Up-regulation of the glutathione S-transferase system in human liver by polycyclic aromatic hydrocarbons; comparison with rat liver and lung

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The cytosolic glutathione S-transferases (GSTs) comprise a pivotal enzyme system protecting the cell from electrophilic compounds. It plays a major role in the detoxification of the primary and dihydrodiol epoxides of polycyclic aromatic hydrocarbons (PAHs), so that modulation of this enzyme system by PAHs will impact on their carcinogenic activity. The potential of six structurally diverse PAHs, namely benzo[a]pyrene (B[a]P), fluoranthene, benzo[b]fluoranthene (B[b]F), dibenz[a]pyrene, dibenzo[a,h]anthracene (D[a,h]A) and 1-methylphenanthrene, to modulate hepatic GST activity was investigated in human precision-cut slices and compared to rat slices, a species frequently used in long-term carcinogenicity studies; changes were monitored at the activity, using three different substrates, protein and mRNA levels. When activity was monitored using the α-class selective 7-chloro-4-nitrobenz-2-oxa-1,3-diazole, B[b]F was the only PAH that caused an increase in activity, which was accompanied by a rise in the Ya immunoreacting band. In rat slices, in addition to B[b]F, B[a]P and D[a,h]A also enhanced activity, being paralleled with increased levels of the Ya immunoreacting band. In the rat, all PAHs elevated mRNA levels. In both human and rat liver slices, only B[b]F enhanced activity when 1-chloro-2,4-dinitrobenzene (CDNB) served as substrate. To investigate tissue differences, similar studies were undertaken in precision-cut rat lung slices, incubated with PAHs under identical conditions, using CDNB, as this was the only substrate for which activity could be detected; none of the PAHs studied stimulated activity. It is concluded that some PAHs have the potential to induce GST activity in human liver tissue and that species and tissue differences exist in the induction of this enzyme system in the rat. However, the extent of induction of GST activity is very modest compared with the effect these compounds have on CYP1 expression, the family responsible for their bioactivation, and it is unlikely to compensate for the enhanced production of reactive intermediates.

Introduction

The majority of chemicals manifest their toxicity and carcinogenicity through metabolically generated reactive intermediates. Most frequently these are the products of oxidation, usually catalysed by the cytochromes P450 (1). Being electrophiles, these reactive intermediates can readily interact with vital cellular macromolecules leading to toxicity and carcinogenicity. Such reactive intermediates include epoxides, which have been implicated in the carcinogenicity of numerous chemicals, including polycyclic aromatic hydrocarbons (PAHs) (2), mycotoxins such as aflatoxin B1 (3), and halogenated aliphatic compounds such as vinyl chloride (4).

The principal protective mechanism of living organisms against epoxides is to deactivate them by metabolically converting them to inactive, readily excretable metabolites. One of the most important enzyme systems catalysing the detoxification of epoxides are the cytosolic glutathione S-transferases (GSTs), a superfamily that exists as a number of isoenzymes, and is widely distributed into various tissues but predominates in the liver (5). These enzymes catalyse the conjugation of epoxides and other reactive intermediates with glutathione, a nucleophile tripeptide present in cellular cytosol. In fact, in the search for naturally occurring chemopreventive agents, induction of the GST system is considered a very desirable characteristic (6). Many putative chemopreventive phytochemicals act, at least partly, by stimulating the detoxification of reactive intermediates through conjugation with glutathione (7,8).

Although a number of studies have shown that GST is an inducible enzyme in cell lines derived from human tissues, to our knowledge the regulation of this enzyme in human tissues, such as the liver, has not been investigated. The advent of precision-cut slices, which can be generated automatically by a slicing apparatus and can be maintained viable in culture, makes it possible to undertake such studies (9,10). Since the GSTs play a pivotal role in the bioactivation of PAHs, a major class of environmental carcinogens, by detoxifying both the primary and the dihydrodiol epoxides, up-regulation of this enzyme system was investigated in precision-cut human liver slices following incubation with six structurally diverse PAHs characterized with different carcinogenic activity. As the rat is the most commonly used species in carcinogenicity studies, with the findings extrapolated to humans, it was of interest and relevance to compare the response in rat liver slices with that observed in human slices exposed to the same PAHs under the same conditions. Finally, since the lung is a principal target organ in PAH-induced carcinogenesis, the up-regulation of the GST system by the six PAHs was evaluated in rat lung slices and compared to the liver.

