Metabolic activation of carcinogens and expression of various cytochromes P450 in human prostate tissue

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Epidemiological evidence suggests a link between meat consumption and prostate cancer. In this study, benign prostatic hyperplasia tissues, obtained by transurethral resection or radical retropubic prostatectomy from UK-resident individuals (n = 18), were examined for CYP1 expression and for their ability, in short-term organ culture, to metabolically activate carcinogens found in cooked meat. Semi-quantitative RT–PCR analysis of CYP1 expression detected CYP1A2 mRNA transcripts in the prostates of four individuals, as well as mRNA transcripts from CYP1A1 and CYP1B1. The compounds tested for metabolic activation were 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhIP; 500 µM, n = 9) and its metabolite N-hydroxy PhIP (20 µM, n = 8), 2-amino-3-methylimidazo[4,5-f]quinoline (IQ; 500 µM, n = 6) and benzo[a]pyrene (B[a]P; 50 µM, n = 5). After incubation (PFMR medium, 22 h, 37°C), DNA was isolated from tissue fragments and DNA adducts were detected and quantified by 32P-postlabelling analysis. DNA adduct formation was detected in all samples incubated with PhIP (mean, adducts per 10⁸ nucleotides), N-hydroxy-PhIP (2736/10⁸) or B[a]P (1/10⁸). IQ–DNA adducts were detected in 5/6 tissues (mean, 1/10⁸). The CYP1 inhibitor α-naphthoflavone (10 µM) reduced B[a]P–DNA adduct formation in tissues from two individuals by 96 and 64%, respectively. This pilot study shows that human prostate tissue can metabolically activate ‘cooked meat’ carcinogens, a process that could contribute to prostate cancer development.

Introduction

The World Health Organization lists meat and animal fat intake as known risk factors for the development of prostate cancer (1). A high intake of well cooked meat has also recently been linked with increased risk of breast cancer in North American women (2) and with increased risk of pancreatic cancer (3,4). Epidemiological evidence linking high meat consumption with an elevated risk of prostate cancer has led us to investigate the metabolic activation of mutagens present in cooked meat. These mutagens include the heterocyclic amines (5), of which 2-amino-3-methylimidazo[4,5-f]quinoline (IQ) and 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhIP) are widely studied examples. These compounds are formed when protein-rich foods (particularly meat and fish) are cooked at high temperatures (6). Other mutagens present in cooked meat include polycyclic aromatic hydrocarbons (PAHs), e.g. benzo[a]pyrene (B[a]P), that are formed by the pyrolysis of fat (6).

Heterocyclic amines are metabolically activated to DNA-binding products via the exocyclic amino group. This can proceed via a two-step pathway involving cytochrome P450 (CYP)-catalysed N-hydroxylation (7,8) followed by an O-esterification step catalysed by N-acetyltransferase(s) (NATs) (9–11) and/or sulfotransferase(s) (SULTs) (11–13). The ultimate reactive intermediates of heterocyclic amines are probably the nitrone ions (14–16), which are formed after dissociation of the N-acetoxy or N-sulfoxy leaving groups. B[a]P can be metabolically activated via a combination of CYP enzymes and epoxide hydrolase, and the ultimate reactive metabolite is a dihydrodiol epoxide (BPDE) (17). Heterocyclic amines and B[a]P can also be metabolically activated by peroxides (8,16,18).

Nearly all malignant tumours of the prostate originate in the epithelium, mainly in the peripheral zone of the gland (19). There is an association between age and increasing prostate size. The increase in prostate volume averages 2% per annum in men between the ages of 55 and 74 years, indicating a doubling time of 35 years (20). Interestingly, benign prostatic hyperplasia (BPH) occurs in the transition zone and not in the peripheral zone. In the normal gland the transition zone makes up only 5% of the total volume of the prostate, but may account for 90% of a benignly enlarged prostate. In most cases, increases in prostatic volume are due to hyperplasia (20). The incidence of prostate cancer is highest in those countries with a high risk of developing BPH (20).

