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DNA adduct formation of 14 heterocyclic aromatic amines in mouse tissue after oral administration and characterization of the DNA adduct formed by 2-amino-9H-pyrido[2,3-b]indole (AαC), analysed by ^{32}P-HPLC

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Heterocyclic aromatic amines (HAAs) are produced during cooking of proteinaceous food such as meat and fish. Humans eating a normal diet are regularly exposed to these food-borne substances. HAAs have proved to be carcinogenic in animals and to induce early lesions in the development of cancer. DNA adduct levels in mouse liver have been measured by ^{32}P-HPLC after oral administration each of 14 different HAAs. The highest DNA adduct levels were detected for 3-amino-1-methyl-5H-pyrido[4,3-b]-indole (Trp-P-2), 3-amino-1,4-dimethyl-5H-pyrido[4,3-b]indole (Trp-P-1) and 2-amino-9H-pyrido[2,3-b]indole (AαC), respectively. To assess a relative risk in a human population, a relative risk index was calculated by combining the DNA adduct levels in mouse liver with human daily intake of heterocyclic amines in a US and in a Swedish population. Such calculations suggest that AαC presents the highest risk for humans, e.g. nine-fold higher compared with the most abundant amines in food, 2-amino-1-methyl-6-phenylimidazo[4,5-b]-pyridine (PhIP). Therefore, the distribution of DNA adducts in different tissues of mouse was investigated after oral administration of AαC. The highest AαC–DNA adduct levels were found in liver (137 adducts/10^8 normal nucleotides) followed by heart, kidney, lung, large intestine, small intestine, stomach and spleen, in descending order. To characterize the chemical structure of the major DNA adduct, chemical synthesis was performed. The major DNA adduct from the *in vivo* experiments was characterized by five different methods. On the basis of these results, the adduct was characterized as N^2-(deoxyguanin-8-yl)-2-amino-9H-pyrido[2,3-b]indole. Considering the abundance of AαC not only in grilled meat, but also in other products like grilled chicken, vegetables and cigarette smoke and in light of the results of the present study, it is suggested that the human cancer risk for AαC might be underestimated.

Keywords: DNA adduct, cancer, food, amine, food mutagen.

Introduction

Heterocyclic aromatic amines (HAAs) are formed during cooking of proteinaceous food such as meat and fish at high temperatures (Johansson and Jägerstad 1994). Humans consuming a normal diet are regularly exposed to these food-borne mutagens and epidemiological studies show a positive correlation between ingestion of well-done meat and incidence of colon cancer in man (Lang *et al.* 1994, Butler *et al.* 2003).

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HAAs are reported to be carcinogenic to animals and to induce early pre-carcinogenic DNA lesions (Turesky 2002). The presence of HAA–DNA adducts in human tissues has been demonstrated (Friesen *et al.* 1994, Totsuka *et al.* 1996, Turteltaub *et al.* 1997) as well as mutational fingerprints of HAAs in the *APC* gene (Nagao *et al.* 1997, Nagao 1999), in which mutations occur at an early stage of colon carcinogenesis (Powell *et al.* 1992). HAA–DNA adducts are believed to play an important role in the initial stages of chemical carcinogenesis (Cui *et al.* 1995, Turesky 2002).

The human intake of HAAs depends upon the amount and type of meat in the diet, the frequency of consumption, the heating temperature and the duration of food processing, etc. (Bogen and Keating 2001). Several studies have been performed to establish daily intake of HAAs in a US (Layton *et al.* 1995, Bogen and Keating 2001) and a Swedish population (Augustsson *et al.* 1997). These studies have shown that daily intake of HAAs differ significantly between countries according to lifestyle and food habits. Among the HAAs, 2-amino-9H-pyrido[2,3-b]indole (AαC) was shown to be present in human diet at high levels; 5.17 ng kg^{-1} day^{-1} in the US population (Layton *et al.* 1995).

AαC was first isolated and identified in the pyrolysis products of soybean globulin (Yoshida *et al.* 1978). It was also detected at significant levels not only in grilled food like beef, fish, vegetables (up to 180 ng g^{-1}), but also in cigarette smoke (42 ng/cigarette) (Matsumoto *et al.* 1981, Eisenbrand and Tang 1993, Layton *et al.* 1995).

CDF mice fed AαC showed tumours in liver (97% in female, 39% in male) and in blood vessels (18% in female, 53% in male) (Ohgaki *et al.* 1984). Furthermore, after treatment with AαC, DNA adducts were detected in rat salivary glands and liver (Yamashita *et al.* 1986). Studies on mouse, rat and human hepatic microsomes have suggested the metabolic pathways that result in formation to AαC–DNA adducts (Raza *et al.* 1996, Frederiksen and Frandsen 2003, Baranczewski and Möller 2004). AαC is metabolically activated mostly by cytochrome P4501A2 to the corresponding hydroxylamine, which after esterification may form DNA adducts. The major hepatic DNA adduct found in rat hepatocytes *in vitro* and in male F344 rats as well as male ICR mice after treatment with AαC *in vivo* was identified as N^2-(deoxyguanosin-8-yl)-2-amino-9H-pyrido[2,3-b]indole (Pfau *et al.* 1997, Frederiksen *et al.* 2004, Majer *et al.* 2004).

