

Short Communication

Relationship between Content and Activity of Cytochrome P450 and Induction of Heterocyclic Amine DNA Adducts in Human Liver Samples *In vivo* and *In vitro*

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Abstract

This study was designed to estimate a correlation between metabolic activation phenotypes and formation of DNA adducts by heterocyclic amines (HCA) in 15 liver samples from healthy donors. The correlation between the amount of endogenous DNA adducts and the content of cytochrome P450 in human liver samples *in vivo* was statistically significant at $r^2 = 0.71$ and $P < 0.005$. Furthermore, the isolated human liver microsomes were treated *in vitro* with two HCAs, 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (PhIP) and 2-amino-9H-pyrido[2,3-*b*]indole (A α C), which have been recognized to induce two DNA adducts: 3',5'-diphosphate-N-(2'-deoxyguanosin-8-yl)-PhIP (3',5'-pdGp-C8-PhIP) and 3',5'-diphosphate-N-(2'-deoxyguanosin-8-yl)-A α C (3',5'-pdGp-C8-A α C). The correlations between the amount of DNA adducts induced by both compounds *in vitro* and the content of cytochrome P450 in human microsomes are statistically significant at $r^2 = 0.69$ and $r^2 = 0.62$ ($P < 0.001$), respectively. Furthermore, the level of DNA adducts after treatment

with PhIP and A α C correlated with the activities of three isozymes of cytochrome P450: CYP1A1, CYP1A2, and CYP3A4. Therefore, three chemical inhibitors were used in the experiments: ellipticine against CYP1A1, furafylline against CYP1A2, and troleandomycin against CYP3A4. The highest inhibition levels in the formation of 3',5'-pdGp-C8-PhIP and 3',5'-pdGp-C8-A α C adducts were estimated to occur in the presence of furafylline at 56% and 69%, respectively. Ellipticine was involved in the inhibition of 40% of 3',5'-pdGp-C8-PhIP adducts and in only 18% of the inhibition of 3',5'-pdGp-C8-A α C adducts. Troleandomycin did not significantly inhibit the formation of 3',5'-pdGp-C8-PhIP adducts under these conditions, but it inhibited the formation of 31% of the 3',5'-pdGp-C8-A α C adducts. We conclude that the formation of DNA adducts can be used as a relevant marker of interindividual variability in the metabolic activation of HCAs in humans. (Cancer Epidemiol Biomarkers Prev 2004; 13(6):1071–8)

Introduction

Epidemiological studies show a positive correlation between the consumption of well-done meat and the incidence of colon cancer in humans (1). Heterocyclic amines (HCA) are formed during cooking at high temperatures of proteinaceous food, such as meat and fish (2, 3). Humans who consume a normal diet are regularly exposed to these food-borne compounds. HCAs are also reported to be carcinogenic to animals and to induce the formation of HCA-DNA adducts in human tissues (4–6). In chronic animal studies about carcinogens, a correlation has been shown between early DNA adduct formation and later appearance of tumors (7). In common with other genotoxic carcinogens, HCAs

require metabolic activation before becoming mutagenic or carcinogenic (8). Studies involving human volunteers and furafylline—a specific inhibitor of cytochrome P450 (CYP) 1A2 isozyme—have suggested the general metabolic pathways of HCAs in humans *in vivo* (9). HCAs are metabolically activated by CYP1A2 to the corresponding *N*-hydroxylamine, which may bind to DNA or may be further metabolized by acetyltransferase NAT2 (8, 10). Currently, it is suggested that other isozymes of cytochrome P450 may also be involved in the *N*-hydroxylation of HCAs and in the induction of DNA adducts. Three isozymes of cytochrome P450, namely, CYP1A2, CYP1A1, and CYP1B1, have been shown to be involved in the *N*-hydroxylation of 2-amino-1-methyl-6-phenylimidazo [4,5-*b*]pyridine (PhIP) and other HCAs (11, 12). Also, for 2-amino-9H-pyrido[2,3-*b*]indole (AC), two isozymes—CYP1A2 and CYP1A1—together with other isozymes of cytochrome P450, such as CYP2C10, have been suggested to be responsible for *N*-hydroxylation of the amine (13).

The aim of the present study was to investigate the correlation between DNA adduct formation by HCAs

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and human metabolic activation phenotypes *in vivo* and *in vitro*. In addition, the aim was to show the capacity of human liver microsomes with different phenotypes *in vitro* to activate two HCAs, PhIP, and AC, to form DNA adducts: 3',5'-diphosphate-N-(2'-deoxyguanosin-8-yl)-PhIP (3',5'-pdGp-C8-PhIP; ref. 14) and 3',5'-diphosphate-N-(2'-deoxyguanosin-8-yl)-AC (3',5'-pdGp-C8-AC), respectively (15).