Materials and methods

Benzo[a]pyrene (B[a]P), fluoranthene (F), benzo[b]fluoranthene (B[b]F), dibenzo[a,h]anthracene (D[a,h]A), 1-chloro-2,4-dinitrobenzene (CDNB) and 1,2-dichloro-4-nitrobenzene (DCNB) (Sigma–Aldrich Co. Ltd, Poole, Dorset, UK); dibenzo[a]pyrene (D[a]P) and 1-methylphenanthrene (1-MP) (LCG Promochem, Middlesex, UK); 7-chloro-4-nitrobenzo-2-oxa-1,3-diazole (Fluka, Buchs SG, Switzerland); Qiagen RNeasy Mini kits (Crawley, West Sussex, UK),

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Fig. 1. Concentration-dependent induction of GST activity, monitored using 7-chloro-4-nitrobenzo-2-oxa-1,3-diazole, by PAHs in precision-cut rat liver slices. Precision-cut rat liver slices were incubated in the absence and presence of the various PAHs, dissolved in dimethyl sulphoxide, at a range of concentrations (0–100 μM) for 24 h. At the end of the incubation period, slices were removed from the media, cytosol prepared and GST activity determined using 7-chloro-4-nitrobenzo-2-oxa-1,3-diazole as substrate. Results are expressed as mean ± SD of triplicate pools of slices; *P < 0.05, **P < 0.01 and ***P < 0.001. B[a]P data from Pushparajah et al. (10).
Absolute™ QPCR Mix (Abgene, Epsom, Surrey, UK) and Earle’s balanced salt solution, foetal calf serum, gentamycin and RPMI with l-glutamine culture media (Gibco BRL Life Technologies, Paisley, Scotland) were all purchased. Twelve-well plates were obtained from Bibby Sterilin (Helena Biosciences, Sunderland, UK). Antibody to the Ya subunit of the α-family of GSTs was obtained from Oxford Biomedical Research (Oxford, MI, USA).

Preparation and incubation of precision-cut tissue slices
Liver sections from two human cadaveric livers that could not be used for transplantation purposes were obtained from the UK Human Tissue Bank (The Innovation Centre, Oxford Street, Leicester, UK). The particulars of the donors and metabolic viability of slices have already been described (11). Male Wistar albino rats (200 g) were obtained from B&K Universal Ltd (Hull, East Yorkshire, UK). The animals were housed at 22 ± 2°C, 30–40% relative humidity in an alternating 12-h light:dark cycle with light onset at 7 h. Rats were killed by cervical dislocation, and liver was immediately excised.

Rat and human liver slices (250 μm) were prepared from 8-mm cylindrical cores using a Krumdieck tissue slicer (Alabama Research and Development Corporation, Munford, AL, USA) as previously described (10). The multi-well plate procedure, using 12-well culture plates, was used to culture the slices. The culture medium was essentially that described by Lake et al. (12) and comprised RPMI 1640 Glutamax II containing foetal calf serum (5%), l-methionine (0.5 mM), insulin (1 μM), gentamycin (50 μg/ml) and hydrocortisone 21-hemisuccinate (0.1 mM). One slice was placed in each well in 1.5 ml of culture medium. Slices were incubated under sterile conditions on a reciprocating plate shaker housed in a humidified incubator, at a temperature of 37°C and under an atmosphere of 95% air/5% CO2. The slices were initially pre-incubated for 30 min in order to slough off any dead cells due to the slicing process. For the production of lung slices, animals were killed by an overdose of sodium pentobarbital, and lungs were perfused intratracheally with agarose (0.75% v/v) at 37°C. Agar was allowed to solidify, and lung slices (600 μm) were prepared from cylindrical cores (8 mm) as described for the liver (13). A pre-incubation of 60 min was carried out. Three different slice pools,

Fig. 2. Concentration-dependent induction of GST activity, monitored using CDNB, by PAHs in precision-cut rat liver slices. Precision-cut rat liver slices were incubated in the absence and presence of the various PAHs, dissolved in dimethyl sulphoxide, at a range of concentrations (0–100 μM) for 24 h. At the end of the incubation period, slices were removed from the media, cytosol prepared and GST activity determined using CDNB as substrate. Results are expressed as mean ± SD of triplicate pools of slices, *P < 0.05.

