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Evidence for the aldo-keto reductase pathway of polycyclic aromatic *trans*-dihydrodiol activation in human lung A549 cells

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Polycyclic aromatic hydrocarbons (PAHs) are tobacco carcinogens implicated in the causation of human lung cancer. Metabolic activation is a key prerequisite for PAHs to cause their deleterious effects. Using human lung adenocarcinoma (A549) cells, we provide evidence for the metabolic activation of ()-*trans*-7,8-dihydroxy-7,8-dihydrobenzo[*a*]pyrene (B[*a*]P-7,8-*trans*-dihydrodiol) by aldo-keto reductases (AKRs) to yield benzo[*a*]pyrene-7,8-dione (B[*a*]P-7,8-dione), a redox-active *o*-quinone. We show that B[*a*]P-7,8-*trans*-dihydrodiol (AKR substrate) and B[*a*]P-7,8-dione (AKR product) lead to the production of intracellular reactive oxygen species (ROS) (measured as an increase in dichlorofluorescein diacetate fluorescence) and that similar changes were not observed with the regioisomer ()-*trans*-4,5-dihydroxy-4,5-dihydrobenzo[*a*]pyrene or the diol-epoxide, ()-*anti*-7,8-dihydroxy-9,10-epoxy-7,8,9,10-tetrahydro-B[*a*]P. B[*a*]P-7,8-*trans*-dihydrodiol and B[*a*]P-7,8-dione also caused a decrease in glutathione levels and an increase in NADP /NADPH ratios, with a concomitant increase in single-strand breaks (as measured by the comet assay) and 7,8-dihydro-8-oxo-2'-deoxyguanosine (8-oxo-dGuo). The specificity of the comet assay was validated by coupling it to human 8-oxoguanine glycosylase (hOGG1), which excises 8-oxo-Gua to yield single-strand breaks. The levels of 8-oxodGuo observed were confirmed by an immunoaffinity purification stable isotope dilution ([¹⁵N5]-8-oxo-dGuo) liquid chromatography electrospray ionization/multiple reaction monitoring/mass spectrometry (LC-ESI/MS) assay. B[*a*]P-7,8-*trans*-dihydrodiol produced DNA strand breaks in the hOGG1-coupled comet assay as well as 8-oxo-dGuo (as measured by LC-ESI/MS) and was enhanced by a catechol *O*-methyl transferase (COMT) inhibitor, suggesting that COMT protects against *o*-quinone-mediated redox cycling. We conclude that activation of PAH-*trans*-dihydrodiols by AKRs in lung cells leads to ROS-mediated genotoxicity and contributes to lung carcinogenesis.

C Ran, Q Dai, Q Ruan, TM Penning, IA Blair, RG Harvey

Strategies for synthesis of adducts of *o*-quinone metabolites of carcinogenic polycyclic aromatic hydrocarbons with 2'-deoxyribonucleosides

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Polycyclic aromatic hydrocarbons (PAHs) are major environmental carcinogens produced in the combustion of fossil fuels, tobacco, and other organic matter. Current evidence indicates that PAHs are transformed enzymatically to active metabolites that react with DNA to form adducts that result in mutations. Three activation pathways have been proposed: the diol epoxide path, the radical-cation path, and the quinone path. The latter involves aldo-keto reductase mediated oxidation of PAH dihydrodiol metabolites to catechols that enter into redox cycles with quinones. This results in generation of reactive oxygen species (ROS) that attack DNA, and the PAH quinones also react with DNA to form adducts. Several strategies for synthesis of the stable adducts formed by the *o*-quinone metabolites of carcinogenic PAHs with 2'-deoxyribonucleosides were investigated and compared. The PAH quinones studied were benz[*a*]anthracene-3,4-dione and its 7-methyl- and 7,12-dimethyl- derivatives. The parent PAHs represent a range of carcino-

genicity from inactive to highly potent. Two synthetic methods were devised that differ in the catalyst employed, Pd(OAc)₂ or CuI. The Pd-mediated method involved coupling a protected amino-catechol PAH derivative with a halo-2'-deoxyribonucleoside. The copper-mediated method entailed reaction of a halo-PAH catechol derivative with a 2'-deoxyribonucleoside. Adducts of benz[*a*]anthracene-3,4-dione (and its 7-methyl- and 7,12-dimethyl- derivatives) with 2'-deoxyadenosine and 2'-deoxyguanosine were prepared by these methods. Availability of adducts of these types through synthesis makes possible for the first time biological studies to determine the role of these adducts in tumorigenesis. The copper-mediated method offers advantages of economy, adaptability to large-scale preparation, utility for synthesis of ¹³C- or ¹⁵N-labeled analogues, and nonformation of bis-adducts as secondary products.

