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ORIGINAL CONTRIBUTION

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Contrasting effects of acute and chronic dietary exposure to 2-amino-3-methyl-imidazo[4,5-*f*]quinoline (IQ) on xenobiotic metabolising enzymes in the male Fischer 344 Rat: implications for chemoprevention studies

Abstract *Background* 2-Amino–3-methyl-imidazo[4,5-*f*]quinoline (IQ) is a mutagen produced in cooked food. It is commonly present in the human diet and often used as a (pro)carcinogen for chemoprevention studies. Many foodstuffs act as chemopreventers by altering xenobiotic metabolising enzyme expression in favour of detoxication over bioactivation pathways. However, IQ itself can also affect enzyme expression, which may be a confounding factor in chemoprevention studies. *Aim of study* Chronic low dose IQ exposure is intuitively closest to the human dietary situation. The aim was to investigate the effects of chronic dietary exposure to IQ on the expression of enzymes involved in the bioactivation and detoxification of xenobiotics and to compare this with acute exposure, often used in chemoprevention studies. *Methods* Male Fischer rats received IQ (300 ppm) in the diet (AIN–76) for 52 weeks or were given IQ ($20\,mg \cdot kg^{-1}$) orally for 3 days. Animals were killed, livers removed and subcellular fractions prepared. A range of enzymes was selected to allow investigation of several cellular mechanisms. Enzyme expression and activity were determined by Western blotting and the use of selective probe substrates as appropriate. *Results* Chronic exposure to IQ led to an increase in phase II detoxifying enzymes. Both the activity and expression of glutathione S-transferase (GST-A1/2) were increased, as were NADPH: Quinone oxidoreductase (NQO), UDP-glucuronosyl transferase (UGT) and β-glucuronidase activities. There were no statistically significant changes in the potential for bioactivation by three cytochrome P450s. In contrast, acute IQ exposure significantly increased the expression and activity of some cytochrome P450 (CYP1A1 and CYP1A2), UGT and β-glucuronidase, but significantly decreased glutathione *S*-transferase expression and activity. There was a non-significant decrease in NQO but no change in CYP3A2 and CYP2E1 activities. *Conclusions* The changes after acute exposure suggest an interaction through the A*h* receptor and xenobiotic response element, modified by the glucocorticoid response element. In contrast, the pattern of effects after chronic exposure suggests activation of the antioxidant response element (ARE). Although the acute model is more practically convenient for short-term chemoprevention screening, the data suggest that an entirely new mechanism is being invoked that completely masks effects of the ARE that occur during chronic exposure. There is a danger that chemopreventive strategies developed using acute models may be misleading, since the mechanism is unlikely to occur during human dietary exposures.