In the present study, we have investigated the expression of CYP1A1, CYP1A2 and CYP1B1 in BPH tissues. We have also examined the ability of these tissues to metabolically activate IQ, PhIP and its N-hydroxy derivative, and B[a]P. A preliminary report of our findings has appeared in abstract form (21).

Materials and methods

Chemicals

PhIP, N-hydroxy-PhIP and IQ were obtained from Toronto Research Chemicals, Canada. Unless otherwise stated, other chemicals were obtained from the Sigma Chemical Company, Poole, UK. External PhIP–DNA and BPDE–DNA standards previously used in an interlaboratory trial (22) were employed for identification of adducts in experimental samples and to calculate the efficiency of the 32P-postlabelling techniques.

Prostate tissues

Adult human prostate tissue was obtained either from transurethral resection samples (from patients undergoing surgery for outflow obstruction due to BPH) or from radical prostatectomy samples in patients undergoing treatment for localized prostate cancer (macroscopically benign tissue from the transition zone was excised immediately after prostate removal). Samples were labelled human prostate (HP)-HP18. Resection in all cases was performed as part of normal clinical practice. Transurethral prostatectomy was carried out using an
Olympus 27 Ch resectoscope with continuous balanced irrigation of the bladder using aqueous glycine (1.5% v/v) and resection was by pure cut diathermy using a Valleylab Force 2 generator at 165 W pure cutting current. Tissues were immediately placed in cold HEPES-buffered saline (HBS) buffer in sterile vessels (23). A sliver from each sample was removed to confirm the histology by conventional light microscopy. Within 4 h, tissues were either frozen for subsequent gene expression analysis and stored at −70°C or transferred from the collection vessels, rinsed in fresh cold HBS and placed in sterile vials containing ‘growth’ medium (medium PFMR-4A) as described previously (23).

Analysis of gene expression by RT–PCR

Tissues from four selected individuals (HP3 and HP6, HP7 and HP8) were investigated for expression of CYP1A1, CYP1A2 and CYP1B1 genes, using β-actin gene expression for comparison, according to a previously published method (8). Positive controls for CYP1A1 and CYP1B1 were derived from benz[a]anthracene-treated human mammary epithelial cells (8), and the positive control for the CYP1A2 gene was derived from human liver [a gift from Dr Alastair Strain, University of Birmingham, UK (8)]. Levels of the CYP1 mRNA transcripts of interest were expressed as a ratio of the level of β-actin mRNA transcripts as standard units, from a single sample of cDNA. Negative (no cDNA) and positive controls were used for each amplification reaction. The sizes of the PCR products were: CYP1A1, 146 bp; CYP1A2, 243 bp; CYP1B1, 489 bp; and β-actin, 661 bp.

Incubation of tissues with carcinogens

Prostatic tissues (50–400 mg) were chopped into small pieces (~1–6 mm³), and five to 10 were suspended in PFMR medium. Tissue fragments were incubated (22 h at 37°C in air) in medium on a rotary shaker with PhIP (500 µM), IQ (500 µM), N-hydroxy-PhIP (20 µM) or B[a]P (50 µM), which were added as solutions in dimethyl sulfoxide (final concentration 1% v/v). Dimethyl sulfoxide (1% v/v) was added to control incubations. Co-incubations of B[a]P with the CYP1A1/CYP1B1 inhibitor α-naphthoflavone (10 µM) were carried out with tissues HP3 and HP6.

Isolation of DNA

Tissue incubation mixtures were centrifuged (500 g for 5 min) and the tissue pellet was homogenized in buffer (0.5 ml of 10 mM EDTA, 50 mM Tris, pH 8) using an Ultra-Turrax tissue homogenizer with ‘ION’ dispersing tool (two 50 s bursts at 3000 r.p.m.), and then subjected to RNase A and T1 and proteinase K digestion (2 mg/ml for 2 h at 37°C) before DNA isolation (24).