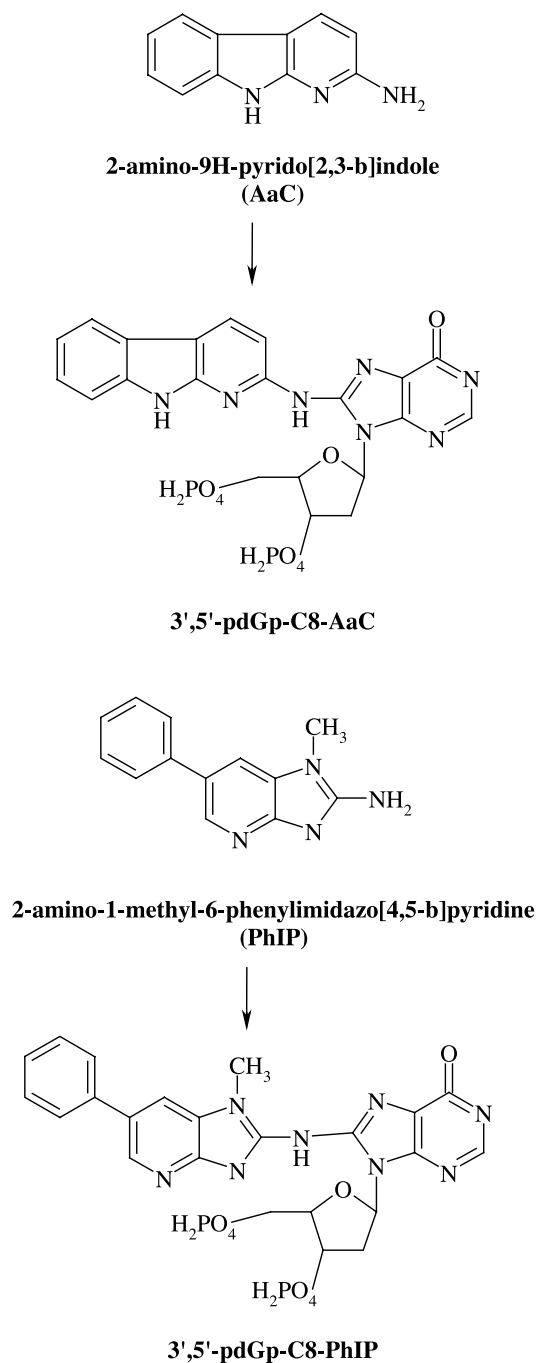


Figure 1. Chemical structures of AαC and PhIP and their corresponding DNA adducts formed by human liver microsomes: 3',5'-pdGp-C8-PhIP and 3',5'-pdGp-C8-AαC.

Materials and Methods

Chemicals. The HCAs AαC and PhIP were purchased from Toronto Research Chemicals, Toronto, Canada. Chemical structures are shown in Fig. 1. Inhibitors were obtained from the following sources: Salford Ultrafine Chemicals & Research Ltd., Manchester, United Kingdom (furaflavone) and Sigma Chemical Co., St. Louis, MO (ellipticine and troleandomycin). The dGp-C8-PhIP from an *in vitro* reaction was a generous gift from Dr. Henrik Frandsen (Institute of Toxicology, National Food Agency, Denmark). The sources of all other enzymes and chemicals have been described elsewhere (16). All solvents and salts were of analytic grade and all water used was run through a MilliQPLUS system (Millipore, Milford, MA).

Synthesis of 2-Nitro-9H-Pyrido[2,3-b]Indole and Reaction with Calf Thymus DNA *In vitro*. The synthesis of 2-nitro-9H-pyrido[2,3-b]indole (NC) and reaction with calf thymus DNA *in vitro* were done as described previously (15) with the following changes: AC (40 mg) was dissolved in 1.0 ml of 50% acetic acid and added to a solution of sodium nitrate (600 mg) in 1.2 ml of water. The mixture was stirred for 1 hour and the yellow precipitate was separated by centrifugation. Calf thymus DNA was dissolved (1 mg/ml) in 100 mmol/L sodium dihydrogen phosphate buffer (pH 5.5) and 2.5 mg of NC in acetonitrile were added in the presence of 5 mg zinc chloride. The reaction occurred overnight at room temperature under continuous shaking. DNA was precipitated by addition of 5 mol/L NaCl and cold 96% ethanol.

Human Liver Samples and Microsomes. The human liver samples and microsomes were obtained from Human Cell Culture Center (Laurel, MA). The characterization of the donors is shown in Table 1. The concentration of cytochrome P450 was determined and the microsomes were characterized for the following enzyme activities: 7-ethoxyresorufin *O*-deethylase (CYP1A1), 7-ethoxycoumarin *O*-deethylase (CYP1A2), 5-mephenytoin 4'-hydroxylase (CYP2C9), bufuralol 1'-hydroxylase (CYP2D6), chlorzoxazone 6'-hydroxylase (CYP2E1), and testosterone 6-hydroxylase (CYP3A4) as described previously (17).