Key words IQ – bioactivation – chemoprevention

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Introduction

Xenobiotic metabolising enzymes (XME) are responsible for the biotransformation of various compounds. Reactions include the oxidative cytochrome P450-dependent monooxygenase (CYP450) pathway, as well as enzymes such as NADPH: Quinone oxidoreductase (NQO), glutathione *S*-transferase (GST) and UDP-glucuronosyl transferase (UGT). These reactions generally result in the formation of polar metabolites that can be cleared easily from the body. There is considerable interest in regulation of these enzymes by dietary chemopreventive agents [1]. Although the expression of individual CYP and GST enzymes may be specifically induced or repressed, it seems likely that many such effects may occur through a more generalised response element. The xenobiotic response element (XRE) [also known as the Ah receptor RE (AhRE)] is present in the promotor region of several enzymes involved in xenobiotic metabolism, including the murine Aromatic Hydrocarbon (A*h*) battery of enzymes [2]. This includes six genes: *cyp1a1*, *cyp1a2*, *Nmo1*, *Ahd4*, *Ugt1.06* and *Gsta1*, coding for two phase I enzymes: CYP1A1 and CYP1A2; and four phase II enzymes: NAD(P)H-menadione oxidoreductase, aldehyde dehydrogenase, UDP-glucuronosyl transferase (UGT) and glutathione *S*-transferase (GST) Ya subunit. Activation of the XRE occurs via ligand binding directly to the A*h* receptor, which is translocated to the nucleus and binds to the XRE, leading to transcriptional upregulation of the specific XME genes [3]. In contrast, an antagonist to the A*h*-receptor decreases the basal levels of the six enzymes [2]. The XRE is also present in the promotor region of fos and jun genes, whose expression may be stimulated with formation of activator protein–1 (AP–1) and consequent upregulation of expression of other genes [4]. A functional antioxidant response element (ARE) is present in promotor elements of GST and NADPH:Quinone oxidoreductase (NQO). This response element is thought to be important in the basal expression of enzymes such as GST [5]. The glucocorticoid response element (GRE) is present in the promoter region of all six of the enzymes on the A*h* battery [6]. This response element is activated by glucocorticoid and glucocorticoid-like structures by glucocorticoid receptor-dependent or independent binding to modulate a range of enzymes by many unclear, complex mechanisms in the mouse, rat and human [6–8]. Linder *et al.* suggested that the glucocorticoid interacted with the glucocorticoid receptor to produce and attract elements that interfere with the A*h*-receptor mediated regulation of the enzymes [6]. It appears that cellular stress, in the form of heat or chemical shock, can potentiate the expression of these genes in mouse L929 cells (heat shock potentiation effect, or HSPE) [9]. However, such responses may also be seen as beneficial to the organism and some agents thought to be chemopreventive, e. g. quercetin may stimulate these response elements in MCF7 human breast carcinoma cells [10].

Heterocyclic amines (HCA), formed during high temperature and long cooking of protein-rich foods such as meat [2], are compounds that require metabolic bioactivation for their toxicity. These compounds are known mutagens in the Ames test [11] and there is a relationship between dose and rate of cancer in laboratory animals. Human data are inconclusive, since it is hard to control for personal cooking preferences in epidemiological studies [11–13]. One such heterocyclic amine, 2-amino–3-methylimidazo[4,5-*f*]quinoline (IQ), is a colon carcinogen thought to be present in the diet of 35 % of New Zealanders [14]. Although there is no epidemiological evidence specifically linking IQ to increased rates of cancer in humans, it is one of the five most common HCA in cooked meats [11] and the International Agency of Research on Cancer (IARC) concluded that IQ was a probable human carcinogen [12]. There is considerable interest in identifying dietary compounds that can inhibit the genotoxicity and carcinogenicity of such heterocyclic compounds [15].

IQ has been used as a model carcinogen in both acute [16, 17] and chronic [14, 18] rat models to assess the ability of chemopreventive agents and foodstuffs to protect animals from cancer. Dietary components that alter the expression of xenobiotic metabolising enzymes in mouse, rat and human [19–21] can potentially decrease the bioactivation of procarcinogens such as IQ, which is *N*-hydroxylated by human and rat CYP1A2 [22] and CYP1B1 [23] to a reactive electrophilic compound that can react with polyguanylic acids in DNA in a non-enzymatic fashion [24, 25]. IQ is also thought to be bioactivated by NQO since dicoumarol, an inhibitor of NQO, inhibits the activation of IQ [26]. IQ also undergoes 5-hydroxylation, catalysed by CYP, plus conjugation reactions catalysed by UGT and sulphotransferases to yield non-toxic metabolites in the rat and human [12, 27]. There is evidence that IQ itself can modulate the expression of the CYP enzymes involved in its own metabolism in the rat [28, 29] at doses (20 –130 mg · kg^{-1}) and exposure regimes typically used in short-term chemoprevention studies [16, 30]. As it is a planar molecule, IQ was proposed as a ligand for the A*h*-receptor and a subsequent agonist for the xenobiotic response element (XRE) [28]. XRE induction by the carcinogen being tested against could well confound interpretation of chemoprevention studies. In contrast, the effects of IQ on enzyme expression in long-term (40 –112 week) carcinogenicity studies, which often use doses of 100–800 ppm fed continuously in the diet [31], have not been investigated. Such models are, at least intuitively, closer to the human feeding situation than acute exposure situations.