32P-postlabelling of DNA adducts and HPLC analysis

DNA adduct analysis was carried out on 4 µg DNA for each sample. The 32P-postlabelling method employed for measurement of heterocyclic amine–DNA adducts was using the ATP-deficient conditions described by Hall et al. (25). Further enzymatic digestion of the 32P-postlabelled mixture of mono-, di- and oligonucleotide 3’,5’-bisphosphates was carried out to produce 5’-mononucleotide adducts as described by Ochiai (26). The nuclease P1 method was employed for analysis of B[a]P-DNA adducts (27). 32P-postlabelling analyses of prostate DNA were carried out in duplicate or in triplicate. Experimental values obtained from multiple determinations of the same DNA sample showed variations of up to 40 and 20% for heterocyclic amine–DNA and B[a]P–DNA adducts, respectively.

32P-labelled DNA digests from heterocyclic amine-treated tissues were chromatographed on polyethyleneimine (PEI)-cellulose TLC plates using the following solvents as described previously (8,28): D1, 1.0 M sodium dihydrogen orthophosphate, pH 6.0; D2, 2.3 M lithium formate, 5.5 M urea, pH 3.5; D3, 0.8 M lithium chloride, 0.5 M Tris–HCl, 8.5 M urea, pH 8.0. Solvents for separation of labelled DNA digests from B[a]P-treated tissues were the same as for heterocyclic amine-treated tissues, except for D2 (3.5 M lithium formate, 8.5 M urea, pH 3.5); DNA adducts were detected and quantified with a two-dimensional scanner (Instant Image; Canberra Packard, Pangbourne, UK). The level of DNA adducts was determined by relating levels of radioactivity in adducts to the specific activity of the [γ-32P]ATP and expressed as relative adduct labelling (RAL adducts per 10⁶ nucleotides). The PhIP–DNA standard (350 adducts/10⁶ nucleotides measured by 3H incorporation (22)) was used to calculate the efficiency of PhIP–DNA adduct labelling.

For HPLC analysis, PhIP–DNA adduct spots containing mono- and oligonucleotide 5’-phosphates, cut out from the PEI-cellulose plates after two-dimensional chromatography, were eluted with pyridinium formate (0.5 ml, 4 M, pH 4.5). After centrifugation at 10 000 g for 10 min to pellet contaminating solids, the supernatant was evaporated and the residue was dissolved in water (200 µl). HPLC analysis was carried out using a Waters

<table>
<thead>
<tr>
<th>Gene expressed</th>
<th>CYPβ-actin ratio (standard units)</th>
<th>HP3</th>
<th>HP6</th>
<th>HP7</th>
<th>HP8</th>
<th>Mean ± SD (n = 4)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CYP1A1</td>
<td>0.23</td>
<td>0.04</td>
<td>0.17</td>
<td>0.08</td>
<td>0.13±0.09</td>
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<tr>
<td>CYP1A2</td>
<td>0.25</td>
<td>0.38</td>
<td>0.36</td>
<td>0.28</td>
<td>0.32±0.06</td>
<td></td>
</tr>
<tr>
<td>CYP1B1</td>
<td>0.70</td>
<td>0.37</td>
<td>0.62</td>
<td>0.36</td>
<td>0.51±0.17</td>
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</table>

β-Actin expression varied by <7% between the four samples.

Results

CYP gene expression in BPH tissues

CYP1A1, CYP1B1 and β-actin mRNA transcripts were detectable in tissues from four out of four individuals (Figure 1; Table I). CYP1A1 and CYP1B1 were also expressed in benz[a]anthracene-treated human mammary epithelial cells (not shown). Expression of the β-actin gene varied only 7% in the four samples. There was a 6-fold variation between levels of CYP1A1 mRNA expression between HP3 and HP6 (mean value 0.13 standard units; Table I). CYP1A2 mRNA transcripts were also expressed in each of the four samples (Figure 1; Table I) and human liver cDNA (not shown). The mean level of expression of CYP1A2 mRNA (0.32 standard units) was 150% higher than that of mean CYP1A1 levels (Table I). CYP1A2 expression levels varied only 1.5-fold among these four samples (Figure 1; Table I). The mean level of CYP1B1 expression (at 0.51 standard units, Table I) was higher than CYP1A1 or CYP1A2 but varied only 2-fold (Figure 1) between the highest (0.70, HP3) and the lowest values (0.36, HP8). The highest levels of CYP1A1 mRNA transcripts were observed in individuals with the highest levels of CYP1B1 (HP3 and HP7): mean CYP1A1 and CYP1B1 levels in these individuals were 3.3- and 1.8-fold higher than mean levels in HP6 and HP8. The number (n = 4) of tissue samples analysed.
expressions in human prostate