Incubation Conditions. Calf thymus DNA (Sigma) was dissolved in 100 mmol/L potassium phosphate buffer (pH 7.4) at a concentration of 1 mg/ml, and 0.5 ml of the solution was used for incubation with human liver microsomes. All incubations with liver microsomes were done at a protein concentration corresponding to 1 mg/ml in 100 mmol/L potassium phosphate buffer (pH 7.4), 0.1 mol/L glucose 6-phosphate, 0.15 mol/L potassium chloride, 0.04 mol/L magnesium chloride hexahydrate, and 0.08 mol/L b-NADPH at 37°C for 1 hour. The different inhibitors were dissolved in methanol and then added in 10-μL aliquots (1% v/v final concentration) to the microsomes. Ten microliters of methanol were used in control experiments. Furaflavone and ellipticine were added at final concentrations of 25, 100, and 200 μmol/L, and troleandomycin at 50, 200, and 300 μmol/L. Incubations containing inhibitors were preincubated with microsomes for 15 minutes before addition of AαC or PhIP. AαC and PhIP were used at

Table 1. Characterization of human liver donors and microsomes as well as the phenotypes of the microsomes

Donor no.	Sex	Race	Age (y)	Smoking history	Total P450	CYP1A2 activity	CYP1A1 activity	CYP3A4 activity	CYP2D6 activity	CYP2E1 activity	CYP2C19 activity
1	M	Cauc.	32	NS	398	355	91.4	20.4	401	608	4.2
2	M	Black	40	S	374	306	587	17.3	180	431	5.1
3	M	Black	52	S	265	231	413	7.39	167	514	5.2
4	F	Cauc.	53	NS	265	357	49.2	16.7	89.1	351	5.4
5	F	Cauc.	34	S	226	344	138	6.08	112	392	22.4
6	F	Black	53	NS	214	183	87.8	6.02	77.6	195	9.4
7	F	Cauc.	49	NS	199	125	37.2	11.8	83.2	215	13.8
8	F	Black	55	S	170	306	66.4	5.14	102	252	9.9
9	M	Cauc.	39	NS	170	253	74.2	2.73	71.4	309	6.5
10	F	Cauc.	42	NS	131	281	107	2.79	113	363	6.8
11	F	Cauc.	44	S	95	207	27.2	4.26	56.4	453	0.0
12	F	Cauc.	52	S	69	165	94.2	5.52	36.8	186	0.0
13	F	Cauc.	65	S	43	490	145	20.0	291	373	18.3
14	F	Cauc.	59	NS	35	4	48.4	3.21	99.3	245	3.2
15	M	Cauc.	51	NS	30	56	27.2	0.28	47.9	242	4.0

NOTE: The total level of cytochrome P450 is given as picomoles per milligram. The activities of isozymes CYP1A2, 1A1, 3A4, 2D6, 2E1, and 2C19 are shown as pmol/mg of protein/min.

Abbreviations: M, male; F, female; Cauc., Caucasian; NS, nonsmoker; S, smoker.

a concentration of 10 $\mu\text{mol/L}$. The reactions were terminated by precipitation of DNA with 5 mol/L sodium chloride and cold 96% ethanol.

DNA Preparation. DNA was isolated from human liver samples by a phenol-chloroform extraction procedure according to previously published reports (18). DNA concentration and purity were determined spectrophotometrically at 260 and 280 nm. DNA was split into 10- μg aliquots and stored at -80°C until analysis. Samples of 10 μg of DNA were hydrolyzed by using micrococcal nuclease and spleen phosphodiesterase. The digested DNA was extracted for adducted nucleotides by using the butanol extraction enhancement method following a procedure described previously (16). Evaporated butanol extracts were stored at -80°C until analysis.

^{32}P -Postlabeling. Evaporated butanol-extracted samples (20 μg of DNA) were dissolved in 5.0 μL of water. PNK buffer (0.5 μL , 400 mmol/L, pH 9.6), T4 polynucleotide kinase (1.0 μL , 10 units), and [^{32}P]ATP (3.5 μL , 35 μCi) were added to a final volume of 10 μL . The mixture was incubated for 30 minutes at 37°C followed by dilution with water to 170 μL and storage at -20°C until analysis.

^{32}P -HPLC Analysis. The HPLC system consisted of a 600E-multisolute delivery system, a DeltaPak 5 $\mu\text{C}18$ 100A main column (Waters Chromatography, Milford, MA), a NewGuard RP18 precolumn (Brownlee Laboratories, Santa Clara, CA), and an on-line A280 radioactivity detector that used a 0.5-ml cell and scintillation fluid FloScint IV (both Radiomatic Instruments & Chemicals Co., Tampa, FL). The energy window was set to 8 to 600 keV with a counting efficiency of 60% for ^{32}P . Counting was done in 12-second cycles.

^{32}P -HPLC analysis was done according to earlier published methods (16, 18) with minor modifications. Briefly, after injection into the HPLC, the samples were eluted with 0.5 ml/min of 2.0 mol/L ammonium formate, 0.4 mol/L formic acid (pH 4.5), and a linear gradient of 0% to 35% acetonitrile from 0 to 70 minutes. A three-way valve was included between the precolumn

and the main column. For the first 5 minutes after injection of the samples, the flow was only allowed to go through the precolumn; then the three-way valve was switched to let the flow go through the main column and the detector. The ^{32}P -HPLC system is described in detail elsewhere (16, 18).

Statistical Analysis. For statistical analyses, SigmaStat statistical software (SPSS Inc., Chicago, IL) was used. Correlations between all variables were analyzed using the Pearson Product Moment Correlation test. Values at $P < 0.005$ and $P < 0.001$ were considered to be statistically significant.

