32P-Postlabeling analysis of IQ, MeIQx and PhIP adducts formed in vitro in DNA and polynucleotides and found in vivo in hepatic DNA from IQ-, MeIQx- and PhIP-treated monkeys

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The 32P-postlabeling method was used to examine the adducts in DNA, polynucleotides, and mononucleotides reacted in vitro with the N-hydroxy and N-acetoxy derivatives of 2-amino-3-methylimidazo[4,5-f]quinoline (IQ), 2-amino-3,8-dimethylimidazo[4,5-f]quinoline (MeIQx) or 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhIP). Adduct profiles were compared to those found in vivo in liver of cynomolgus monkeys fed IQ, MeIQx or PhIP. The N-acetoxy derivatives of IQ, MeIQx and PhIP (generated in situ from the corresponding N-hydroxylamine in the presence of acetic anhydride) each formed three principal adducts in DNA. Adduct 1 of IQ, MeIQx and PhIP was chromatographically identical to the 32P-labeled bis(phosphate) derivative of N-(deoxyguanosin-8-yl)-IQ, N-(deoxyguanosin-8-yl)-MeIQx, and N-(deoxyguanosin-8-yl)-PhIP respectively, and this adduct comprised ~65% of total adduct levels found in DNA in vitro. The C8-guanine adduct and the two minor adducts were also found in poly(dt-G-dC).poly(dt-G-dC), suggesting that the two minor adducts of IQ, MeIQx and PhIP are also formed on the guanine base. The N-acetoxy derivatives of IQ, MeIQx, and to a much lesser extent PhIP, also formed adducts with adenine-containing polynucleotides including poly(dA), poly(dA).poly(dtT) and poly(dA-dT), poly(dA-dT), but these adenine adducts were chromatographically different from those found in DNA. The three guanine adducts of N-acetoxy-IQ, -MeIQx and -PhIP found in vitro in DNA and in guanine-containing polynucleotides were also found in the liver of monkeys fed IQ, MeIQx or PhIP respectively, indicating that metabolic activation via N-hydroxylation and esterification occurred in vivo in monkeys. With each compound, the C8-guanine adduct was the predominant adduct found in vivo. The results indicate similarities among IQ, MeIQx and PhIP in the DNA adducts formed in vitro and in vivo and substantiate the use of the 32P-postlabeling method for comparative adduct studies.

Introduction

2-Amino-3-methylimidazo[4,5-f]quinoline (IQ*), 2-amino-3,8-dimethylimidazo[4,5-f]quinoline (MeIQx) and 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhIP) belong to a family of heterocyclic amine compounds that are formed during the ordinary cooking of meats such as beef, fish and fowl (1). All the three compounds are potent carcinogens (2–5). IQ and MeIQx cause tumors in the liver, lung, Zymbal’s gland, clitoral gland and skin of F344 rats, and tumors in liver and lung of CDF1 mice (2). IQ has also been shown to cause tumors in the small and large intestine of F344 rats (2) and in the mammary gland of female Sprague–Dawley rats (4). In contrast to IQ and MeIQx, PhIP is not a hepatocarcinogen in adult rodent models but causes lymphomas in CDF1 mice (5), and colon and mammary adenocarcinomas in F344 rats (3). The carcinogenicity of all three compounds in non-human primates is currently under study (6). To date, IQ has been shown to be a potent hepatocarcinogen in nonhuman primates (6, 7).

IQ, MeIQx and PhIP form DNA adducts in vivo and in vitro following metabolic activation to N-hydroxylamines and/or to more reactive N-acetoxy derivatives (8–12). Other reactive esters, such as the sulfate, also appear to play a role in the further activation of the N-hydroxylamines (8–12). In vitro studies with DNA and 2′-deoxyguanosine have shown that the reactive metabolite of IQ, MeIQx and PhIP form an adduct at the C8 atom of guanine that is a major adduct for each compound (13–16). Recently Turesky et al. (14) showed that N-acetoxy-IQ and N-acetoxy-MeIQx each form a N2′-guanine adduct in vitro that constitutes ~4% and ~10% respectively of total adduct levels. In rodents administered the parent compound, the C8-guanine adduct of IQ (17) and PhIP (15, 16) has been identified in tissue DNA. To date, the structural identity of MeIQx–DNA adducts formed in vivo in animals administered MeIQx has not yet been reported.