Fig. 3. Concentration-dependent induction of GST activity, monitored using DCNB, by PAHs in precision-cut rat liver slices. Precision-cut rat liver slices were incubated in the absence and presence of the various PAHs, dissolved in dimethyl sulphoxide, at a range of concentrations (0–100 μM) for 24 h. At the end of the incubation period, slices were removed from the media, cytosol prepared and GST activity determined using DCNB as substrate. Results are expressed as mean ± SD of triplicate pools of slices, *P < 0.05 and **P < 0.01.
comprising 4–10 slices, were used per time point. Both liver and lung slices were incubated with the various PAHs for 24 h, in accordance with our previous studies (10,11).

**Enzyme assays**

Following incubation, slices were removed from the medium, homogenized and cytosolic fractions were prepared by differential centrifugation. Cytosolic GST activity (14) was monitored using three accepting substrates whereas protein concentration was determined using bovine serum albumin as standard (15). The incubation conditions used and the range of concentrations of the various PAHs were based on our previous optimization studies (10). The three substrates used to monitor GST activity in the various tissues were 7-chloro-4-nitrobenzo-2-oxa-1,3-diazole whose conjugation is selectively catalysed by the \( \alpha \)-class of transferases; CDNB which is a non-specific substrate, whose conjugation with glutathione is catalysed by a number of GST isoenzymes, including the \( \alpha \)-, \( \mu \)- and \( \pi \)-classes and DCNB which is particularly catalysed primarily by the \( \mu \)-class (5,16).

Finally, in order to determine changes in enzyme protein expression, hepatic cytosolic proteins were resolved by electrophoresis and incubated with the primary antibody (antibody to the rat Ya subunit of the \( \alpha \)-family raised in goat) and the corresponding peroxidase-linked anti-goat IgG. Immunoblots were quantitated by densitometry using the GeneTool software (Syngene Corporation, Cambridge, UK).

**Transcript level measurement**

Two slices were used for total RNA extraction, and for each sample triplicate extractions were carried out. RNA was extracted using the Qiagen RNeasy Mini kit and was quantified using a Nanodrop Spectrophotometer. Total RNA was treated with RNase-free DNase to remove genomic contamination. Reverse transcription was primed with random hexamers and carried out by Superscript II according to the manufacturer’s instructions. To ensure that DNase-treated
samples were free from genomic contamination, a reverse transcription control was carried out for every RNA sample. cDNA generated from 50 ng was amplified using Absolute™ QPCR Mix with 400 nM primers and 100 nM fluorogenic probe in a total reaction volume of 25 μl. Quantitative polymerase chain reactions were run on the ABI7000 SDS instrument and quantitation was carried out using the ABI proprietary software against a standard curve generated from rat genomic DNA.

For the quantitative reverse transcription–polymerase chain reaction, the primer and TAMRA/FAM dual-labelled probe (10) were designed using the Primer Express software (Applied Biosystems, Warrington, CA, USA) and purchased from MWG (Ebersberg, Germany). The 5′-primer (CCATGGCCAA-GACTACCTTGTA), 3′-primer (AGGCTGGCATCAAACCTCTTCA) and probe (CCCGGTGAGACATCCACCTGGAAC) were designed to amplify sequences within a single exon, so that genomic DNA could be used as a standard.

Induction potency for individual PAHs was calculated from the linear part of the concentration–activity graph plot, using the Prism software (version 4.03, GraphPad Inc., San Diego, CA, USA), and values are expressed as fold-increase per micromole.

Statistical evaluation was carried out using the Student’s t-test.

Results

When GST activity was monitored using 7-chloro-4-nitrobenzo-2-oxa-1,3-diazole as the substrate, B[a]P (80%), and to a lesser extent D[a,h]A (40%), and B[b]F (15%) were the only PAHs that caused a statistically significant increase in activity in rat liver slices; maxima were reached at a PAH concentration of about 5–10 μM (Figure 1). When DCNB or DCNB served as the accepting substrates, only B[b]F caused a weak (15%), but statistically significant rise in GST activity (Figures 2 and 3). B[a]P, B[b]F and D[a,h]A, but not the other PAHs, decreased GST activity at the highest concentrations of 50–100 μM, whatever the substrate used (Figures 1–3). Immunoblot analysis revealed a single immunoreactive protein; quantification by densitometry showed a clear rise in protein levels in the liver microsomes from B[b]F-, B[a]P- and, to a lesser extent, D[a,h]A-treated rat liver slices (Figure 4). All PAHs studied caused an increase in GST mRNA levels, with B[b]F being clearly the most potent (Figure 5); potency of induction by individual PAHs is shown in Table 1.