Results

Estimation of *In vivo*-Generated DNA Adducts in Human Liver Tissues.

The total level of DNA adducts

Table 2. Total endogenous DNA adduct levels from the human livers of the donors shown in Table 1

Donor no.	Total level of DNA adducts* (DNA adducts/ 10^8 NN)
1	26.8 \pm 6.7
2	19.7 \pm 3.4
3	17.1 \pm 1.5
4	20.0 \pm 2.9
5	10.2 \pm 3.4
6	17.3 \pm 4.1
7	16.2 \pm 5.8
8	23.0 \pm 4.7
9	16.1 \pm 5.1
10	14.5 \pm 3.1
11	13.0 \pm 2.9
12	15.5 \pm 5.2
13	12.3 \pm 3.3
14	10.5 \pm 3.2
Mean value (all)	16.6 \pm 4.7
Mean value, females	15.2 \pm 4.8
Mean value, males	19.9 \pm 4.1

*Determined by ^{32}P -HPLC.

was analyzed in liver tissue from the 14 donors by using the ^{32}P -HPLC technique (Table 2). The donors were an average age of 48 years, with a range of 32 to 65 years. Of the group of 14, 10 were females and 4 were males. It was not possible to analyze the DNA adducts in the liver tissue from donor 15. The mean value of DNA adducts was calculated to be $16.6 \pm 4.7/10^8$ normal nucleotides (NN), with a range of 10.5 ± 3.2 to 26.9 ± 6.9 DNA adducts/ 10^8 NN. The correlation between the total amount of human liver DNA adducts and the content of cytochrome P450 in the liver samples was statistically significant ($r^2 = 0.71$ and $P < 0.005$), as calculated by using a linear regression model from data shown in Fig. 2. No statistically significant difference was detected in DNA adduct levels between females and males: 15.2 ± 4.8 versus $19.9 \pm 4.1/10^8$ NN, respectively. There was no significant difference in liver DNA adducts when smokers and nonsmokers were compared. No information was available on exposure of the donors to passive smoking or about dietary patterns. Because the size of the study cohort is limited to 15 individuals, the absence of significance might not reflect the true situation in the population.

For donor 13, a peak with a retention time (t_R) of 58.6 minutes (Fig. 3B) at a DNA adduct level of $1.4/10^8$ NN was detected. The peak was characterized as possibly being the $3',5'$ -pdGp-C8-PhIP adduct ($3',5'$ -pdGp-C8-PhIP) by using a $3',5'$ -pdGp-C8-PhIP standard synthesized *in vitro* (14). This step was followed by co-chromatography in two different systems by using HPLC and TLC for separation of ^{32}P -ATP-labeled DNA adducts (Fig. 3). This peak, with an identical retention time as the $3',5'$ -pdGp-C8-PhIP in two different chromatographic systems, was only found in donor 13, which represented an individual with generally high levels of several P450 isozymes (Table 1).

DNA Adduct Provocation by PhIP and A α C, *In vitro*, in the Presence of Human Liver Microsomes. The human liver microsomes from the 15 donors (Table 1) were incubated with calf thymus DNA *in vitro* in the presence of PhIP or AC for 1 hour, after which the DNA adduct levels were measured by the ^{32}P -HPLC technique.

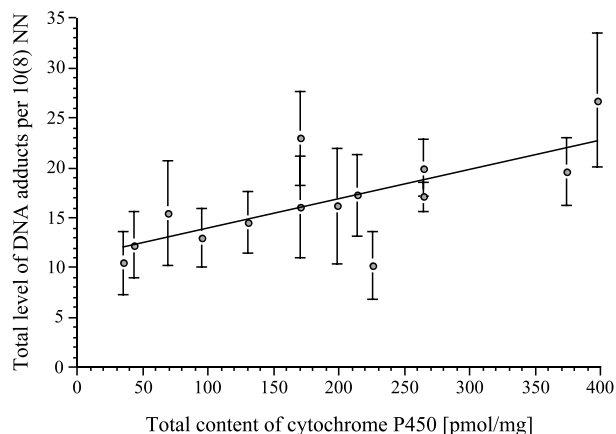


Figure 2. Curve, correlation between total content of cytochrome P450 and total level of endogenous DNA adducts (DNA adducts/ 10^8 NN) of human livers. Point, one individual. The correlation is statistically significant ($r^2 = 0.71$, $P < 0.005$).

