Ferguson et al. [27]. Facilitated absorption and the weak binding properties of RS materials would also impact on enterohepatic recycling as this would enhance reabsorption of IQ following biliary or intestinal excretion. In addition, the potential effect of RS diets on gut microfloral enzymes must be considered and whether they can convert biliary metabolites back to parent IQ where it would be available for reabsorption.

Overall, these studies have shown that RS and NSP diets affect the metabolism and disposition of the dietary carcinogen IQ. The implications of our results (as presented in the previous paper [3]) are that RS diets could enhance carcinogenic events whereas diets high in LPCW sources will be the most likely to show protective properties. Of foremost importance in future investigations would be to establish whether these diets can modulate other hepatic, colonic or bacterial phase 2 enzymes such as UDP-glucuronosyl-transferase and sulfotransferase. In addition, the use of LC-MS-MS technology with ion trap or triple quadrupole technology for better metabolite identification and more selective and sensitive detection [28] is required together with more selective extraction methods

that involve materials derived from molecular imprinted polymers or employ immunoaffinity purification procedures [29,30].

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