The aim of the present study was to do the following:

- Compare the *in vivo* DNA adducts formation of 14 HAAs.
- Make a relative risk comparison between the 14 HAAs.
- Investigate AαC-induced DNA adducts in relation to other HAA–DNA adducts as measured by the ^{32}P-HPLC method, *in vivo* in mice.
- Investigate AαC–DNA adduct formation in different tissues of mice.
- Characterize the major DNA adduct of AαC formed *in vivo* in mice.

Materials and methods

Chemicals

The heterocyclic amines: 3-amino-1,4-dimethyl-5H-pyrido[4,3-b]indole (Trp-P-1), 3-amino-1-methyl-5H-pyrido[4,3-b]indole (Trp-P-2), 2-amino-9H-pyrido[2,3-b]indole (AαC), 2-amino-3-methyl-9H-pyrido[2,3-b]indole (MeAαC), 2-amino-6-methyldipyrido[1,2-a:3′,2′-d]imidazole (Glu-P-1), 2-aminodipyrido[1,2-a:3′,2′-d]imidazole (Glu-P-2), 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhIP), 2-amino-3-methylimidazo[4,5-f]quinoxaline (IQx), 2-amino-3,8-dimethylimidazo[4,5-f] quinoxaline (MeIQx), 2-amino-3,4,8-trimethyl-3H-imidazo[4,5-f]quinoxaline (4,8-diMeIQx), 2-amino-3,7,8-trimethyl-3H-imidazo[4,5-f]quinoxaline (7,8-diMeIQx), 2-amino-3,4,7,8-tetramethyl-3H-imidazo[4,5-f] quinoxaline (4,7,8-triMeIQx), 2-amino-3-methylimidazo[4,5-f]quinoline (IQ) and 2-amino-3,4-dimethylimidazo[4,5-f]quinoline (MeIQ) were purchased from Toronto Research Chemicals (Toronto, Canada). Enzymes and chemicals were obtained from the following sources: RNAse A, RNAse T1, micrococcal nuclease and calf thymus DNA (Sigma, St Louis, MO, USA), spleen phosphodiesterase (Boehringer, Mannheim, Germany) and protein kinase K (Merck, Darmstadt, Germany). T4 Polynucleotide kinase and adenosine 5′[γ-^{32}P]triphosphate (3000 Ci mmol^{-1}) were from Amersham (Little Chalfont, UK). All solvents and salts were of analytical grade and all water used was run through a MilliQ$_{PLUS}$ system (Millipore, Milford, MA, USA).

Treatment of animals

Male mice of the strain NMRI (age 8 weeks) with an average body weight of 31 g were used in all experiments. The animals were kept under barrier conditions with water and diet *ad libitum* and a light–dark cycle of 12 h. The animals were divided into 15 groups, with three animals in each group. Each group was given an oral dose of 40 mg kg^{-1} bw of one of the following 14 HAAs: Trp-P-1, Trp-P-2, AαC, MeAαC, Glu-P-1, Glu-P-2, PhIP, IQx, MeIQx, 4,8-diMeIQx, 7,8-diMeIQx, 4,7,8-triMeIQx, IQ or MeIQ, three times during 3 consecutive days. The amines were dissolved in 200 µl 50% ethanol (v/v). The control group was given carrier only. The animals were sacrificed 24 h after the last treatment by cervical dislocation and the livers were removed and stored at −80°C until analysis.

To study tissue distribution of AαC, three groups of mice were given 20, 40 and 100 mg AαC kg^{-1} bw orally three times during 3 consecutive days. The control animals were given carrier only. The animals were sacrificed 24 h after the last treatment by cervical dislocation. The following tissues were removed and stored at −80°C until analysis: liver, heart, kidney, lung, large and small intestines, stomach and spleen.

DNA preparation and DNA enrichment

DNA was isolated from tissues by a phenol/chloroform extraction procedure according to previously published reports (Möller *et al.* 1993). 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 DNA were hydrolysed using micrococcal nuclease and spleen phosphodiesterase. The digested DNA was extracted for adducted nucleotides using the butanol extraction enhancement method following an earlier described procedure (Baranczewski and Möller 2004). Evaporated butanol extracts were stored at −80°C until analysis.

^{32}P-post-labelling

Evaporated butanol extracted samples (20 µg DNA) were dissolved in 5.0 µl water. PNK buffer (0.5 µl, 400 mM, 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 min 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 multisolvent delivery system, a DeltaPak 5 µ C18 100A main column (Waters Chromatography, Milford, MA, USA), a NewGuard RP18 precolumn (Brownlee Laboratories, Santa Clara, CA, USA), and an online A280 radioactivity detector using a 0.5 ml cell and scintillation fluid FloScint IV (both Radiomatic Instruments & Chemicals Co., Tampa, FL, USA). The energy window was set to 8–600 KeV with a counting efficiency of 60% for ^{32}P. The counting was performed in 12 s cycles. ^{32}P-HPLC analysis was performed according to published methods (Möller *et al.* 1993, Zeisig and Möller 1995, Baranczewski and Möller 2004) with minor modifications. Briefly, after injection of the total unrefined labelling mixture into the HPLC, the samples were eluted with 0.5 ml min^{-1} of 2.0 M ammonium formate, 0.4 M formic acid (pH 4.5), and a linear gradient of 0–35% acetonitrile between 0 and 70 min. A three-way valve was included between the precolumn and the main column. During the first 5 min 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.