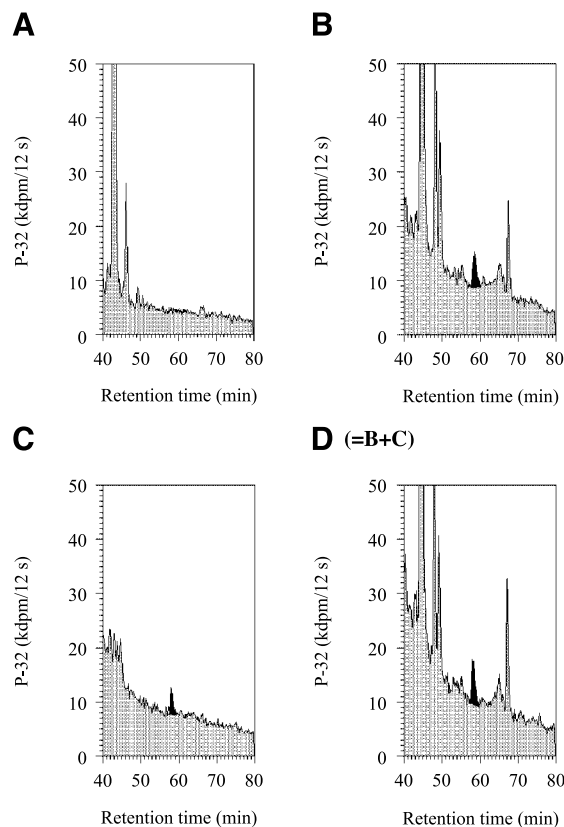


Figure 3. ^{32}P -HPLC chromatograms of control, calf thymus DNA after treatment *in vitro* with DMSO (A), DNA isolated from the liver of donor 13 (B), and $3',5'$ -pdGp-C8-PhIP synthesized *in vitro* (C; ref. 14). Panel D shows co-chromatography of DNA from the liver of donor 13 and $3',5'$ -pdGp-C8-PhIP generated *in vitro*. The black peak shows the position of the $3',5'$ -pdGp-C8-PhIP adduct.

AC induced DNA adducts for all donors under these conditions, but for PhIP-DNA adducts, only samples from 13 donors were available. For PhIP, the DNA adduct level was detected at one specific peak ($t_R = 58.6$ minutes). The peak was characterized as the $3',5'$ -pdGp-C8-PhIP adduct by using a $3',5'$ -pdGp-C8-PhIP standard and co-chromatography on the ^{32}P -HPLC (data not shown). One peak was also found for AC ($t_R = 53.0$ minutes). The peak was characterized as the $3',5'$ -pdGp-C8-AC adduct ($3',5'$ -pdGp-C8-AC) by using calf thymus DNA-AC standard and co-chromatography by ^{32}P -HPLC (data not shown). The correlations between the amount of DNA adduct induced by the two compounds and the cytochrome P450 isozymes were as follows. DNA adduct levels from provocation by PhIP or AC and the total content of cytochrome P450 in human liver microsomes were statistically significant ($r^2 = 0.69$, $P < 0.001$, and $r^2 = 0.62$, $P < 0.001$, respectively) (Fig. 4). For all donors, except donor 13, the AC-induced formation of DNA adducts was higher when compared with $3',5'$ -pdGp-C8-PhIP. No differences in DNA adduct levels induced by PhIP and AC were found for gender or smoking.

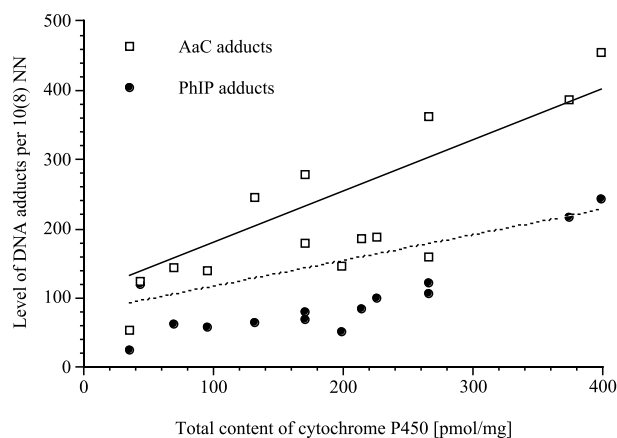


Figure 4. Correlation between total content of cytochrome P450 in human liver microsomes and DNA adduct formation after provocation of calf thymus DNA *in vitro* with PhIP ($r^2 = 0.69$, $P < 0.001$) or A α C ($r^2 = 0.62$, $P < 0.001$) in the presence of human liver microsome preparations (Table 1).

Furthermore, the induction of 3',5'-pdGp-C8-PhIP and 3',5'-pdGp-C8-A α C DNA adducts detected under the conditions used in this study with human microsomes showed a statistically significant correlation with the activities for three isozymes of cytochrome P450: CYP1A1 ($r^2 = 0.93$ for 3',5'-pdGp-C8-PhIP and $r^2 = 0.82$ for 3',5'-pdGp-C8-AC DNA, $P < 0.001$), CYP1A2 ($r^2 = 0.76$ for 3',5'-pdGp-C8-PhIP and $r^2 = 0.80$ for 3',5'-pdGp-C8-AC DNA, $P < 0.001$), and CYP3A4 ($r^2 = 0.84$ for 3',5'-pdGp-C8-PhIP and $r^2 = 0.80$ for 3',5'-pdGp-C8-AC DNA, $P < 0.001$) (Fig. 5).

Effect of Enzyme Inhibitors on DNA Adduct Formation. The effect of the enzyme inhibitors ellipticine, furafylline, and troleandomycin on the formation of 3',5'-pdGp-C8-A α C DNA adducts induced by the human liver microsomes of donor 1 is shown in Fig. 6. Ellipticine, an inhibitor of CYP1A1, and furafylline, an inhibitor of CYP1A2, both significantly inhibited the induction of 3',5'-pdGp-C8-A α C adducts (Fig. 6A and B, respectively). A significant inhibition was also observed for the formation of 3',5'-pdGp-C8-A α C DNA adducts with troleandomycin, which is known to specifically interact with CYP3A4 isozymes (Fig. 6C).