The 32P-postlabeling method is a sensitive method for the detection of a multitude of chemical carcinogen DNA adducts (18, 19). By this method, adducts are resolved as fingerprints on TLC sheets after ion-exchange chromatography and autoradiography (18, 19). This method has been used by a number of laboratories for the detection of IQ, MeIQx and PhIP adducts formed in vivo and in vitro (10, 11, 16, 17, 20–29). While interlaboratory differences in adduct profiles exist, in our hands, we have consistently seen three principal adducts of IQ, MeIQx and PhIP by 32P-postlabeling analysis of DNA from rodents and monkeys given these compounds (17, 23, 30, 31). Using a synthetic C8-guanine-IQ adduct standard in the 32P-postlabeling assay, we have confirmed the presence of dG-C8-IQ in DNA from cynomolgus monkeys fed IQ (17, 22). However, the other adducts of IQ, and the adducts of MeIQx and PhIP, have not yet been identified in tissue DNA from cynomolgus monkeys fed these compounds. As a means of further characterizing the adducts of IQ, MeIQx and PhIP found by our 32P-postlabeling assay, and in particular the adducts in cynomolgus monkeys undergoing carcinogenicity testing, we have examined the adducts in DNA, polynucleotides and mononucleotides reacted in vitro with the N-hydroxy or N-acetoxy derivatives of IQ, MeIQx, PhIP, and compared these adducts to those found in vivo in liver of heterocyclic amine-fed monkeys. Our results substantiate the use of the 32P-postlabeling method as a means to analyze heterocyclic amine adducts in monkeys undergoing carcinogenicity bioassay.
Materials and methods

Chemicals
IQ, MelQx and PhIP were obtained from the Nard Institute, Osaka, Japan. N-Hydroxy-IQ, N-hydroxy-MelQx and N-hydroxy-PhIP were synthesized as described previously (16,31,32). Poly(dA), poly(dC), poly(dG) and poly(dT) were purchased from Pharmacia, Piscataway, NJ. Poly(dA-dT), poly(dT-dA), poly(dG-dC), poly(dC-dG), poly(dA), poly(dT) and poly(dG) poly(dC) were purchased from Boehringer-Mannheim, Indianapolis, IN. 2'-Deoxyguanosine and 2'-deoxyguanosine 3'-monophosphate were purchased from Sigma, St Louis, MO. The 3'-phospho-dG-C8-IQ adduct standard was synthesized as described previously (13). Chinese hamster ovary cell DNA was purified as described previously (34). [32P]ATP was prepared as described (23).

Instrumentation
HPLC analysis and purifications were carried out on a Gilson model 715 system equipped with a model 621 data module, model 116 UV detector, two model 303 pumps, a model 802 monometric module and a model 811 dynamic mixer. UV spectra were obtained on a Shimadzu UV-2101PC scanning spectrophotometer after HPLC. Fast atom bombardment (FAB) mass spectra were acquired on a VG 7070E-HF mass spectrometer equipped with a xenon atom bombardment ionization source and a VG 11/250 data system. The FAB sample matrix was glycerol for negative-ion spectra and 1-diisoglycerol/glycerol (1:1) containing 5% acetic acid for positive ion spectra.

Modification of DNA, polynucleotides, nucleosides and nucleotides with N-hydroxy- and N-acetoxy derivatives of IQ, MelQx and PhIP
Nucleic acids were dissolved in degassed 10 mM Tris/1 mM EDTA buffer (pH 7.5) at a concentration of 0.2 mg/ml. Five nanomoles of N-hydroxy-IQ, N-hydroxy-MelQx or N-hydroxy-PhIP were then added, followed by the addition of a 10-fold molar excess of acetic anhydride (3.5 µl) to generate the N-acetoxy-derivatives in situ. The total incubation volume was 500 µl. After a 30 min incubation at room temperature, the samples were extracted twice with chloroform/isomyl alcohol (24:1) and the nucleic acids were precipitated with ethanol.