Synthesis of 2-nitro-9H-pyrido[2,3-b]indole (NαC) and reaction with calf thymus DNA in vitro

The synthesis of NαC and reaction with calf thymus DNA *in vitro* were performed as described by Hashimoto *et al.* (1982) and Grivas (1988). AαC (40 mg) was dissolved in 1.0 ml 50% acetic acid and added to a solution of sodium nitrate (600 mg) in 1.2 ml water. The mixture was stirred for 1 h and then the yellow precipitate was separated by centrifugation. Calf thymus DNA was dissolved (1 mg ml^{-1}) in 100 mM sodium dihydrogen phosphate buffer (pH 5.5) and 2.5 mg NαC in acetonitrile was added in the presence of 5 mg zinc chloride. The reaction was performed overnight at room temperature under continuous shaking. DNA was precipitated by addition of 5 M NaCl and cold 96% ethanol.

Synthesis of N^2-(guanin-8-yl)-2-amino-9H-pyrido[2,3-b]indole

The synthesis of N^2-(guanin-8-yl)-2-amino-9H-pyrido[2,3-b]indole was performed in analogy to protocols by Hashimoto *et al.* (1982) and by Pfau *et al.* (1997). Guanine N^3-oxide (11.2 mg) dissolved in 2 ml DMSO and dimethylformamide (3:1, v/v) was cooled to 0°C. Acetic anhydride (6 µl) was added followed by addition of AαC (2.5 mg). The reaction was performed overnight at room temperature under continuous shaking. The reaction product was concentrated to 100 µl in a vacuum centrifuge. A total of 1 ml water was added and the precipitate was isolated. The precipitate was dissolved in 0.1 M hydrochloric acid and analysed by HPLC.

Analysis of modified bases

The solution of AαC modified DNA (200 µg) was hydrolysed with 1 M hydrochloric acid (final concentration of 0.1 M) for 60 min at room temperature. After extraction with *n*-butanol, the extract was dried in a vacuum centrifuge and redissolved in methanol. The solution was injected into the HPLC with a diode array detector, on an Alltima ODS column (4.6 × 250 mm, Alltech Assoc., Deerfield, IL, USA). Analysis was performed at a flow rate of 1 ml min^{-1} with a linear gradient of acetonitrile (5–60% over 40 min) in water.

Results

Comparison between heterocyclic aromatic amines regarding their induction of DNA adducts

Fourteen HAAs (Trp-P-1, Trp-P-2, AαC, MeAαC, Glu-P-1, Glu-P-2, PhIP, IQx, MeIQx, 4,8-diMeIQx, 7,8-diMeIQx, 4,7,8-triMeIQx, IQ and MeIQ) were tested using the ^{32}P-HPLC technique regarding their induction of DNA adducts in mouse liver after oral administration of the same dose.

Using this technique, 13 amines were found to generate DNA adducts in the liver: Trp-P-2, Trp-P-1, AαC, MeIQ, 7,8-diMeIQx, IQx, MeIQx, 4,8-diMeIQx, IQ, 4,7,8-triMeIQx, MeAαC, Glu-P-1 and PhIP, in descending order (table 1 and figure 1). No DNA adducts were detected in livers of animals treated with Glu-P-2 (figure 1). The ^{32}P-HPLC technique allowed a detection limit of 0.1 DNA adducts per 10^8 normal nucleotides (NN). The highest level of DNA adducts was detected for Trp-P-2 ($199.5 \pm 10.3/10^8$ NN), Trp-P-1 ($105.8 \pm 6.9/10^8$ NN) and AαC ($76.5 \pm 11.9/10^8$ NN).

The DNA adduct levels induced *in vivo* were multiplied with daily intake of amines in the US and the Swedish populations (table 1) to obtain a relative risk index (RRI), defined as the DNA adduct level in mouse liver × human daily intake of the amine (ng/person/day). The highest RRI was calculated for AαC, RRI = 27 693, which was nine-fold higher compared with PhIP (RRI = 3254) and 14-fold higher compared with MeIQx (RRI of 1921). IQ and MeIQ showed the lowest RRIs (table 1).

Table 1. Total DNA adduct levels in mouse liver, human daily intake and a relative risk index for 14 HAAs tested.

HAAs	Total level of DNA adducts per 10^8 NN ± SD	Daily intake of HAAs in the US population[1,2] (ng/person/day)	Daily intake of HAAs in the Swedish population[3] (ng/person/day)	Relative risk index (RRI)[4] USA	Relative risk index (RRI)[4] Sweden
Trp-P-2	199.5 ± 10.3	–	ND	–	–
Trp-P-1	105.8 ± 6.9	–	–	–	–
AαC	76.5 ± 11.9	362[1] 105[2]	–	27693[1] 8032[2]	–
MeAαC	5.1 ± 0.3	–	–	–	–
Glu-P-1	1.9 ± 0.3	–	–	–	–
Glu-P-2	ND	–	–	–	–
PhIP	2.8 ± 0.3	1162[1] 434[2]	72	3254[1] 1215[2]	202
IQ	8.8 ± 3.4	20[1] 13[2]	1	176[1] 114[2]	9
MeIQ	25.6 ± 2.4	–	1	–	26
IQx	15.8 ± 5.7	–	–	–	–
MeIQx	10.5 ± 0.3	183[1] 84[2]	72	1921[1] 882[2]	756
4,8-DiMeIQx	10.4 ± 5.8	20[1] 15[2]	16	208[1] 156[2]	166
7,8-DiMeIQx	23.4 ± 7.7	–	–	468	374
4,7,8-TriMeIQx	3.0 ± 0.4	–	–	–	–

[1]Layton *et al.* (1995).
[2]Bogen and Keating (2001) estimated mean HAAs intake as TWA = time-weighted average for males.
[3]Augustsson *et al.* (1997).
[4]DNA adduct formation (mouse, *in vivo*) multiplied by human daily intake.
–, Data not available; ND, not detected; NN, normal nucleotides.