D Xu, Y Duan, IA, Blair, TM Penning, RG Harvey

Synthesis of dibenzo[*def,p*]chrysene, its active metabolites, and their ¹³C-labeled analogues.

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Dibenzo[*def,p*]chrysene (DBC), commonly referred to by the obsolete name dibenzo[*a,l*]pyrene, is the most carcinogenic polycyclic aromatic hydrocarbon currently known.

DBC is suspected to be involved in initiation of lung cancer in cigarette smokers.

Efficient new syntheses of DBC, its active metabolites [DBC diol (1), DBC dione (2), DBC diol epoxide (3)], and their previously unknown ¹³C₂-labeled analogues are reported. Although several syntheses of DBC and its active metabolites have been described, they entail relatively long multistep sequences not considered suitable for preparation of the ¹³C-labeled analogues. The isotopically-labeled analogues were needed as standards for sensitive methods of analysis of the metabolites of DBC and their DNA adducts in human cells using stable isotope dilution liquid chromatography/tandem mass spectrometry. The synthesis was initially carried out with unlabeled precursors. It entailed Pd-catalyzed Suzuki–Miyaura coupling of phenanthrene 9-boronic acid with 2-bromophenylacetone followed by TiCl₄-catalyzed cyclization to yield 9-methylbenzo[*g*]chrysene. The latter was transformed to DBC via bromination and conversion to 9-formylbenzo[*g*]chrysene, followed by Wittig reaction with methoxymethylene triphenylphosphine, and mild acid-catalyzed cyclization.

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Oxidation of PAH *trans*-dihydrodiols by human aldo-keto reductase AKR1B10

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The AKR1B10 enzyme has been identified as a potential biomarker for human non-small cell lung carcinoma and as a tobacco exposure and response gene. AKR1B10 functions as an efficient retinal reductase *in vitro* and it may regulate retinoic acid homeostasis.

AKR1B10 was found to oxidize a wide range of PAH *trans*-dihydrodiol substrates *in vitro* to yield PAH *o*-quinones. These reactions exhibited unexpected stereoselectivity. In the case of benzo[*a*]pyrene-7,8-dihydrodiol the enzyme was specific for the minor (+)-benzo[*a*]pyrene-7*S*,8*S*-dihydrodiol diastereomer formed *in vivo*. However, AKR1B10 displayed reasonable activity in the oxidation of both the (-)-*R,R* and (+)-*S,S* stereoisomers of benzo[*g*]chrysene-11,12-dihydrodiol and oxidized the potentially relevant, albeit minor, (+)-benz[*a*]anthracene-3*S*,4*S*-dihydrodiol. It is likely that AKR1B10 plays a

contributing role in the activation of PAH trans-dihydrodiol metabolites in human lung. AKR1B10 retinal reductase activity in human lung cells was confirmed in vitro and found to be 5- to 150-fold greater than the oxidation of PAH trans-dihydrodiols examined. AKR1B10 was highly expressed at the mRNA and protein levels in human lung adenocarcinoma A549 cells, and robust retinal reductase activity was measured in lysates of these cells. The much greater catalytic efficiency of retinal reduction compared to PAH trans-dihydrodiol metabolism suggests AKR1B10 may play a greater role in lung carcinogenesis through dysregulation of retinoic acid homeostasis than through oxidation of PAH trans-dihydrodiols.

C Ran, D Xu, Q Dai, TM Penning, IA Blair, RG Harvey

Synthesis of $^{13}\text{C}_2$ -benzo[*a*]pyrene and its 7,8-dihydrodiol and 7,8-dione implicated as carcinogenic metabolites

Tetrahedron Letters, 2008, Vol 49, Iss 29, pp 4531-4533.