The present study has investigated the effects of chronic dietary exposure to IQ on the expression of en-

zymes involved in the bioactivation and detoxification of procarcinogens such as IQ and compared this with acute exposure, more typical of short-term chemoprevention studies. These experiments have been carried out in the male Fischer 344 rat, a strain often used for cancer studies, in order to construct a suitable animal model for use in chemoprevention studies, more reflective of the natural human diet.

Materials and methods

Chemicals

2-Amino-3-methylimidazo[4,5-*f*]quinoline (IQ) was from Toronto Research Chemicals (Canada). Glutathione and 2,6-dichloroindophenol (DCPIP) were obtained from ICN (Sydney, Australia). Resorufin and dicoumarol were from Sigma (Castle Hill, Australia). 3,4-Dichloronitrobenzene (DCNB) was purchased from Lancaster Chemicals (Eastgate, UK). Uridine diphosphate glucuronic acid (UDPGA), nicotinamide adenine dinucleotide (NADH) and nicotinamide adenine dinucleotide phosphate (NADPH) were from Boehringer Mannheim (Auckland, NZ). 1-Chloro–2,4-dinitrobenzene (CDNB) and 4-nitro–2-benzylchloride (NBC) were obtained from ACROS (New Jersey, USA). Ethoxyresorufin and methoxyresorufin were synthesised by Dr. Brian Palmer, Auckland Cancer Society Research Centre, Auckland, NZ.

Dosing regimens

Inbred Fischer 344 rats were made available from the Animal Laboratories at the University of Auckland Faculty of Medicine & Health Sciences and all experiments received ethical approval from the University of Auckland Animal Ethics Committee (AEC No. 499). Acute studies involved 5–6 week old male Fischer rats (n = 4/group) fed control diet (AIN–76A, [32]) for 4 weeks prior to IQ ($20\,mg \cdot kg^{-1}$) administration in 0.01M NaOH/0.1M HCl ($4\,mL \cdot kg^{-1}$) or vehicle control gavaged daily for 3 days. Animals were killed 24 hours after final exposure.

For the chronic study, male Fischer F344 rats (20 per group) were fed defined AIN–76A control diet ± IQ (125 ppm for the first 26 weeks then 300 ppm for the remaining 26 weeks). The dose was increased after 26 weeks when no pathological signs of disease, necessary for future chemoprevention studies, were seen in the IQ exposure group. Animals were killed after 52 weeks by CO_2 euthanasia in line with institution guidelines [33], the organs removed and examined for tumours. The incidence was noted and histological analysis on the organs and pathological analysis of the tumours performed. Animals showing signs of suffering were killed prior to the end of the experiment and the details noted (Table 1).

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 [34]. Protein content was determined by the bicinchoninic acid assay using bovine serum albumin as the standard [35].

Western immunoblots

Hepatic cytosolic and microsomal samples were diluted to $1\,mg.mL^{-1}$ with MilliQ water and then further diluted 1:4 with sample buffer. Samples were then boiled at 70 °C for 20 minutes. SDS/PAGE was performed on 10 % acrylamide gels using the BioRad Mini-Protean III apparatus. Sample (20 µL) was added to each lane and run for 40 minutes at 200 V. Proteins were then transferred at 110 V for 60 minutes 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 a monoclonal antibody to GST-A1 (Ya) or GST-A2 (Yc), purchased from Biotrin (Dublin, Ireland); or CYP1A1/2, CYP2E1 or CYP3A2 purchased from Gentest (USA). Secondary probing was done with the Elite ABC Vectastain kit (Vector Labs, USA) and the proteins were visualised by Enhanced Chemiluminescence (ECL) (Amersham, UK) and the film (Kodak Scientific Imaging Film) exposed for 60 seconds. Quantitative analysis of the bands (area/density relationships) was achieved by the MD30 image analysis system (Leading Edge, Adelaide, Australia).