DNA adduct formation from AαC in different mouse tissues

The levels of DNA adducts (^{32}P-HPLC) induced by AαC were analysed in the following tissues: liver, heart, kidney, lung, large and small intestines, stomach and spleen. AαC was orally administered once a day on 3 consecutive days. In all tested tissues, one major peak was detected with a retention time of 53.0 min (figure 1). No DNA adduct peak in that region was detectable in DNA from controls. The highest DNA adduct levels were found in the liver (136.7 ± 37.4/10^8 NN) followed by heart, kidney, lung, large intestine, small intestine, stomach and spleen, in descending order (figure 2). In all examined tissues there was an increase of the AαC–DNA adducts in a dose-dependent manner. In the small and large intestines as well as in the stomach, DNA adduct analyses were performed on the epithelium (not of the whole tissue).

Characterization of DNA adducts formed from AαC in vivo

In all analysed tissues of mouse after oral administration of AαC, one major peak was found with a retention time of 53.0 min (figures 1 and 3). For characterization of the DNA adduct formed from AαC *in vivo* 2-nitro-9H-

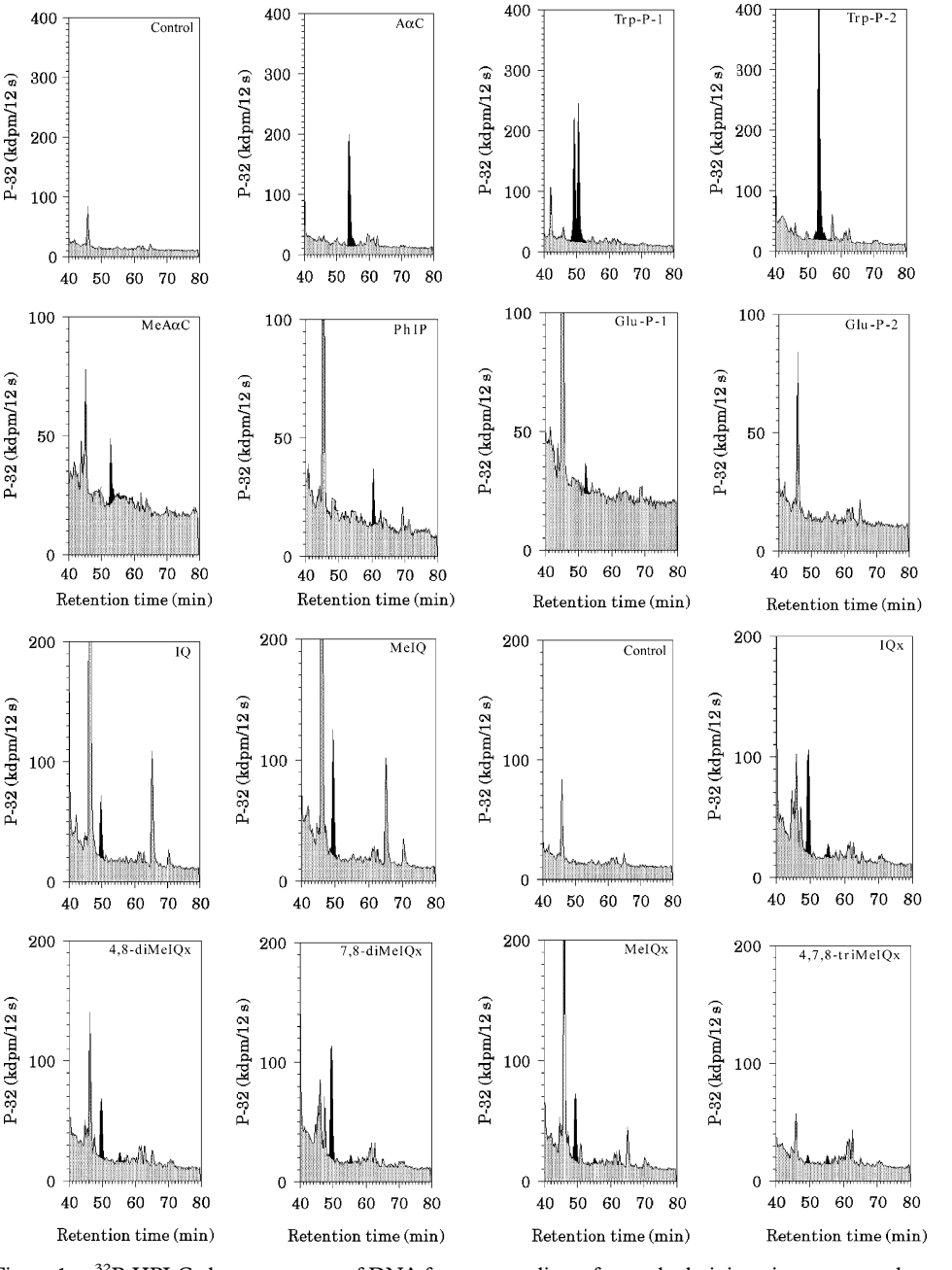

Figure 1. ^{32}P-HPLC chromatograms of DNA from mouse liver after oral administration once per day during 3 consecutive days, with 40 mg kg^{-1} bw of each amine: AαC, MeAαC, PhIP, Trp-P-1, Trp-P-2, Glu-P-1, Glu-P-2, IQ, MeIQ, 4,8-diMeIQx, 7,8-diMeIQx, IQx, MeIQx and 4,7,8-triMeIQx. The control was administered carrier only, 50% ethanol (v/v). The animals were sacrificed 24 h after the last treatment and DNA was analysed by using the ^{32}P-HPLC technique.