Fig. 2. $^{32}$P-postlabelling analysis of heterocyclic amine–DNA and B[a]P–DNA adducts in human prostatic tissues. Heterocyclic amine–DNA adduct patterns are shown after exposure to the following compounds (using tissue from the following individuals) at 37°C for 22 h: (a) vehicle only (HP1); (b) PhIP (500 µM, HP12); (c) N-hydroxy-PhIP (20 µM, HP12); (d) IQ (500 µM, HP1); and postlabelling using the ATP-deficient method. The PhIP–DNA adducts shown in (b) and (c) were further enzymatically digested (26), giving rise to adduct spot 1 shown in (e) (88% of the total radioactivity) and (f) (94% of the total radioactivity), respectively. Using the nuclease P1 postlabelling technique: (g) DMSO vehicle only (HP2); (h) B[a]P–DNA standard (adduct spot 2, see text); (i) after incubation with B[a]P (adduct spot 2, 50 µM, HP2). The origin of the radioactive spot in (g), and to the right of adduct spot 2 in (i) is unknown (see results).

in the current study is, however, too low to make definitive conclusions about co-ordinate regulation of CYP1 genes in this tissue.

Metabolic activation of heterocyclic amines by BPH tissues in organ culture

No adduct spots were observed in digests of the DNA of untreated control tissues (Figure 2a). After incubation with PhIP at 500 µM, PhIP–DNA adducts (as 3',5'-bisphosphates) were formed in BPH tissues from all of nine individuals (Table II; Figure 2b). PhIP–DNA adduct levels varied 40-fold (range 0.18–7.25 adducts per $10^8$ nucleotides) (Table II; Figure 3). For tissues incubated with N-hydroxy PhIP at 20 µM concentration (i.e. 25 times lower concentration than for the parent compound), PhIP–DNA adducts were detectable in eight out of eight tissues (Table II; Figure 2c); there was a 145-fold range in adduct levels (68–9889 per $10^8$ nucleotides; Figure 3), and the mean adduct level (2736 per $10^8$ nucleotides) was 900-fold higher compared with tissues incubated with the parent compound PhIP (3 adducts per $10^8$ nucleotides). Our results suggest that the enzymes catalysing N-hydroxylation enzymes are rate limiting in PhIP–DNA adduct formation in these prostatic tissues. The greater variation between individuals for metabolic activation of N-hydroxy-PhIP compared with PhIP, suggests larger inter-individual differences in the activity of O-esterification enzymes, for example NATs and SULTs, than for the enzymes that catalyse PhIP N-hydroxylation. IQ–DNA adducts were detectable (Table I; Figure 2d) in five out of six separate tissue incubations. IQ–DNA adduct formation ranged from below the limit of detection (less than 0.1 adduct per $10^8$ nucleotides) to 2.21 adducts per $10^8$ nucleotides, with a mean of 1 adduct per $10^8$ nucleotides (Figure 3). The patterns of heterocyclic amine–DNA adduct spots (Figure 2b–d) observed were similar, in each case, to those observed previously following incubations with human mammary epithelial cells (28). These patterns show a mixture of mono-, di- and oligonucleotide 3',5'-bisphosphates generated by incomplete digestion of the DNA by micrococcal nuclease and spleen phosphodiesterase before labelling.