Synthesis of the $^{13}\text{C}_2$ -labelled analogues of benzo[*a*]pyrene (BP) and its active 7,8-dihydrodiol, 7,8-dione, and *anti*- and *syn*-diolepoxide metabolites is reported. These compounds were needed as standards for sensitive methods of LC-MS analysis of the metabolites of BP formed in human bronchoalveolar H358 cells. Although syntheses of all twelve of the mono- ^{13}C -labelled analogues of benzo[*a*]pyrene with a ^{13}C -atom at each of the peripheral carbon atoms of the BP ring system have been reported, the classical synthetic methods entailed large numbers of steps and were unsuitable for preparation of the $^{13}\text{C}_2$ -labelled analogues. The synthetic method entailed Pd-catalyzed Suzuki–Miyaura coupling of a naphthalene boronic acid with 2-bromobenzene-1,3-dialdehyde followed by double Wittig reaction of the product with $^{13}\text{CH}_2\text{PPh}_3$, bis-epoxidation, and mild acid-catalyzed cyclization. The methodology is readily adaptable to the synthesis of the active metabolites of other PAH carcinogens.

CA Shultz, NT Palackal, D Mangal, RG Harvey, IA Blair, TM Penning

Fjord-region benzo[*g*]chrysene-11,12-dihydrodiol and benzo[*c*]phenanthrene-3,4-dihydrodiol as substrates for rat liver dihydrodiol dehydrogenase (AKR1C9): structural basis for stereochemical preference

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This study demonstrates that benzo[*g*]chrysene-11,12-dihydrodiol (B[*g*]C-11,12-dihydrodiol) derived from the *fjord*-region parent hydrocarbon B[*g*]C is oxidized by rat AKR1C9 with a *k*_{cat}/*K*_m 100 times greater than that observed with the commonly studied *bay*-region benzo[*a*]pyrene-7,8-dihydrodiol (B[*a*]P-7,8-dihydrodiol). Conversely, despite its strikingly similar structure to B[*g*]C-11,12-dihydrodiol, benzo[*c*]phenanthrene-3,4-dihydrodiol (B[*c*]Ph-3,4-dihydrodiol) is consumed by AKR1C9 at sluggish rates comparable to those observed with B[*a*]P-7,8-dihydrodiol. CD spectroscopy revealed that only the (+)-B[*g*]C-11,12-dihydrodiol stereoisomer was oxidized, while AKR1C9 oxidized both stereoisomers of B[*a*]P-7,8-dihydrodiol and B[*c*]Ph-3,4-dihydrodiol. The (+)-*S,S*- and (-)-*R,R*-stereoisomers of B[*g*]C-11,12-dihydrodiol were purified by chiral RP-HPLC. The 11*S*,12*S*-stereoisomer was oxidized at the same rate as the racemate. The 11*R*,12*R* stereo-isomer did not act as an inhibitor to AKR1C9, indicating that the (-)-*R,R*-stereoisomer was excluded from the active site. To understand the basis of stereochemical preference, we screened alanine-scanning mutants of active site residues of AKR1C9. These studies revealed that in comparison to the wild type, F129A, W227A, and Y310A enabled the oxidation of both the B[*g*]C-11*S*,12*S*-dihydrodiol and the B[*g*]C-11*R*,12*R*-dihydrodiol. Molecular modeling revealed that unlike B[*a*]P-7,8-dihydrodiol and B[*c*]Ph-3,4-dihydrodiol, B[*g*]C-11,12-

dihydrodiol enantiomers are significantly bent out of plane. As a consequence, the (-)-*R,R*-stereoisomer was prevented from binding to the active site because of unfavorable interactions with F129, W227, or Y310. Additionally, LC/MS validated that the product of the reaction of B[*g*]C-11,12-dihydrodiol oxidation catalyzed by AKR1C9 was B[*g*]C-11,12-dione, which was trapped *in Vitro* with the nucleophile 2mercaptoethanol. The similarity between rates of *trans*-dihydrodiol oxidation by the rat and human liver specific AKRs (AKR1C9 and AKR1C4) implicate these enzymes in hepatocarcinogenesis in rats observed with the *fjord*-region PAH.

Q Ruan, S. Gelhaus, TM Penning, RG Harvey, IA Blair

Aldo-keto reductase- and cytochrome P450-dependent formation of benzo[*a*]pyrene-derived DNA adducts in human bronchoalveolar cells