Enzyme activity assays

CYP1A1/2

Ethoxyresorufin or methoxyresorufin (0–100 µM) were incubated in triplicate with sample microsomes (1 mg of protein) ± 1 mM NADPH in 67 mM phosphate buffer, pH 7.4, containing 25 µM $MgCl_2$ in a final volume of 200 µL for 20 minutes at 37 °C. The reaction was stopped by the addition of ice cold methanol (1 mL), the precipitate removed by centrifugation and the fluorescence of the supernatant assessed using excitation 530 nm, emission 585 nm. The formation of resorufin was determined from a standard curve (0–200 µM) [36].

CYP2E1 activity assay

para-Nitrophenol (0–100 mM) was incubated in triplicate with sample microsomes (0.2 mg of protein final

concentration) ± 1 mM NADPH in 67 mM phosphate buffer, pH 7.4, containing 25 µM $MgCl_2$ and 1mM ascorbic acid in a final volume of 200 µL and the change in absorption was recorded at 420 nm for 5 minutes at 37 °C. The formation of *para*-nitrocatechol was determined from a standard curve (0–100 µM) [37].

GST activity assays

The substrates 1-chloro–2,4-dinitrobenzene (CDNB), 3-4-dichloronitrobenzene (DCNB) and 4-nitro-2-benzylchloride (NBC) were used to measure the activity of general GST, the GST-mu class and the GST-theta class, respectively. Substrate (0.02–1 mM) was added to 1mM glutathione and cytosolic protein (0.02 mg) in 100 mM potassium phosphate buffer, pH 6.5 to a final volume of 1 mL. The reaction was followed spectrophotometrically at 340, 345 or 310 nm for CDNB, DCNB and NBC with extinction coefficients of 9.6 $mM^{-1} \cdot cm^{-1}$, 8.5 $mM^{-1} \cdot cm^{-1}$ and 1.9 $mM^{-1} \cdot cm^{-1}$ respectively [38, 39].

NQO activity assay

NQO activity was assessed in triplicate by adding 4 mM DCPIP (10 µL) to 50 mM Tris-HCl buffer, pH 7.5, containing 0.1 % BSA solution, 0.5 mM NADH, cytosolic protein (0.2 $mg.mL^{-1}$) ± 100 µL of the inhibitor dicoumarol. The final incubation volume was 1 mL. The loss of absorbance at 600 nm was monitored. NQO activity was determined as the activity in the absence of dicoumarol minus the activity in the presence of dicoumarol [40]. A standard curve of 0–80 nM of DCPIP was used.

UGT activity assay

The microsomal activity of UGT was measured by incubating 0.1 mM 4-methyl umbelliferone with 0.5 mg of sample microsomes and 0.1M tris-HCl, pH 7.4 with the addition of 20 mM UDPGA in 0.05 % BSA tris-HCl pH 7.4, 5 mM MgCl2. The decay of 4-methyl umbelliferone fluorescence was measured [41].

β-Glucuronidase activity assay

The activity of β-glucuronidase was determined by a modification of the UGT assay described previously [42]. 4-Methyl umbelliferone glucuronide (0.1 mM) was incubated with 0.5 mg of sample microsomes and 0.1 M tris-HCl, pH 7.4. The increase in fluorescence due to the formation of the 4-methyl umbelliferone was measured at excitation 355 nm and emission 460 nm and determined from a standard curve (0–100nM) as described above.

Analysis of data

The parameters Vmax and Km for each sample were calculated using the Grafit® programme (Erithacus, UK). The data shown in the text and the figures are mean ± s. e. m. Statistical analysis was by Student's non-paired *t*-test, taking $P < 0.05$ as significant.

Results.