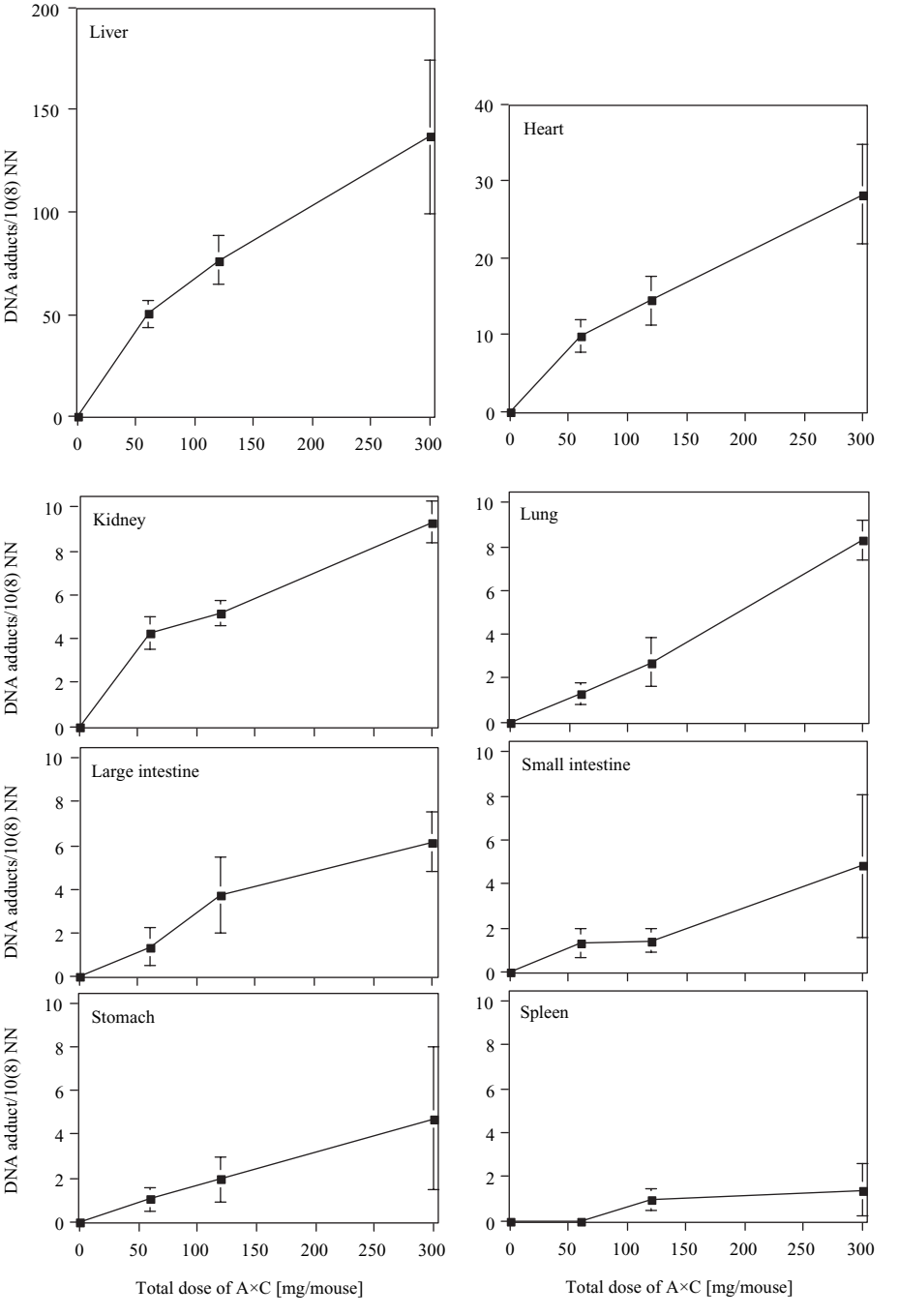

Figure 2. Dose-dependent formation of DNA adducts in tissues of mouse administered once a day during 3 consecutive days with 0, 20, 40 or 100 mg kg^{-1} bw AαC. The animals were sacrificed 24 h after the last administration and the tissues removed. DNA was analysed using the ^{32}P-HPLC technique. Points show means of three experiments ± standard deviations.