For the determination of differences in metabolic activation and in the induction of DNA adducts for PhIP and A α C, the human liver microsomes from donors 1, 4, and 13 were used. The microsomes of the donors represented high, medium, and low content of cytochrome P450 (Table 1). The human liver microsomes were incubated *in vitro* in the presence of calf thymus DNA with PhIP or AC, and DNA adduct levels were estimated by the 32 P-HPLC technique.

For all three donors, the highest inhibition in the formation of 3',5'-pdGp-C8-PhIP and 3',5'-pdGp-C8-A α C DNA adducts was found in the presence of furafylline (inhibitor of CYP1A2) at the levels of 56% and 69%, respectively (Fig. 7). Ellipticine (inhibitor of CYP1A1) inhibited DNA adduct formation with 40% of 3',5'-pdGp-C8-PhIP adducts and with 18% of 3',5'-pdGp-C8-A α C

adducts (Fig. 7). Troleandomycin (inhibitor of CYP3A4) did not significantly inhibit the formation of 3',5'-pdGp-C8-PhIP adducts under these conditions. However, this concentration of troleandomycin inhibited the formation of 31% of the 3',5'-pdGp-C8-A α C DNA adducts (Fig. 7).

In addition, 3',5'-pdGp-C8-PhIP and 3',5'-pdGp-C8-A α C DNA adduct formation did not show any correlation with the activity of acetyltransferase NAT2 (data not shown).

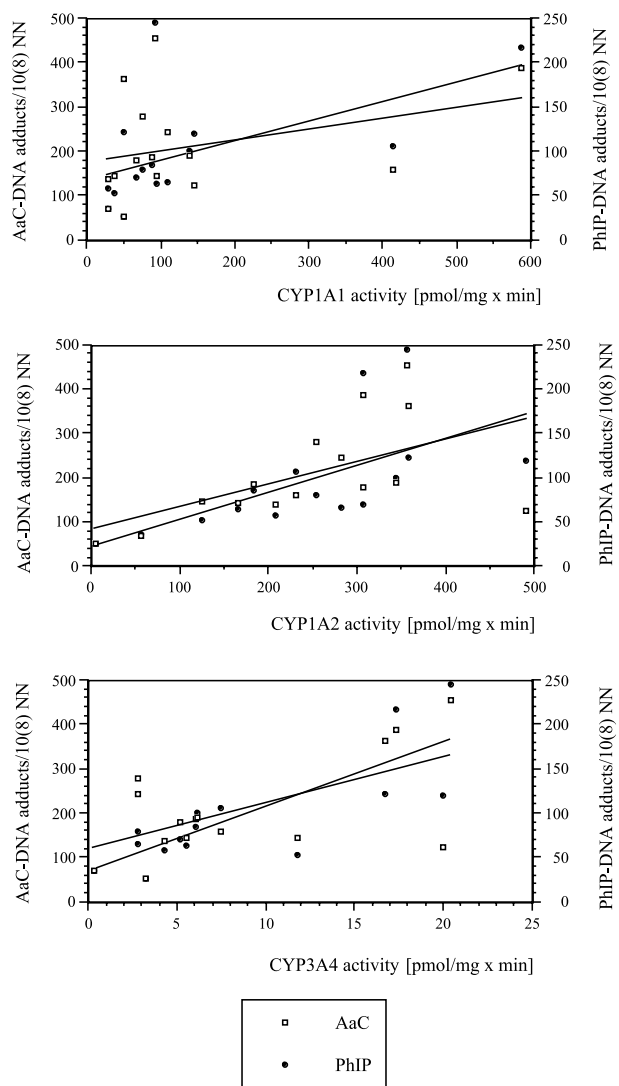


Figure 5. Curves, correlation between the activities of isozymes of cytochrome P450 (CYP1A1, CYP1A2, CYP3A4) and DNA adduct levels after provocation of calf thymus DNA *in vitro* with PhIP ($n = 13$, $r^2 = 0.93$, $r^2 = 0.82$, $r^2 = 0.84$, $P < 0.001$, respectively) or A α C ($n = 15$, $r^2 = 0.82$, $r^2 = 0.80$, $r^2 = 0.80$, $P < 0.001$, respectively) in the presence of human liver microsome preparations. DNA adduct levels are shown as DNA adducts/ 10^8 NN. For PhIP-DNA adducts, only samples from 13 donors were available. Open squares, 3',5'-pdGp-C8-A α C adducts; black circles, 3',5'-pdGp-C8-PhIP adducts.