Effects of IQ exposure on Glutathione-S-transferase activity and expression

A significant ($p < 0.01$) increase of 101.7 ± 56.0 $nmol \cdot mg^{-1} \cdot min^{-1}$ from 166.3 ± 13.5 $nmol \cdot mg^{-1}$ $min^{-1} \cdot$ in the Vmax occurred in the IQ-exposed group after chronic exposure. No significant change was detected for GST-M (control value of 15.9 ± 2.7 $nmol.mg^{-1} \cdot min^{-1}$) or GST-T (control value of 61.4 ± 5.6 $nmol.mg^{-1} \cdot min^{-1}$) activity. The increase in total GST activity matched the trend shown by the expression data of the alpha class (Fig. 1). The expression of GST-A1 and GST-A2 increased significantly ($p < 0.01$) to 245 ± 67 % and 256.7 ± 97 % of control values (Fig. 2). In contrast, there was a decrease from 191.5 ± 3.7 $nmol \cdot mg^{-1} \cdot min^{-1}$ to 178.2 ± 1.1 $nmol \cdot mg^{-1} \cdot min^{-1}$ ($p < 0.05$) in total GST activity after acute exposure to IQ. With no change in the activity of GST-M or GST-T, it is assumed that the decrease in activity is due to the decreased alpha class expression (Fig. 1). Acute exposure significantly ($p < 0.01$) decreased the expression of GST-A1 by 81.3 ± 10 % of control and a non-significant decrease of 38 % was seen for the expression of GST-A2 (Fig. 2).

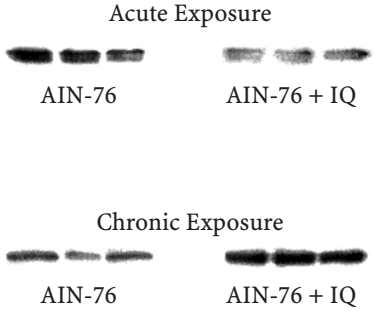

Fig. 1 Western blot showing the effects of chronic and acute exposure to IQ on the expression of glutathione *S*-transferases, GST-A1, in male Fischer 344 rats (n=3 per group).

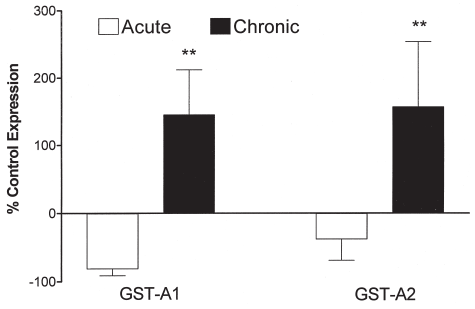

Fig. 2 Effects of acute high dose and chronic low dose exposure to IQ on the expression of glutathione *S*-transferase (GST-A1 and GST-A2), as determined by area/density relationships of Western blots. Values shown are mean ± s. e. m. (n = 4 for acute study; n = 15 for chronic study). ** P < 0.01 compared with relevant control (-IQ).

Effect of IQ exposure on CYP activity and expression

Chronic exposure resulted in non-significant decreases in CYP1A1 (from 11.4 ± 2.4 pmol · mg^{-1} · min^{-1} to 5.9 ± 0.7 pmol · mg^{-1} · min^{-1}) and CYP1A2 (a decrease of 5.1 ± 0.2 pmol · mg^{-1} · min^{-1} from 10.2 ± 2.5 pmol · mg^{-1} · min^{-1}) activity, with no significant change in the expression of CYP1A1/2 (121 ± 17.4 % of control). Acute exposure to IQ resulted in a significant ($p < 0.001$) increase in activity of CYP1A1 from 19.5 ± 2.0 to 52.9 ± 1.2 pmol · mg^{-1} · min^{-1} and an increase ($p < 0.01$) in CYP1A2 from 19.2 ± 2.0 to 58.5 ± 4.6 pmol · mg^{-1} · min^{-1}. The increase in activity was reflected by a significant ($p < 0.001$) increase in the expression of CYP1A1/2, to 224 ± 4 % of control. No significant changes were observed in either expression of CYP3A2 (102.7 ± 3 % of control for acute and 72.8 ± 7 % of control for chronic) or CYP2E1 activity (control levels were 6.8 ± 0.3 nmol · mg^{-1} · min^{-1} for acute and 9.6 ± 1.6 nmol · mg^{-1} · min^{-1} for chronic).