Further enzymatic digestion (26) of PhIP–DNA adducts formed after incubation of tissues with PhIP (500 µM) or N-hydroxy-PhIP (20 µM) to generate mononucleotide 5'-phosphates gave rise to a major adduct spot 1 (Figure 2e) which constituted 88 and 94% (Figure 2f) of the total radioactivity, respectively. HPLC analysis of adduct spot 1 derived from N-hydroxy PhIP and the PhIP–DNA standard showed that these radioactive peaks co-eluted at 44 min (not shown). This strongly indicates the identity of the adduct formed in prostatic tissues as N-(deoxyguanosin-8-yl)-2-amino-1-methyl-
Table II. DNA adduct formation in human prostate tissue after incubation (22 h) with heterocyclic amines or B[a]P

<table>
<thead>
<tr>
<th>Prostate tissue identifier</th>
<th>Mean adducts per 10^8 nucleotides</th>
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<tr>
<td></td>
<td>PhIP (500 µM)</td>
</tr>
<tr>
<td>HP1 R</td>
<td>–</td>
</tr>
<tr>
<td>HP2 R</td>
<td>–</td>
</tr>
<tr>
<td>HP3 R</td>
<td>–</td>
</tr>
<tr>
<td>HP4 R</td>
<td>–</td>
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<tr>
<td>HP5 R</td>
<td>–</td>
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<tr>
<td>HP6 T</td>
<td>–</td>
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<tr>
<td>HP7 T</td>
<td>–</td>
</tr>
<tr>
<td>HP8 T</td>
<td>–</td>
</tr>
<tr>
<td>HP9 T</td>
<td>–</td>
</tr>
<tr>
<td>HP10 T</td>
<td>2.50</td>
</tr>
<tr>
<td>HP11 T</td>
<td>3.43</td>
</tr>
<tr>
<td>HP12 T</td>
<td>3.95</td>
</tr>
<tr>
<td>HP13 T</td>
<td>7.25</td>
</tr>
<tr>
<td>HP14 T</td>
<td>0.18</td>
</tr>
<tr>
<td>HP15 T</td>
<td>0.32</td>
</tr>
<tr>
<td>HP16 T</td>
<td>3.64</td>
</tr>
<tr>
<td>HP17 T</td>
<td>1.23</td>
</tr>
<tr>
<td>HP18 T</td>
<td>4.23</td>
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</tbody>
</table>

α-N, α-naphthoflavone; UD, undetectable; –, not determined.
^aTissues were obtained by radical retropubic prostatectomy (R) or transurethral prostatectomy (T).
^bValues for DNA adduct formation are means of single incubations, and adduct levels were analysed in duplicate, except those below. Values are not adjusted for labelling efficiency.
^cAdducts from individuals HP2 and HP3 were analysed in triplicate.

Metabolic activation of benzo[a]pyrene by prostatic tissues in organ culture

Analysis of DNA from a control (DMSO vehicle only) incubation, ^32^P-postlabelled using the nuclease P1 digestion method, is shown in Figure 2g (HP2). A faint background radioactive spot was detected near the bottom of the TLC plate that did not interfere in the subsequent analysis of DNA from B[a]P-treated tissues. The BPDE–DNA adduct standard (22) produced a single strong adduct spot (labelled 2 in Figure 2h) well-resolved from that in Figure 2g. DNA from prostate tissues incubated with B[a]P (50 µM) also gave rise to a strong adduct spot chromatographically identical to that obtained with the BPDE–DNA standard (labelled 2 in Figure 2i). This strongly indicates that adduct formation by B[a]P in prostate tissue is via formation of BPDE. B[a]P–DNA adducts were formed in tissues from all five individuals (Table II). The efficiency of labelling for the BPDE–DNA adduct is ~93% (22). The B[a]P–DNA adduct levels calculated are therefore likely to be close to the actual levels. B[a]P–DNA adduct formation varied 8-fold (Table II), ranging from 0.26–2.09 adducts per 10^8 nucleotides (mean 1.34; Figure 3). Addition of the CYP1 inhibitor α-naphthoflavone (29) (10 mM) to the incubation mixture reduced B[a]P–DNA adduct formation by 96% (0.08 ± 0.03 adducts/10^8 nucleotides in the presence of α-naphthoflavone compared with 1.92 ± 0.19 in its absence; P < 0.001) and 64% (0.69/10^8 compared with 1.91/10^8) in prostatic tissues HP3 and HP6, respectively (Table II, mean inhibition, 80%).