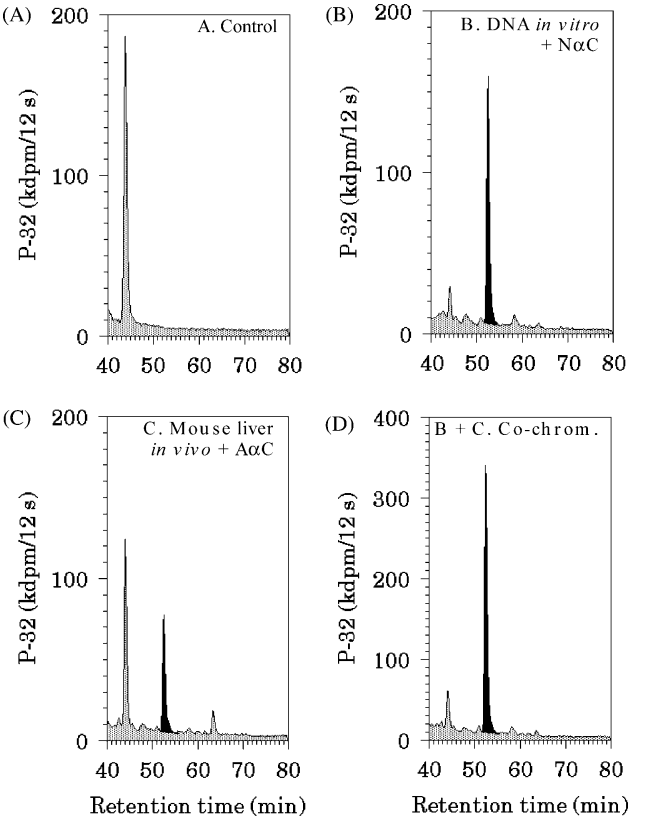

Figure 3. ^{32}P-HPLC chromatograms of liver DNA: (A) liver of a control mouse, after administration only with carrier; (B) calf thymus DNA after treatment *in vitro* with NαC; (C) mouse liver DNA after administration of AαC; and (D) co-chromatography of calf thymus DNA after treatment *in vitro* with NαC and DNA from mouse liver after treatment *in vivo* with AαC.

pyrido[2,3-b]indole was reacted with calf thymus DNA *in vitro* to synthesize N^2-(deoxyguanosin-8-yl)-2-amino-9H-pyrido[2,3-b]indole. The reaction product had after ^{32}P-post-labelling a retention time of 53.0 min (figure 3), identical to the ^{32}P-label DNA adduct formed *in vivo* from AαC. The retention time comparison was performed by co-chromatography of the *in vivo* formed DNA adduct and the synthesized N^2-(deoxyguanosin-8-yl)-2-amino-9H-pyrido[2,3-b]indole. The result was a single homogeneous peak (figure 3). The co-chromatography procedure was also carried out using ion-exchange TLC of ^{32}P-post-labelled DNA adducts. In addition, in this case the synthesized N^2-(deoxyguanosin-8-yl)-2-amino-9H-pyrido[2,3-b]indole co-migrated with the *in vivo* formed DNA adduct of AαC (figure 4). To characterize further the structure of the DNA adduct, AαC was reacted with the N^3-oxide of guanine. At the same time, the *in vivo* formed DNA adduct was hydrolysed with 1 N hydrochloric acid, and the modified base was dissolved in methanol. Both modified bases were analysed using HPLC, on C-18 modified silica gel column with a gradient of acetonitrile in water. The reaction products had the same retention time 24.1 min (figure 5). Moreover, a UV/VIS spectrum for both modified bases was used for confirmation of identity. The UV/

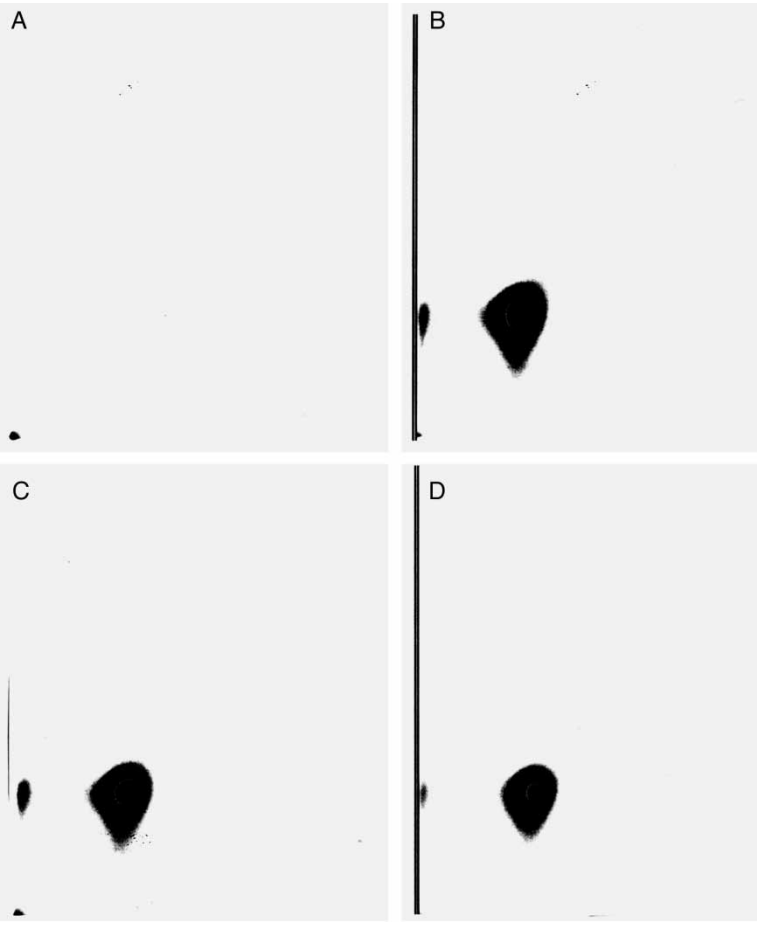

Figure 4. ^{32}P-TLC chromatograms of: (A) DNA from liver of control animals, after administration only with carrier; (B) calf thymus DNA after treatment *in vitro* with NαC; (C) DNA from mouse liver after administration of AαC; and (D) co-chromatography of calf thymus DNA after treatment *in vitro* with NαC and DNA from mouse liver after treatment *in vivo* with AαC.