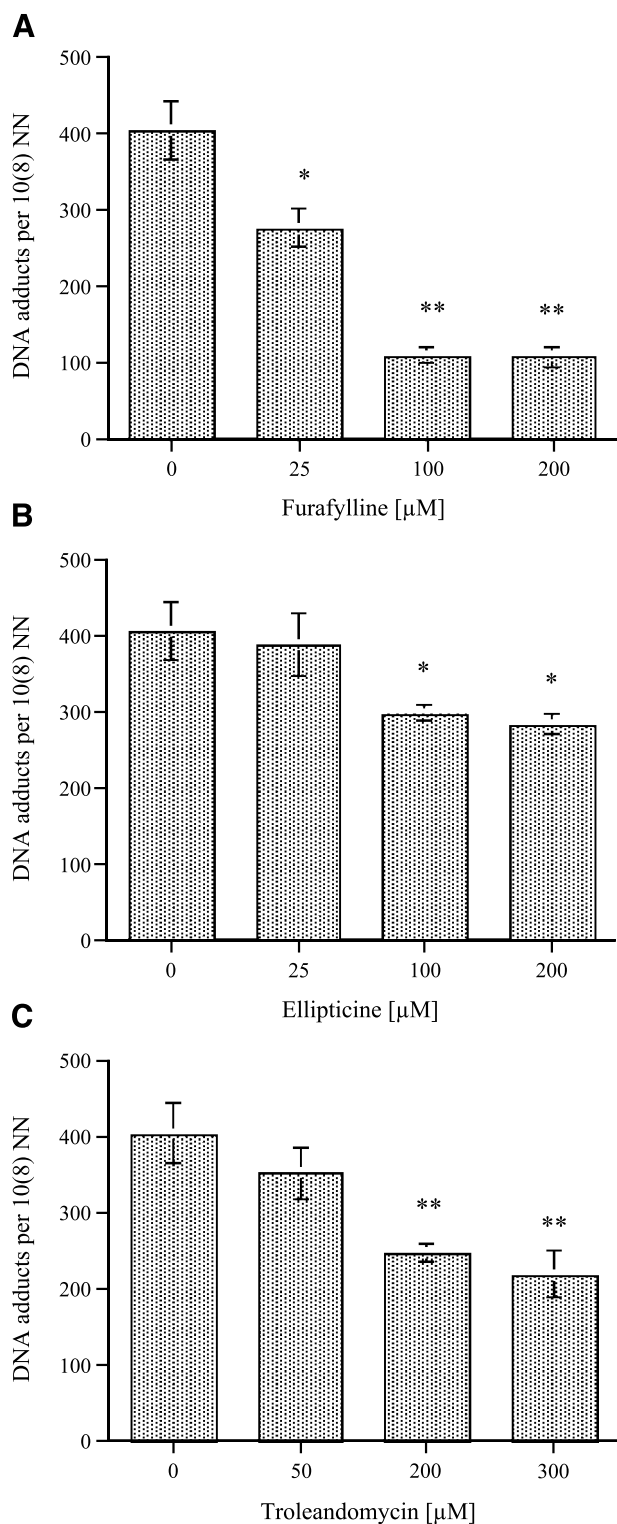


Figure 6. The influence of concentrations of specific inhibitors: furafylline (A), ellipticine (B), and troleandomycin (C) on induction of DNA adducts after treatment of calf thymus DNA *in vitro* with A α C in the presence of human liver microsomes of donor 1. Columns, mean; bars, SD. *, $P < 0.05$; **, $P < 0.01$.

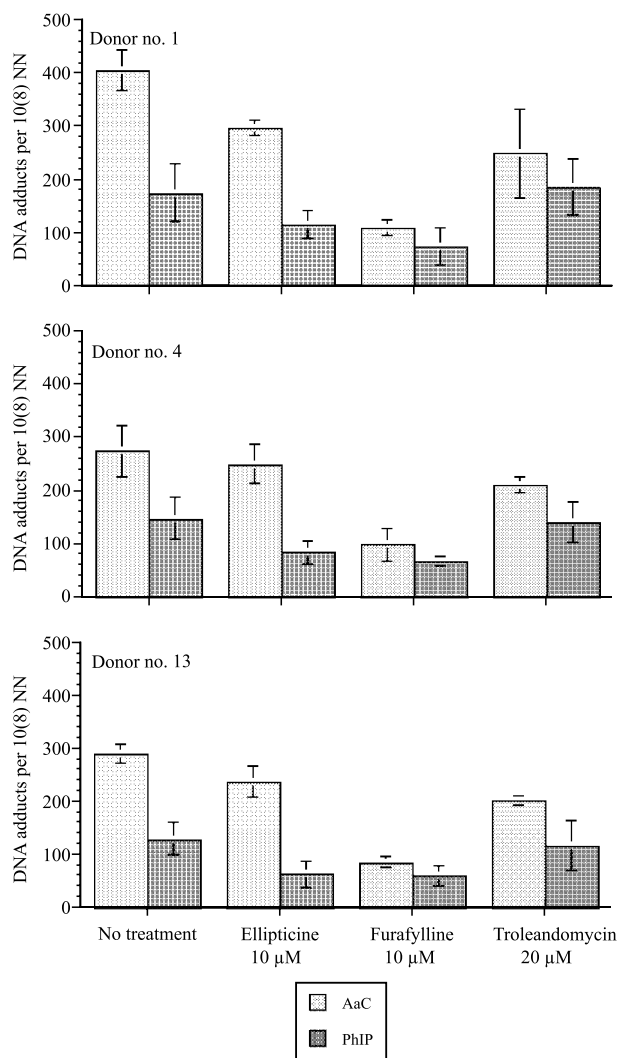


Figure 7. Inhibition of DNA adducts after provocation of calf thymus with A α C or PhIP in the presence of human liver microsomes (donors 1, 4, and 13, respectively) and specific inhibitors of isozyme CYP1A1 (*Ellipticine*), CYP1A2 (*Furafylline*), and CYP3A4 (*Troleandomycin*). Columns, mean; bars, SD.