When GST was assayed using either DCNB or CDNB as substrates, activity in the two human liver samples was similar to that seen in the rat; in the case of 7-chloro-4-nitrobenzo-2-oxa-1,3-diazole, however, activity was higher in the two human livers. When human liver slices were incubated with the various PAHs under identical conditions to the rat slices and 7-chloro-4-nitrobenzo-2-oxa-1,3-diazole was employed as the substrate, B[b]F was the only PAH that caused a statistically significant rise in activity (25%) (Figure 6); a similar picture emerged when DCNB was utilized as the substrate (Figure 7). None of the PAHs studied modulated GST activity significantly when DCNB was the substrate used to monitor activity (Figure 8). None of the compounds impaired GST activity at 50 μM, the highest concentration employed, whatever the substrate (Figures 6–8), which contrasts with the rat studies. Antibodies to the Ya protein recognized a single immunoreacting band, which was elevated only in the slices exposed to B[b]F (Figure 9).

No activity was detectable in rat lung slices incubated for 24 h, when DCNB or 7-chloro-4-nitrobenzo-2-oxa-1,3-diazole were used. However, activity was detectable in the presence of CDNB but it was only ~10% of that observed in the liver; none of the PAHs used elevated GST activity (Figure 10).

Discussion

The studies with rat liver slices indicate that GST is an inducible enzyme when exposed to PAHs, but the extent of induction is low when compared to enzyme systems such as the CYP1 family of cytochromes P450 (11). Moreover, not all PAHs stimulated activity at the concentrations studied. Of the six PAHs investigated, B[b]F, B[a]P and D[a,h]A stimulated GST activity, when assessed using as substrate 7-chloro-4-nitrobenzo-2-oxa-1,3-diazole. The fact that the effects were absent or less pronounced when GST activity was monitored using as substrate CDNB or DCNB would imply that PAHs may display selectivity towards the α-class. In concordance with the present findings, when liver slices were exposed to the same series of PAHs and changes in gene expression were assessed using oligonucleotide microarrays an increase in the gene expression of the αi isoence was noted only in the case of B[a]P, B[b]F and D[a,h]A (17). In addition to increases in transcript expression, we demonstrate that these are paralleled by an increase in protein expression indicating that higher enzyme availability is likely to be responsible for the observed rise in activity. This is consistent with previous work that 3-methylcholanthrene, another PAH, increases selectively the expression of the Ya subunit (18,19). From the activity level, employing DCNB as substrate, 3-methylcholanthrene elevated activity in mice, but failed to do so in Sprague–Dawley rats (19,20); a modest increase was only noted when activity was monitored using the non-specific CDNB as substrate (21). Moreover, in the present studies, an increase in the mRNA levels of the α-class of GSTs elicited by these three PAHs.

Table I. Induction potency of GST mRNA levels by PAHs in precision-cut rat liver slices

<table>
<thead>
<tr>
<th>PAH</th>
<th>GST mRNA levels (fold-increase/μmol of PAH)</th>
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<tbody>
<tr>
<td>B[b]F</td>
<td>4.35</td>
</tr>
<tr>
<td>D[a,h]P</td>
<td>3.17</td>
</tr>
<tr>
<td>B[a]P</td>
<td>2.48</td>
</tr>
<tr>
<td>D[a,h]A</td>
<td>2.11</td>
</tr>
<tr>
<td>Fluoranthene</td>
<td>1.24</td>
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<tr>
<td>1-Methylphenanthrene</td>
<td>0.59</td>
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Induction potency values of the PAHs were calculated from the linear part of the concentration–activity graph plot, using the Prism software version 4.03 (GraphPad Inc.).