VIS spectra were identical with three characteristic maxima of absorption at 249, 290 and 360 nm, respectively (figure 6). Taken together results suggest that the DNA adduct formed *in vivo* in mouse after administration of AαC most likely is N^2-(deoxyguanosin-8-yl)-2-amino-9H-pyrido[2,3-b]indole.

Discussion

Several studies have been attempted to estimate a cancer risk for heterocyclic amines based on daily intake and experimental data (Layton *et al.* 1995, Bogen and Keating 2001, Rohrmann *et al.* 2002). One aim of the present study was to estimate relative risk regarding a target tissue in humans based on DNA adduct formation and daily intake of HAAs. In a previous lifetime tumour study on 2-nitrofluorene (metabolized to amine) in rats, the amount of DNA adducts in a

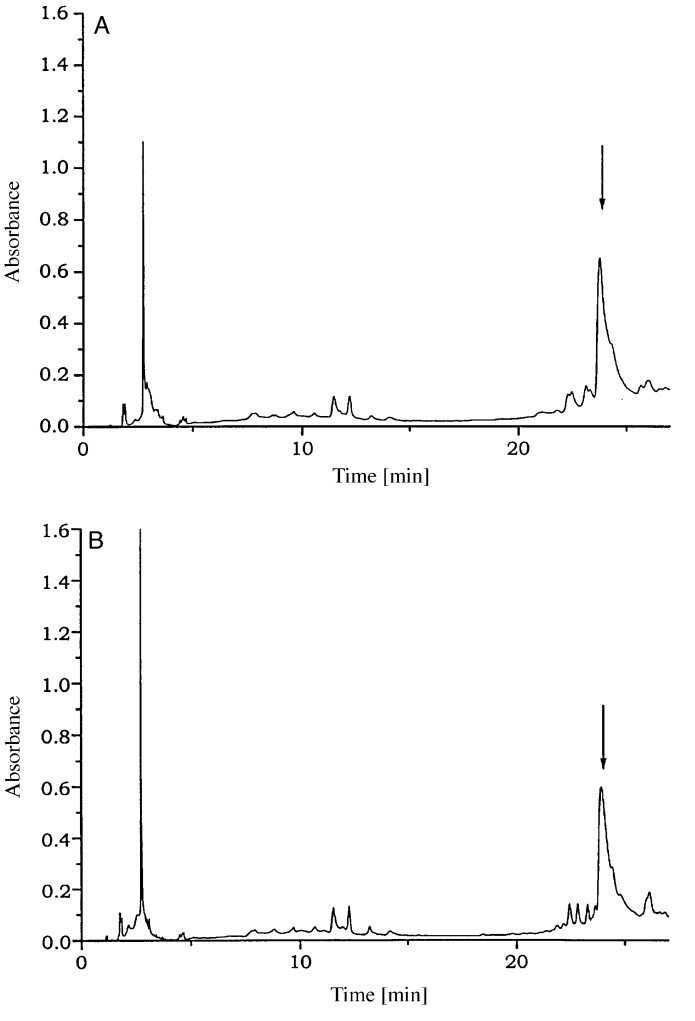

Figure 5. HPLC analysis of: (A) the reaction product between guanine N^3-oxide and AαC; and (B) guanine modified *in vivo* with AαC. Arrows indicate the adequate peaks for the N^2-(guanin-8-yl)-2-amio-9H-pyrido[2,3-b]indole adduct.

tissue was correlated to tumour formation up to 2 years later. This correlation was very clear in the forestomach and glandular stomach. In other tissues, it was more of a character that the presence of DNA adducts was correlated to tumour formation (Cui *et al.* 1995). It was therefore assumed in this study that the level of DNA adducts was possible to use as a relative risk marker. The doses used were in line with what is normally used in the literature (Turesky and Vouros 2004). A two- to three-fold difference in DNA adduct levels was reported in the liver of Muta®Mice and *c-myc/lacZ* double transgenic mice for AαC versus IQ and MeIQx (Davis *et al.* 1996). The results of this study concerning 14 HAAs showed that the HAAs induced very different levels of DNA damage, at the same oral dose, with a 105-fold difference between the highest and lowest values (table 1). The differences were especially pronounced for γ- and α-carbolines when compared with