Effect of IQ exposure on NQO, UGT and β-glucuronidase activity

Following chronic exposure to IQ, all animals had a large and significant ($p < 0.001$) increase in NQO activity from 166.9 ± 35.7 nmol · min^{-1} · mg^{-1} to 1321 ± 256.3 nmol · min^{-1} · mg^{-1}, despite variability (274 %–1400 %). In contrast, after acute exposure to IQ there was a non-significant decrease in the activity of NQO (200.6 ± 66.4 nmol · min^{-1} · mg–1 to 156.0 ± 36.2 nmol · min^{-1} · mg^{-1}). After both acute and chronic exposure to IQ, UGT activity increased significantly (Fig. 3). An increase of 27 ± 5 nmol.min^{-1} · mg^{-1} ($p < 0.001$) from 18 ± 5 nmol · min^{-1} · mg^{-1} occurred after chronic exposure, whilst a smaller increase of 12 ± 4 nmol · min^{-1} · mg^{-1} ($p < 0.01$) from 43 ± 5 nmol · min^{-1} · mg^{-1} occurred after acute exposure to IQ.

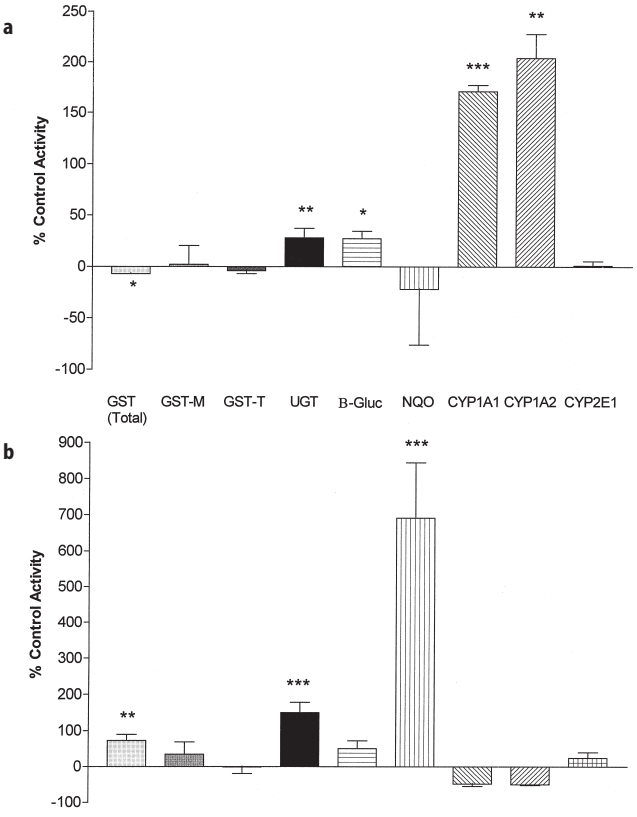

Fig. 3 Effect of acute (**a**) and chronic (**b**) exposure of male Fischer 344 rats to IQ on glutathione *S*-transferase (GST), UDP-glucuronosyl transferase (UGT), β-glucuronidase, NAD(P)H: Quinone oxidoreductase (NQO), cytochrome P450 (CYP) 1A1/2 and CYP2E1 activity. Values shown are mean ± s. e. m. (n = 4 for acute study; n = 15 for chronic study). * P < 0.05; ** P < 0.01; *** P < 0.001 compared with control (-IQ).

β-glucuronidase activity also increased after both chronic (from 4.0 ± 0.7 nmol · min^{-1} · mg^{-1} to 6.0 ± 0.9 nmol · min^{-1} · mg^{-1}) and acute (from 8.1 ± 0.2 nmol · min^{-1} · mg^{-1} to 10.3 ± 0.6 nmol · min^{-1} · mg^{-1}, $p < 0.05$) exposure.