Fig. 5. Concentration-dependent changes in GST mRNA levels in precision-cut rat liver slices incubated with PAHs. Precision-cut rat liver slices were incubated in the absence and presence of the various PAHs, dissolved in dimethyl sulphoxide, at a range of concentrations for 24 h. At the end of the incubation period, slices were removed from the media and total RNA extracted. GST mRNA levels were determined by quantitative reverse transcription–polymerase chain reaction (TaqMan). Changes are shown as fold-increases compared to control slices incubated with dimethyl sulphoxide only. Results are expressed as mean ± SD where n = 4; *P < 0.05, **P < 0.01 and ***P < 0.001. B[a]P data from Pushparajah et al. (10).
raises the possibility of transcriptional activation. It should be pointed out that, as the Q-PCR technology measures transcript pool size, it is not possible to rule out mRNA stabilization as a possible mechanism. Induction by planar compounds of the α-class of the GST isoenzymes is believed to be regulated by the aryl hydrocarbon receptor, a transcriptional factor residing in the cellular cytosol (22). Indeed, of the six PAHs employed in the present study, B[\(b\)F], B[\(a\)P] and D[\(a,h\)]A are the best ligands for this receptor and the most potent inducers of CYP1, the up-regulation of which is also regulated by the aryl hydrocarbon receptor (11). As PAHs lose their planarity, the Ah affinity diminishes, and a good correlation is evident (\(R = 0.74\), Figure 11) between rise in mRNA levels and the calculated area/depth\(^2\) (11), an index of planarity (23). D[\(a,l\)]P also caused an increase in the GSTs mRNA levels, despite being a poor ligand to the aryl hydrocarbon receptor, but this was translated to neither increased protein expression nor activity. Such a discrepancy between mRNA levels and enzyme expression and activity has been reported also for other enzymes involved in xenobiotic metabolism (11). It has been proposed that a threshold exists that has to be exceeded in order for mRNA to be translated, so that activation of the gene at the mRNA level does not always lead to elevated protein levels (24). However, the fact that the mRNA levels were monitored in the complete slice but activity/protein in the isolated microsomes could have contributed to this lack of correlation as translation products of mRNA could have been missed. Furthermore, as mRNA levels were determined only at a single time point, differences in temporal response may be partly responsible.

We believe that the fact that both liver donors were smokers (11) has not impacted on the outcome of these studies; it has already been reported that the PAH 3-methylcholanthrene failed or poorly induced GST activity in

Fig. 6. Concentration-dependent induction of GST activity, monitored using 7-chloro-4-nitrobenzo-2-oxa-1,3-diazole, by PAHs in precision-cut human liver slices. Precision-cut human liver slices were incubated in the absence and presence of the various PAHs, dissolved in dimethyl sulfoxide, at a range of concentrations (0–50\(\mu\)M) for 24 h. At the end of the incubation period, slices were removed from the media, cytosol prepared and GST activity determined using 7-chloro-4-nitrobenzo-2-oxa-1,3-diazole as substrate. Results are expressed as mean ± SD of triplicate pools of slices, *\(P < 0.05\).
rodent tissues, even following the intra-peritoneal administra-
tion of high doses (20,21). In human liver slices, GST ac-
tivity determined using CDNB was markedly higher than
when DCNB served as substrate, in agreement with previous
studies conducted in human liver (25). When human liver
slices were exposed to the same series of PAHs, under
identical incubation conditions, a significant increase in
activity was observed only in the presence of B[b]F and,
similar to rat slices, when GST was monitored using either 7-
chloro-4-nitrobenzo-2-oxa-1,3-diazole or CDNB as sub-
strates. As this rise in activity was accompanied by elevated
levels of immunoreactive Ya, it may be inferred that increased
enzyme levels are responsible for this increase, rather than
activation of pre-existing enzyme. No statistically significant
increase was observed with any of the other PAHs studied.
These observations raise the possibility that the α-class of
GST is more inducible in rat than in human liver; however,
analysis of a larger number of human liver samples would be
required for such a conclusion to be firmly reached. It is
noteworthy to point out that, of the six PAHs studied, B[b]F is
the most avid ligand for the aryl hydrocarbon receptor (11).
GST activity in the rat lung is generally only about 10–15%
of the hepatic activity (26,27). In the present studies, in contrast
to the liver, no GST activity was detectable in the lungs when
7-chloro-4-nitrobenzo-2-oxa-1,3-diazole or CDNB as sub-
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These observations raise the possibility that the α-class of

Fig. 7. Concentration-dependent induction of GST activity, monitored using CDNB, by PAHs in precision-cut human liver slices. Precision-cut human liver slices
were incubated in the absence and presence of the various PAHs, dissolved in dimethyl sulphoxide, at a range of concentrations (0–50 μM) for 24 h. At the end of
the incubation period, slices were removed from the media, cytosol prepared and GST activity determined using CDNB as substrate. Results are expressed as mean ±
SD of triplicate pools of slices, *P < 0.05.
conjugation enzyme activities decline following a 24-h incubation, activity may drop below the detection limit (29). GST activity was detectable only when CDNB was used. CDNB is a non-specific substrate, the conjugation of which with glutathione is catalysed by a number of GST isoenzymes, including the μ- and π-classes (16), which are expressed in the lung (4). It is also pertinent to point out that the rate of conjugation of CDNB in rat lung is far higher when compared with DCNB (27,28). The lack of inducibility of the GST enzyme system by PAHs in the lung may impact adversely as to the sensitivity of this organ to the carcinogenicity of these compounds, especially in view of the high inducibility of CYP1A1/1B1 (11), the enzymes responsible for their bioactivation in this tissue. As the dihydrodiol epoxides, the principal genotoxic metabolites, are not substrates of the epoxide hydrolase, their detoxification is effected primarily by the GSTs (30).