Influence of prostatectomy method on adduct formation

On the basis of a limited number of experiments, our results show that there are no differences in the abilities of tissues obtained from open radical prostatectomy and those obtained by transurethral prostatectomy using hot wire diathermy to metabolically activate IQ and B[a]P. Both methods of prostatectomy therefore seem to be suitable for providing tissues for carcinogen metabolism studies.

Discussion

Gene expression analysis of cDNAs from four individuals detected the expression of CYP1A1, CYP1A2 and CYP1B1 genes in BPH tissues. The expression of CYP1A2 was originally thought to be confined to the liver (30) and is barely detectable in cultured human mammary epithelial cells derived from reduction mammaryplasty tissues (8), but has been detected in chronic pancreatitis tissues (31,32). Expression of CYP1A2 may contribute to elevated carcinogen activation in BPH prostate tissue compared with other extra-hepatic organs where CYP1A2 is not expressed (7). CYP1A2 has 30 times (and CYP1A1 80 times) the catalytic efficiency (V_max/K_m) of CYP1B1 in metabolizing PhIP to its promutagenic metabolite N-hydroxy-PhIP (7). Levels of CYP1A2 and CYP1A1 are thus likely to have more influence than CYP1B1 on N-hydroxylated heterocyclic amine levels in BPH tissues. Close agreement has been found between CYP1A2 mRNA and protein levels in human liver (33,34). Immunohistochemical analysis of prostate tissue sections could confirm translation of CYP1A2 mRNA and would give an indication of CYP1A2 protein localization and expression levels. The CYP1A2 gene is polymorphic (35–37) but the functional consequences of this are unknown. CYP1A1 is also expressed and is inducible in mammary epithelial cells (8,38), and is likely to contribute significantly

6-phenylimidazo[4,5-b]pyridine. The level of PhIP–DNA adducts in the PhIP–DNA standard (22) was determined to be 10.78 ± 2.34 adducts per 10^8 nucleotides (range 7.86–12.32, n = 4 determinations). As the adduct level was estimated by ^3H incorporation to be 350 adducts per 10^8 nucleotides (22), the efficiency of labelling is calculated to be 3%. The levels of experimentally induced PhIP–DNA adduct formation in prostatic tissues in our study can therefore be estimated to be ~30-fold higher than the reported values (e.g. mean levels of 90 adducts per 10^8 nucleotides in tissues incubated with PhIP at 500 µM).
to the metabolic activation of heterocyclic and aromatic amines and PAHs in human mammary and prostate tissues. Further study is required to elucidate the contribution of various CYP enzymes to the metabolic activation of these carcinogens, including as yet unidentified CYP isoforms that may be expressed specifically in the prostate. It is therefore appropriate to point out that the CYP enzymes involved in the metabolic activation of these compounds in the prostate remain to be conclusively identified.

CYP1A1 is inducible in human mammary epithelial cells and in the rat prostate gland by the aryl hydrocarbon receptor ligand 3-methylcholanthrene (39). Larsen et al. (38) reported a 21-fold variation of CYP1A1 mRNA levels in human mammary epithelial cells in seven individuals, whereas CYP1B1 expression varied by only 2.5-fold. CYP1A1 and CYP1B1 genes are not co-ordinately expressed in chorion carcinoma cell lines (40), but it is difficult to establish from our results whether this is also the case in prostate tissues: although the highest levels of CYP1A1 and CYP1B1 expression are in the same individuals, there is much greater variation of CYP1A1 expression (6-fold) compared with CYP1B1 (2-fold).

PhIP is a prostate carcinogen in the F344 rat when administered orally at 400 p.p.m. for 52 weeks, and also causes atypical hyperplasias in both lobes (41). PhIP–DNA adducts are detectable in rat prostate tissues after ingestion, and this compound is suspected of being a prostate carcinogen in man (41). The major site of reaction in DNA of metabolically activated heterocyclic amines is the C8 atom of guanine (15,42). PhIP concentrations in the tissue incubation experiments are four to five orders of magnitude higher than plasma levels of PhIP after a fried hamburger meal (based on ingestion of 2 µg PhIP by a 70 kg male with a volume of distribution of 60/dm³). However, men eating cooked meats are chronically exposed to PhIP, other heterocyclic amines and PAHs over the period of a lifetime (~75 years), indicating an opportunity to accumulate DNA adduct-induced mutations at much lower levels of exposure. Demonstration of the presence of PhIP–DNA adducts in the prostate in vivo would strengthen the hypothesis that cooked meat consumption leads to pre-mutagenic lesions in this tissue, possibly leading to carcinogenesis; meat consumption increases colon cancer risk (43), and PhIP–DNA adducts have been detected in human colon tissue (44).