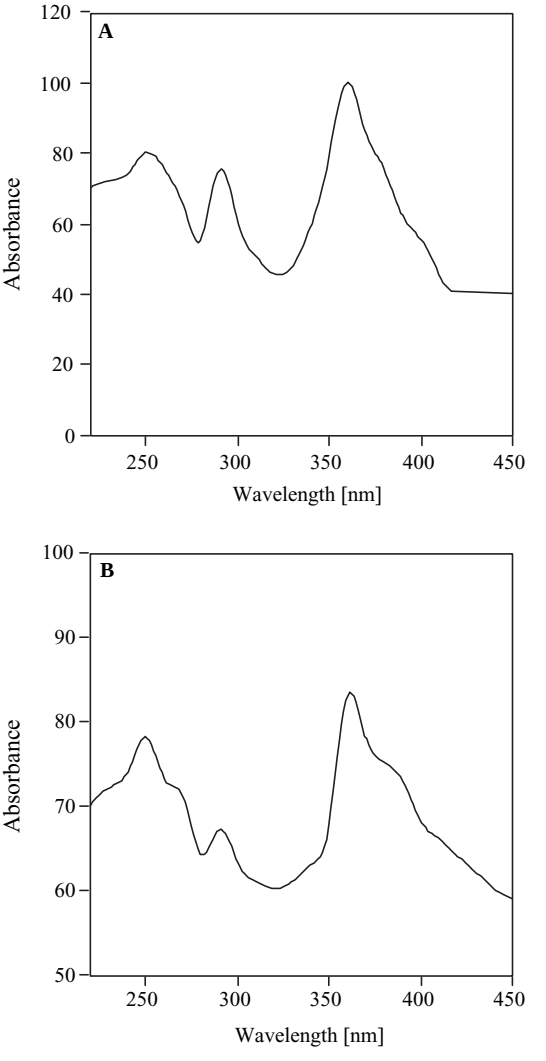

Figure 6. UV/VIS spectra analysis of: (A) the reaction product between guanine N^3-oxide and AαC; and (B) guanine modified *in vivo* with AαC.

aminoimidazoazaarenes (figure 1 and table 1). One of the reasons for the differences in DNA adduct formation can be differences in labelling efficiency and in the recovery of the DNA adducts in the ^{32}P-post-labelling method. Previous experiments performed with two standard adducts: PhIP- and 4,8-diMeIQx–DNA adducts, showed that the differences can be significant (Wohlin *et al.* 1996), but not as high as those presented in this study. In previous experiments when animals were treated with different doses of HAAs (between 10 and 50 mg kg^{-1} bw), the highest level of DNA adducts was detected for AαC, MeAαC, MeIQ, IQ and MeIQx (Turesky and Vouros 2004). These studies were performed at different laboratories and at different concentrations of HAAs. Recently, AαC–DNA adducts were compared in three tissues (colon, liver and lung) of male F344 rats and male ICR

mouse (Majer *et al.* 2004). In both species, the highest level of DNA adducts was detected in livers.

The human daily intake of HAAs not only depend upon differences in culture and food habits, but also upon the amount and type of meat in the diet, the frequency of consumption, the cooking method, the temperature and duration of cooking, etc. (Augustsson *et al.* 1997, Rohrmann *et al.* 2002). To estimate the relative risk better, the DNA adduct level formed from each amine was combined with the calculated human daily intake of the amine in question (RRI). RRI was very high for AαC. Together with the abundance of AαC not only in grilled meat and fish, but also in other products like grilled vegetables (e.g. onions) and in cigarette smoke (Matsumoto *et al.* 1981, Bogen and Keating 2001), it is suggested that this amine probably represents a relatively high risk for humans when compared with other HAAs.

Furthermore, the γ-carbolines, Trp-P-1 and Trp-P-2, induce high levels of DNA adducts compared with other amines. Unfortunately, data are not available concerning the daily intake of γ-carbolines. Additionally, these carcinogens are widely distributed in cooked foods and also in cigarette smoke (Wakabayashi *et al.* 1995), and in the environment, e.g. in airborne particles and river water (Ohe 1997). Therefore, these amines could be involved in the aetiology not only of liver or colon cancer, but also in other tissues. The aim of the comparison between different HAAs is not to establish an absolute cancer risk, but to combine a genotoxic effect with exposure and thereby achieve a better understanding of the potential to induce DNA lesions.

Several investigations have dealt with the formation of HAA–DNA adducts in tissues (Hirose *et al.* 1995, Turesky 2002, Turesky and Vouros 2004). HAAs generally induce high DNA adduct levels in the liver, which for most HAAs is the most prominent tumour target tissue (Turesky 2002). In this study, the highest DNA adduct level following administration of AαC was found in the liver, followed by heart, kidney and lung in descending order. All HAAs, except PhIP, tested for carcinogenic potency in rodents have induced liver tumours (Turesky 2002). No tumour incidence has been shown for AαC in the heart, kidney or lung (Ohgaki *et al.* 1984, Turesky 2002). However, the high level of AαC-derived DNA adducts in the heart and in lung together with the presence of AαC in cigarette smoke might imply that AαC is a risk factor for heart disease and smoking-related tumours.

In this study, one major DNA adduct (N^2-(deoxyguanosin-8-yl)-2-amino-9H-pyrido[2,3-b]indole) was detected in all mouse tissues analysed by ^{32}P-HPLC after administration of AαC. These results indicate that mouse *in vivo* metabolize AαC by a similar pathway as the F334 rat and ICR mouse *in vivo* (Frederiksen *et al.* 2004, Majer *et al.* 2004), in rat hepatocytes (Pfau *et al.* 1997) as well as in rat liver microsomes (Raza *et al.* 1996).

In conclusion, out of 14 different hetrocyclic amines, a 105-fold difference to induce DNA adducts was shown *in vivo* in mice after oral administration. When comparing the DNA adduct formation with human daily intake AαC showed the highest relative cancer risk, nine- and 14-fold higher when compared with PhIP and MeIQx, respectively. The DNA adduct formation induced by AαC in different

tissues was highest in the liver (137 ± 37 DNA adducts 10^8 NN) followed by heart, kidney, large intestine, small intestine, stomach and spleen, in descending order.

The results indicate that the human cancer risk for AαC probably is underestimated due to the high levels of AαC, not only in grilled meat, but also in other products like grilled chicken, vegetables and cigarette smoke.

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