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There is substantial evidence to suggest that polycyclic aromatic hydrocarbons (PAHs) such as benzo-*[a]*pyrene (B[*a*]P) induce lung cancer through metabolic activation. As part of a program to delineate the routes of PAH activation, we have examined DNA adducts that are formed in human lung cells. A stable isotope dilution liquid chromatography/multiple reaction monitoring mass spectrometry method was used to quantify eight *anti*-7,8-dihydroxy-9,10-epoxy-7,8,9,10-tetrahydro-B[*a*]P (B[*a*]PDE)-derived DNA adducts in four H358 human bronchoalveolar cell lines with different phenotypes. In P450 1A1/P4501B1-induced H358 cells exposed to (()-B[*a*]P-7,8-dihydro-7,8-diol (B[*a*]P-7,8-dihydrodiol), (+)-*antitrans*-B[*a*]PDE-*N*2-2 ϕ -deoxyguanosine [(+)-*anti-trans*-B[*a*]PDE-*N*2-dGuo] was the major DNA adduct, and it formed with no lag phase. In AKR1A1-transfected H358 cells, (+)-*anti-trans*-B[*a*]PDE-*N*2-dGuo was also the major adduct with a 3 h lag phase before significant adduct formation was detected. In AKR1A1-transfected H358 cells with induced P450 1A1/P450 1B1, (+)-*anti-trans*-B[*a*]PDE-*N*2-dGuo was formed with no lag phase in amounts similar to those in the H358 cells with up-regulated P4501A1/P450 1B1. Surprisingly, the greatest amount of (+)-*anti-trans*-B[*a*]PDE-*N*2-dGuo was formed in the control H358 cells. Furthermore, (+)-*anti-trans*-B[*a*]PDE-*N*2-dGuo formation was 2-fold higher in (()-B[*a*]P-7,8-dihydrodiol-exposed H358 cells when compared with (()-B[*a*]P-7,8-dihydrodiol-exposed cells. The P450 1A1/1B1 inhibitor 2,4,3',5' tetramethoxy-stilbene did not attenuate DNA adduct formation in the control H358 cells, suggesting that another P450 was responsible. These data raise the intriguing possibility that P450 1A1/P450 1B1 and AKR1A1 may be protective against (+)-B[*a*]PDE-mediated DNA damage.

Q Dai, D Xu, K Lim, RG Harvey

Efficient syntheses of C8-aryl adducts of adenine and guanine formed by reaction of radical cation metabolites of carcinogenic polycyclic aromatic hydrocarbons with DNA
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The synthesis of the C8-aryl adducts of adenine and guanine formed by reaction of the radical cation metabolites of carcinogenic polycyclic aromatic hydrocarbons (PAHs), such as benzo[*a*]pyrene (BP) and dibenzo[*def,p*]chrysene (DBC), with DNA is reported. The synthetic approach involves in the key step direct reaction of a PAH aldehyde with a di- or triamine precursor of a purine. The method is operationally simple, affords good yields of adducts, and is broad in its scope. The C8-aryl adducts of adenine and guanine derived from BP (6-BP-8-Ade and 6-BP-8-Gua) and DBC (10-DBC-8-Ade and 10-DBC-8-Gua) were synthesized in good yields by this method. Analogous C8-aryl adenine and guanine

derivatives of other PAHs (anthracene, benz[*a*]anthracene, and chrysene) were also readily prepared via this approach. This method of synthesis is superior to the only method that is currently available. It entails direct reaction of short-lived PAH radical cations (generated electrochemically or chemically) with 2'-deoxyribonucleosides or the corresponding purine bases. It provides the adducts in low yields accompanied by complex mixtures of secondary products. An alternative synthesis that involves Pd-catalyzed Suzuki-Miyaura coupling of arylboronic acids with 8-bromopurine nucleosides was also investigated. Although the C8-purine adducts of PAHs, such as naphthalene, phenanthrene, pyrene, and chrysene, could be prepared by this method, analogous adducts of carcinogenic PAHs and other structurally related PAHs, e.g., anthracene, benz[*a*]anthracene, benzo[*a*]pyrene, and dibenzo[*def,p*]-chrysene, could not be obtained. This difference was shown to be a consequence of the facility of competing hydrolytic deboration of the corresponding arylboronic acids.