Discussion

From previous studies [28], we had anticipated that a primary effect of chronic IQ exposure would be upregulation of CYP1A and possibly other CYP isozymes. However, no significant changes were observed for CYP1A, CYP3A2 or CYP2E1. CYP3A2 and CYP2E1 are regulated by very different mechanisms compared to the other enzymes investigated [43, 44]. Instead of effects on CYP, chronic exposure to the dietary carcinogen, IQ, caused significant effects on several phase II enzymes, increasing total glutathione transferase (GST) enzyme activity and expression of GST-A1/2, the major hepatic forms of the enzyme. UGT, β-glucuronidase and NQO activities were also increased. No significant changes were ob-

served for GST-M and GST-T, possibly due to the different mechanisms involved in the regulation of the various isozymes of GST [8].

Taken together, the enzymatic changes seen after chronic IQ exposure are not indicative of an XRE response, but instead of the electrophile counter attack response, mediated through the antioxidant response element (ARE) [45]. The ARE controls the basal expression of the detoxification enzymes GST-A, NQO [45] and UGT [46]. These results were not anticipated since such effects are associated with exposure to oxidants, and IQ is not necessarily considered as such. However, although no GSH conjugates have been detected for IQ, it is possible that *N*-hydroxylated metabolites do form semimercaptal conjugates that spontaneously rearrange to sulphinamides which decompose to yield the parent amine [47]. Such redox cycling may deplete intracellular glutathione, which may in turn promote activation of the ARE [48]. Such an event would appear biologically plausible, since a chronic exposure regime would permit an adaptive response that at least partially enables the organism to cope with subsequent doses of the potential dietary carcinogen.

These data, however, contrast with the limited information on IQ-induced changes in XME that are currently described in the literature [28, 29, 49]. Such observations encouraged us to study further the effects of acute exposure in the same animal strain as our chronic studies. Intriguing differences were apparent in the comparative effects of chronic and acute exposure to the dietary carcinogen IQ on the expression and activity of xenobiotic metabolising enzymes. Following acute exposure, the activity and expression of CYP1A2 and CYP1A1, responsible for the bioactivation of IQ, increased confirming previous studies [29, 49]. The level of UGT and β-glucuronidase activity also increased. The effect on glutathione transferase activity was opposite to that after chronic exposure. Total GST activity decreased significantly and this was reflected in the expression data, especially for GST-A1, where a 81% decrease occurred.

The early work into the effects of IQ exposure on XME had led to the proposal that IQ is a ligand for the A*h*-receptor and a subsequent agonist for the xenobiotic response element (XRE) [28]. In the present acute exposure study, we used a dose of 20 $mg.kg^{-1}$, at least 1,000,000 times the concentration at which dioxin (the classical A*h* agonist) shows effects at the A*h* receptor [50]. Even for a weak agonist such as IQ [51], this high dose would be expected to bind to the A*h* receptor and elicit a response through the XRE. However, both our current results as well as recent literature on the mechanisms of IQ induction of CYP1A1/2 support instead a non-A*h* receptor-mediated mechanism for IQ modulation of XME. CYP1A1/2 activity and expression increased after various acute doses of IQ in A*h*-/- mice, suggesting the increase in CYP1A1 and 1A2 was not due to the A*h* receptor [29]. The differential changes in the activities of CYP1A1/2, UGT, GST-A2 and NQO in the present study confirm that it is not a simple XRE-mediated event, but may involve the glucocorticoid receptor and glucocorticoid response element [6].

The glucocorticoid response element (GRE) is present in the promoter region of all six of the enzymes on the *Ah* battery and it has been shown previously to be affected by carcinogenic polycyclic aromatic hydrocarbons (PAHs; [6]). At high doses, these act as A*h* receptor agonists in combination with the synthetic glucocorticoid dexamethasone. Such combinations resulted in differential enzyme regulation: both CYP1A and UGT increased, whilst GST-A2, NQO1 and aldehyde dehydrogenase 3 decreased. The changes did not occur when glucocorticoid was applied alone and they were blocked by the glucocorticoid receptor antagonist RU38486. Thus it was proposed that the glucocorticoid interacted with the glucocorticoid receptor to produce and attract elements that interfere with the A*h*-receptor mediated regulation of the enzymes [6].