Synthesis and purification of dG-C8-MelQx and 3'-phospho-dG-C8-MelQx
N-Hydroxy-MelQx (0.12 µmol in 5 µl 0.05 M KH2PO4) was added to a degassed solution of Sorenson's phosphate buffer (6.7 mM monopotassium phosphate/disodium phosphate, pH 7.0) containing either dG (5 mg/ml) for the synthesis of the dG-C8-MelQx adduct, or dGMP (5 mg/ml) for the synthesis of 3'-phospho-dG-C8-MelQx. The solution was maintained under nitrogen at room temperature while 100 µl of a 160 mM solution of acetic anhydride in acetone was added dropwise over a 1 min period. After 20 min, the major dG or dGMP adduct of MelQx was purified by HPLC on a Supelcosil LC-18-DB semipreparative column (25 cm x 10 mm) (Supelco, Bellefonte, PA) at ambient temperature with UV absorbance monitored at 264 nm. The HPLC solvents were methanol (5-10 min), isocratic (10-15 min), 12-50% methanol (15-30 min), 50-100% methanol (30-40 min), followed by 100% methanol (40-45 min). All gradients were linear and the flow rate was 1.7 ml/min throughout the run. One major dG-MelQx adduct peak was observed that eluted at 36.4 min. After reaction with dGMP, one major MeIQx-dGMP adduct peak was observed which eluted at 33.5 min.

Synthesis and purification of dG-C8-PhIP and 3'-phospho-dG-C8-PhIP
N-Hydroxy-PhIP (0.21 µmol in 2.5 µl DMF/DMSO) was added to a degassed solution of Sorenson's phosphate buffer containing either dG or dGMP and the reaction carried out exactly as described above for MelQx. The major dG or dGMP adduct of PhIP was purified by HPLC on a Supelcosil LC-18-DB semipreparative column (as described above) with UV absorbance monitored at both 254 and 315 nm. The HPLC solvents were methanol/water, and the program was isocratic 40% methanol (0-5 min), 4-12% methanol (5-10 min), isocratic (10-15 min), 12-50% methanol (15-30 min), 50-100% methanol (30-40 min), followed by 100% methanol (40-45 min). All gradients were linear and the flow rate was 1.5 ml/min throughout the run. One major dG-PhIP adduct peak eluting at 34.6 min was observed. One major dGMP-PhIP adduct peak eluting at 30.7 min was observed.

Spectral characterization of dG-C8-MelQx and dG-C8-PhIP and the 3'-phospho derivatives
The major adduct of MeIQx with dGMP was purified by HPLC prior to 32P-postlabeling analysis. The UV spectrum of this adduct was similar to that reported previously for the C8-guanine adduct of MeIQx (14) and identical to the UV spectrum obtained from our major PhIP adduct with dG. FAB mass spectral analysis of the major dG adduct of PhIP showed an ion [M + H]+ at m/z 479.3 and an ion [M − H]+ at m/z 477.2, which agreed well with the calculated mass of the molecular ion of dG-C8-MelQx at m/z 479.2 and 477.2 respectively. The major adduct of PhIP with dGMP was purified by HPLC prior to 32P-postlabeling analysis. The UV spectrum of this adduct was similar to that previously reported for the C8-guanine adduct of PhIP (15,16) and identical to the UV spectrum obtained from our major PhIP adduct with dG. FAB mass spectral analysis of the major dG adduct of PhIP showed an ion [M + H]+ at m/z 490.0, which is in agreement with the calculated mass of the molecular ion for dG-C8-PhIP at 490.2. In addition, a fragment ion at m/z 374.0 corresponding to MH-deoxyribose+ was observed.

Results

32P-Postlabeling analysis of IQ adducts
N-Hydroxy-IQ and N-acetoxy-IQ (generated in situ in the presence of acetic anhydride) react with DNA in vitro to form the same three adducts found in vivo in the liver of cynomolgus monkeys fed IQ (Figure 1A and B). Although the reaction of N-acetoxy-IQ was >35-fold higher than that observed with N-hydroxy-IQ (Table I), the adducts formed were the same (profile shown for N-acetoxy-IQ only). Adducts 1, 2 and 4 were observed upon reaction of N-hydroxy-IQ or N-acetoxy-IQ with poly(dG-dC), poly(dG-dC) (Figure 1C). Using poly(dG), poly(dC), or single-stranded poly(dG), adducts 1, 2 and 4 were also found (Figure 1D: pattern shown for poly(dG), poly(dC) only). No reaction was seen with poly(dC).