Discussion

The results presented in this study provide evidence that the level of endogenous DNA adducts, measured at a target organ like human liver, correlate with the total endogenous content of cytochrome P450. This correlation was also present under *in vitro* conditions, in which DNA adduct formation was provoked with carcinogenic compounds in the presence of human liver microsomes. DNA adduct formation is generally regarded as a critical, initiating event in the multistep process of chemical carcinogenesis (19). Therefore, the results of this study support a hypothesis that the carcinogenic effect of many chemicals depends on their metabolic transformation into active intermediates that can induce modifications in DNA (20). The results also suggest that the enzyme systems, and the changes in activities or expression of

these enzyme systems that are responsible for such activation or metabolism, are very important factors in the interaction between humans and chemicals (8, 21). Therefore, any exogenous or endogenous factors that can alter the activities or capacities of the enzyme systems responsible for metabolism may influence interindividual variability in metabolic activation and human susceptibility to chemical carcinogenesis.

One possible approach to determine the differences in metabolic activation of chemicals, interindividual variability, and human susceptibility is to conduct experiments *in vitro* by using human liver microsomes to detect DNA adducts.

The results presented in this study provide evidence for the involvement of CYP1A2 in the formation of 3',5'-pdGp-C8-PhIP and 3',5'-pdGp-C8-AαC adducts in humans. The formation of 3',5'-pdGp-C8-PhIP and 3',5'-pdGp-C8-AαC adducts is dependent on CYP1A2, from a high correlation with 7-ethoxycoumarin *O*-deethylation and on inhibition by a specific inhibitor of CYP1A2: furafylline (9). The formation of 3',5'-pdGp-C8-PhIP adducts correlates also with 7-ethoxyresorufin *O*-deethylation and inhibition by ellipticine, a selective inhibitor of isozyme CYP1A1 of cytochrome P450. A recent study that compared PhIP activation by CYP1A1, CYP1A2, and CYP1B1 by using a *Salmonella typhimurium* strain to determine DNA damage by activated carcinogens showed that all three P450 isozymes were capable of activating PhIP to form reactive intermediates (11, 12). Recently, it was also reported that isozyme CYP1B1, the newest member of the dioxin-inducible CYP1 family that includes CYP1A1 and CYP1A2, is involved in metabolic activation of PhIP (22). Therefore, further studies will be required to estimate the relative activity of each isozyme of cytochrome P450 in the metabolism of PhIP.

The formation of 3',5'-pdGp-C8-AαC adducts correlates not only with the activity of CYP1A2 but also with testosterone 6β-hydroxylation and inhibition by troleanomycin, and with 7-ethoxyresorufin *O*-deethylation and inhibition by ellipticine. So far, only limited data are available about the metabolism of AC (10, 13). The results of this study confirm that CYP1A2 is associated with high activity for AC metabolism, which is in accordance to recently published data (12). Raza et al. (13) also suggested that the activities of CYP1A1 and CYP2C10 were implicated in the metabolic activation of AC. The present study confirms this finding for isozyme CYP1A1 of cytochrome P450, but a significant activity was also seen for CYP3A4, which has been shown to be present in as much as 60% of the total P450 content in human liver (23).

In conclusion, this study on human microsomes showed the following results:

(a) The human endogenous DNA adduct level was related to the total content of cytochrome P450.

(b) The mean values of total DNA adduct levels in the human livers tested were 10 to 27 DNA adducts/10⁸ NN.

(c) Gender and smoking habits did not show any significant correlation to endogenous DNA adduct levels of human livers. Because the size of the study cohort is limited to 15 individuals, the absence of significance might not reflect the true situation in the population.

(d) One individual with a high expression of several CYP isozymes showed an endogenous DNA adduct that co-chromatographed in two different systems with the food mutagen PhIP (3',5'-pdGp-C8-PhIP).

(e) For *in vitro* reaction of human liver microsomes and DNA, the food mutagens PhIP and AαC formed DNA adducts. The DNA adducts formed were 3',5'-pdGp-C8-PhIP and 3',5'-pdGp-C8-AαC, respectively, from co-chromatography with standards.

(f) In all cases except one, AαC was more potent in inducing DNA adducts after activation of human microsomes when compared with PhIP.

(g) The induction of DNA adducts by AαC and PhIP was significantly correlated to higher levels of CYP1A1, CYP1A2, and CYP3A4 as well as to the inhibition of these isozymes, followed by a lowered capacity to form DNA adducts.

(h) CYP 1A2 was most important for the induction of DNA adducts by AαC and PhIP.

The presented data show that humans with induced phenotypes of various forms of the cytochrome P450 system had a higher level of endogenous DNA adducts. These individuals were also more sensitive in forming DNA adducts when exposed to food mutagens.

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