The high dose of IQ used in the present acute study produced a similar spectrum of XME changes to those previously seen with PAHs and glucocorticoids [6]. We suggest that IQ administered thus may be directly toxic and so disturb the homeostatic mechanisms of the rat to cause a cascade of effects including release of glucocorticoids. The glucocorticoids may then activate the GRE, which in turn may modulate the A*h*-receptor response caused by the high dose of IQ. However, such acute exposure conditions do not reflect the normal dietary situation.

It must be borne in mind that other factors could influence enzyme expression and activity in acute versus chronically exposed animals. IQ is a carcinogen, and chronic exposure resulted in metastatic tumours in the rat. Cytokines such as interleukin–6, released in the cancer process, have been shown to decrease the expression of GST in primary hepatocyte culture [52]. Hayes and co-workers observed increases in GST-A and GST-M in rats with hepatic pre-neoplasia, similar to the changes that occurred with chemoprotection [53]. However, in the present study not all the chronically exposed animals developed cancer: three of the exposure group failed to have any pathological signs of the disease (Table 1) and analysis of the expression and activity data showed there was no difference between them and the rest of the exposure group. Indeed, overall, there was no relationship between tumour incidence and any of the changes in activity or expression observed in the chronically exposed rats.

Care must be taken to avoid the production of misleading data that may result from confounding factors contained in the normal rat diet. The defined diet such as the AIN–76A used in this study eliminates phytoe-

Tab. 1 Total glutathione *S*-transferase (GST), glucuronosyl transferase (UGT) and NADPH: Quinone oxidoreductase (NQO) activities in the liver and pathological lesions and tumours after 52-week dietary IQ exposure in male Fischer 344 rats.

	Enzyme activity ($nmol \cdot mg^{-1} \cdot min^{-1}$)			Pathology			
				Lesions		Tumours	
Animal	GST	UGT	NQO	Hyperplastic	Degenerative	Benign	Malignant
1	203.1	N/A	1114.8	0	0	0	1
2	223.3	7.4	1378.3	0	2	2	0
3	241.22	5.7	3387.3	0	1	1	2
4	278.8	9.1	1475.5	0	0	0	1
5	254.0	4.5	816.1	0	0	0	1
6	262.9	3.9	1579.5	0	0	0	0
7	385.5	3.0	452.8	0	0	1	1
8	293.7	3.4	526.5	0	0	0	2
9	167.6	3.2	800.6	0	2	0	0
10	198.9	3.1	653.6	0	0	0	1
11	317.5	3.9	1444.5	0	0	1	2
12	331.0	3.6	863.5	2	1	3	1
13	306.5	2.9	469.9	0	0	0	3
14	244.2	1.9	895.3	0	0	0	2
15	311.7	7.4	3958.0	0	0	0	2

strogen and vitamin effects that are known to effect CYP levels in the rat [54]. Age effects are also important: CYP and other enzyme levels are known to alter with time [55–57]. Indeed we observed the level of control enzymes alter in all enzyme measured between the acute and the chronic study. All enzymes decreased their expression/activity after 52 weeks, except for CYP2E1, where a 40 % rise from acute control level was observed.

Whilst the levels of IQ in the present study (0.03 %) are several orders of magnitude higher than the typical human exposure [11], the study demonstrates how important it is to regulate the dose, the duration and the diet used in the study of both carcinogens and chemopreventative agents. Acute, high dose carcinogen exposure elicits a response that appears to work through the xenobiotic response element attenuated by the glucocorticoid response element, which may reflect a direct toxic effect to the rat. The danger is that potentially chemopreventive dietary components that appear to be active during such acute exposures to carcinogens may work through a mechanism not applicable to the low dose, chronic situation in humans, giving rise to potential artefacts in both tumour and short-term endpoints such as DNA adducts. Furthermore, it should also be noted that any chemoprevention study must be interpreted with caution and that investigations into the effect of IQ at lower doses must be undertaken. Looking at the present study, the induction of the detoxification enzymes through the activation of the ARE would suggest that IQ is a model monofunctional inducer, and hence ideal chemopreventative agent [58].

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