H Jiang, S. Gelhaus, D Mangal, RG Harvey, IA Blair, TM Penning

Metabolism of benzo[*a*]pyrene in human bronchoalveolar H358 Cells using liquid chromatography–mass spectrometry

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Benzo[*a*]pyrene (B[*a*]P), a representative polycyclic aromatic hydrocarbon (PAH), is metabolically activated by three enzymatic pathways: by peroxidases (e.g., cytochrome P450 peroxidase) to yield radical cations, by P4501A1/1B1 monooxygenation and epoxide hydrolase to yield diol epoxides, and by P4501A1/1B1 monooxygenation, epoxide hydrolase, and aldo-keto reductases (AKRs) to yield *o*-quinones. In humans, a major exposure site for environmental and tobacco smoke PAH is the lung; however, the profile of B[*a*]P metabolites formed at this site has not been well characterized. In this study, human bronchoalveolar H358 cells were exposed to B[*a*]P, and metabolites generated by peroxidase (B[*a*]P-1,6- and B[*a*]P-3,6-diones), from cytochrome P4501A1/1B1 monooxygenation [3-hydroxy-B[*a*]P, B[*a*]P-7,8- and 9,10-*trans*-dihydrodiols, and B[*a*]P-*r*-7,*t*-8,*t*-9,*c*-10-tetrahydrotetrol (B[*a*]P-tetraol-1)], and from AKRs (B[*a*]P-7,8-dione) were detected and quantified by RP-HPLC, with in-line photo-diode array and radiometric detection, and identified by liquid chromatography–mass spectrometry (LC–MS). Progress curves showed a lag phase in the formation of 3-hydroxy-B[*a*]P, B[*a*]P-7,8-*trans*-dihydrodiol, B[*a*]P-tetraol-1, and B[*a*]P-7,8-dione over 24 h. Northern blot analysis showed that B[*a*]P induced P4501B1 and AKR1C isoforms in H358 cells in a time-dependent manner, providing an explanation for the lag phase. Pretreatment of H358 cells with 10 nM 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) eliminated this lag phase but did not alter the levels of the individual metabolites observed, suggesting that both B[*a*]P and TCDD induction ultimately yield the same B[*a*]P metabolic profile. The one exception was B[*a*]P-3,6-dione which was formed without a lag phase in the absence and presence of TCDD, suggesting that the peroxidase responsible for its formation was neither P4501A1 nor 1B1. Candidate peroxidases that remain include PGH synthases and uninduced P450 isoforms. This study shows that the P4501A1/1B1 and AKR pathways are inducible in human lung cells and that the peroxidase pathway was not. It also provides evidence that each of the pathways of PAH activation yields their distinctive metabolites in H358 human lung cells and that each pathway may contribute to the carcinogenic process.

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Substituent effects in benz[*a*]anthracene carbocations: A stable ion, electrophilic substitution (nitration, bromination), and DFT study

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A series of novel carbocations were generated from isomeric monoalkylated and dialkylated benz[*a*]anthracenes (BAs) by low-temperature protonation in FSO₃H/SO₂ClF. With the

monoalkyl derivatives (5-methyl, 6-methyl, 7-methyl, and 7-ethyl) as well as the D-ring methylated analogues (9-methyl, 10-methyl, and 11-methyl), the C-7 or the C-12 protonated carbocations were observed (as the sole or major carbocation) in all cases. Protonation of the 12-methyl derivative (**9**) gave the C-7 protonated carbocation (**9H⁺**) as the kinetic species and the *ipso*-protonated carbocation (**9aH⁺**) as the thermodynamic cation. With the 12-ethyl derivative (**10**), relief of steric strain in the bay-region greatly favors *ipso* protonation (**10aH⁺**). With 3,9-dimethyl (**14**), C-7 protonation (**14H⁺**) is strongly favored (with <10% protonation at C-12), and with 1,12-dimethyl (**15**) the sole species observed is the C-7 protonated carbocation (**15H⁺**). For 7-methyl-12-ethyl, 7-ethyl-12-methyl, and 7,12-diethyl derivatives (**16**, **17**, and **18**), two *ipso*-protonated carbocations were initially formed (C-7/C-12), rearranging in time to give the C-12 protonated carbocations exclusively (**16aH⁺**, **17aH⁺**, and **18aH⁺**). Protonation outcomes are compared with the computed relative energies by DFT. Charge delocalization paths in the resulting carbocations were deduced based on the magnitude of $\phi_{\ddot{a}}^{13C}$ values. For the thermodynamically more stable C-12 protonated carbocations, the charge delocalization path is analogous to those derived based on computed NPA charges for the benzylic carbocations formed by 1,2-epoxide (bay-region) and 5,6-epoxide (K-region) ring opening. Nitration (and bromination) of the 4-methyl, 7-methyl, 7-ethyl, 3,9-dimethyl, and 1,12-dimethyl derivatives resulted in isolation and characterization of several novel derivatives. Excellent agreement is found between low temperature protonation selectivities and the regioselectivities observed in model substitution reactions.