In the studies with rat liver slices, GST activity dropped below control levels at the highest concentrations (50–100 μM) of B[b]F, D[a,h]A and B[a]P. This is unlikely to be due to decreased slice viability as it was not observed at the protein level. Moreover, leakage of lactic dehydrogenase into the culture medium, an index of slice viability, was not influenced by incubation with the various PAHs, at least up to concentrations of 100 μM (results not shown). Most likely the drop in activity represents enhanced generation of metabolically formed electrophiles, such as epoxides and quinones, which, following saturation of deactivating

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Fig. 8. Concentration-dependent induction of GST activity, monitored using DCNB, by PAHs in precision-cut human liver slices. Precision-cut human liver slices were incubated in the absence and presence of the various PAHs, dissolved in dimethyl sulphoxide, at a range of concentrations (0–50 μM) for 24 h. At the end of the incubation period, slices were removed from the media, cytosol prepared and GST activity determined using DCNB as substrate. Results are expressed as mean ± SD of triplicate pools of slices.
pathways, are now available to bind to the enzymes impairing their activity. Indeed, the three PAHs that displayed this behaviour are the most potent inducers of the CYP1 family (11), which is responsible for the bioactivation of PAHs, among the six compounds studied. The fact that this behaviour was not evident when human slices were used may reflect the lower inducibility of the CYP1 family in human liver (11). Similarly, this drop in activity at high PAH concentrations was not seen in the lung since in this organ, even after PAH-mediated induction, CYP1 activity, as exemplified by the O-deethylation of ethoxyresorufin, was much lower in lung compared with liver slices (11).

In summary, the present study has established that (i) the \( \alpha \)-class of GSTs is modestly inducible by PAHs, but the extent depends on the nature of the PAH involved; (ii) up-regulation of this enzyme by PAHs may involve transcriptional activation; (iii) suggests that GST induction potential is related to the activation of the aryl hydrocarbon receptor; (iv) GST activity is not inducible in the rat lung when assessed using CDNB and (v) for the first time, to our knowledge, it is demonstrated that GST activity is inducible in human liver tissue. Although it appears that human hepatic GSTs are less sensitive to induction by PAHs compared with the rat, for such a conclusion to be firmly reached, studies should be extended to include more human liver samples and a larger series of PAHs.

Fig. 9. Concentration-dependent induction of GST protein levels by B[\( \beta \)][F] in precision-cut human liver slices. Precision-cut human liver slices were incubated in the absence and presence of B[\( \beta \)][F] (0–50 \( \mu \)M) for 24 h. At the end of this period, slices were removed from the media and cytosolic proteins were resolved by sodium dodecyl sulphate–polyacrylamide gel electrophoresis before being transferred electrophoretically to nitrocellulose paper. Immunoblot was carried out using anti-rat Ya subunit, followed by peroxidase-labelled anti-rabbit IgG; all lanes were loaded with 5 \( \mu \)g of protein. Figures above the immunoblot represent percentage values compared with control (dimethyl sulphoxide treated) slices which were set at 100%.

Fig. 10. Concentration-dependent induction of GST activity, monitored using CDNB, by PAHs in precision-cut rat lung slices. Precision-cut rat lung slices were incubated in the absence and presence of the various PAHs, dissolved in dimethyl sulphoxide, at a range of concentrations (0–5 \( \mu \)M) for 24 h. At the end of the incubation period, slices were removed from the media, cytosol prepared and GST activity determined using CDNB as substrate. Results are expressed as mean ± SD of triplicate pools of slices.
Fig. 11. Correlation between GST mRNA-induction potency of PAHs and molecular size. Area/depth$^2$ ($a/d^2$) values from Pushparajah et al. (11), determined from the dimensions (length, width and depth) of each molecule. All structural calculations were conducted on the Sybyl 7.0 (Tripos Associates, St Louis, MO, USA) molecular modelling suite of programmes operating under Linux.

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