NATs and SULTs play a major role in the activation of heterocyclic amines in the breast (J.A.Williams, E.M.Stone, G.Fakis, N.Johnson, J.A.Cordell, W.Meini, H.Glatt, E.Sim and D.H.Phillips, manuscript in preparation). NAT1 and NAT2 mRNA transcripts are expressed in prostate epithelium (45). SULT2B1 (46) mRNA transcripts have also been detected in prostate tissues, but full characterization of all SULT genes is required, as there are wide variations in the catalytic efficiency of isoforms in pro-mutagen activation (13). Molecular epidemiology studies assessing the effect of functional NAT or SULT polymorphisms should provide clearer evidence of the effect of cooked meat consumption on prostate cancer risk.

PhIP–, IQ– and B[a]P–DNA adducts at similar levels are formed in BPH tissue. PhIP–DNA adducts were formed at levels three orders of magnitude higher (with large inter-individual variations) when tissues were incubated with N-hydroxy PhIP at 25-fold lower concentration (Figure 3). This strongly suggests that metabolic activation of PhIP occurs via N-hydroxylation in the prostate, as has previously been shown to be the case in human mammary epithelial cells (28), where mean levels of PhIP–DNA adducts after treatment of human mammary epithelial cells with N-hydroxy PhIP were 30–600 times higher than after treatment with PhIP. Wang et al. (45) reported PhIP–DNA adduct formation from N-hydroxy PhIP in prostate epithelial cells after incubation at 10 µM for 2 h (compared with 22 h at 20 µM in the current study), although the levels of adduct formation were an order of magnitude lower than those observed in our experiments.

A study of the metabolites of orally administered [¹⁴C]PhIP in plasma from colon cancer patients identified the parent compound as the major radioactive species in three out of five patients (47). Systemic circulation of PhIP in healthy individuals would present the parent compound for local metabolic activation to DNA-binding derivatives in the prostate. Our results in prostate have shown that N-hydroxy PhIP is a much better substrate for metabolic activation than the parent compound in prostate tissues. N-hydroxy PhIP was not detectable in human plasma after oral administration of the parent heterocyclic amine (47), and hepatic amine reductases have been shown to be able to reduce the N-hydroxylated derivatives of heterocyclic and aromatic amines back to their respective parent compounds (48). Also, after incubation of another heterocyclic amine, 2-amino-3,8-dimethylimidazopyrimidin-4,5-β-lactone (MeIQx), in human hepatocytes, only the N-hydroxy-MeIQx glucuronide was formed, and not the free N-hydroxy metabolite (49). Together with the data presented in this paper, these studies (47–49) represent accumulating weight of evidence to support the notion that metabolic activation occurs at the target site (i.e. the prostate).

Some epidemiological studies suggest a positive association between occupational exposures to PAHs and increased risk of prostate cancer (50–52). Non-occupational exposure to PAHs is mainly in the diet (53) and by inhalation of air-borne pollution and tobacco-smoke. In this study, the PAH B[a]P was metabolically activated in prostatic tissues. The inhibition of B[a]P adduct formation by α-naphthoflavone, an inhibitor of the CYP1 family (54), indicates that CYP1 enzymes are at least partially responsible for the metabolic activation of PAHs in human prostatic tissue.

In summary, our results show expression of CYP1A2, CYP1A1 and CYP1B1 in BPH tissues. We also report the metabolic activation of compounds suspected of being prostate carcinogens in these tissues. These results are consistent with epidemiological evidence associating high levels of cooked meat ingestion and increased risk of prostate cancer.

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