N-Acetoxy-IQ reacted with dGMP, forming adduct 1 (Figure 1E), which co-migrated with the C8-guanine adduct (Figure 1F). Co-migration studies confirmed that adducts 1, 2 and 4 found in poly(dG-dC), poly(dG-dC), poly(dG), poly(dC) and poly(dG) were the same as those found in DNA reacted in vitro with N-hydroxy-IQ or N-acetoxy-IQ, and the same as those found in vivo in liver of monkeys.

N-Acetoxy-IQ reacted with polymers containing adenine alone...
or those containing adenine and thymine including poly(dA), poly(dA),poly(dT) and poly(dA-dT).poly(dA-dT) (Table I). N-Hydroxy-IQ also showed some reactivity with the adenine-containing polymers, though to a much lower extent than N-acetoxy-IQ (Table I). No reaction was observed with poly(dT) with either N-hydroxy-IQ or N-acetoxy-IQ. Under standard conditions of the 32P-postlabeling assay, one main adduct was found in poly(dA) which was located in the upper right quadrant of the autoradiogram (Figure 2A). Under intensification conditions at least two additional adducts were also found (Figure 2B). None of the adenine adducts comigrated with the adducts found in DNA (Figure 2C and D).

**Table 1.** Total adduct levels in nucleic acids reacted with N-hydroxy-IQ, N-hydroxy-MelQx or N-hydroxy-PhIP in the presence or absence of acetic anhydride

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<th>Acetic anhydride</th>
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Nucleic acids were modified with 10 μM N-hydroxyamine in the presence or absence of 100 μM acetic anhydride as described in Materials and methods. 32P-Postlabeling analysis was carried out under standard conditions. The coefficient of variation was < 15% in duplicate determinations.

ND, not detected (level of detection: 1 adduct/10^7 nucleotides).

**Fig. 2.** 32P-Postlabeling profiles of IQ adducts in poly(dA). Poly(dA) adducted in vitro with N-acetoxy-IQ was analyzed under standard conditions (A) and under intensification conditions (B). Pattern of IQ DNA adducts under intensification conditions (C). Co-chromatography of IQ adducts in poly(dA) with those in DNA (intensification conditions) (D). Autoradiography was performed at -70°C for 1-3 h.

**Fig. 3.** 32P-Postlabeling profiles of MelQx adducts in nucleic acids. DNA from liver of monkeys fed MelQx (A). Adducts formed in vitro in DNA (B); poly(dG-dC).poly(dG-dC) (C); poly(dG).poly(dC) (D); and dGMP (E) reacted with N-acetoxy-MelQx. 3′-Phospho-dG-C8-MelQx standard (F). The 32P-postlabeling assay was run under standard conditions. Autoradiography was performed at -70°C for 30 min-2 h (b,d,e,f); 6.5 h (c); and 24 h (a).
The three adducts found in DNA were chromatographically identical to those found in poly(dG-dC),poly(dG-dC) (Table I). N-Acetoxy-PhIP also formed adducts in poly(dC), but these adducts were present at much lower levels and were chromatographically different from those found in poly(dG-dC).poly(dG-dC) or DNA (Figure 6B). In poly(dG).poly(dC) and poly(dG) reacted with N-acetoxy-PhIP, adducts 1 and 2, but not adduct 3, were detected (Figure 5D: profile shown for poly(dG).poly(dC) only). Co-migration studies showed that the PhIP adducts 1 and 2 in poly(dG).poly(dC) or poly(dG) were chromatographically identical to adducts 1 and 2 found in DNA. When N-acetoxy-PhIP was reacted with dGMP, only adduct 1 was observed (Figure 5E). Adduct 1 was chromatographically identical to the 32P-labeled 3'phospho-dG-C8-PhIP standard (Figure 5F).

Under standard conditions of the 32P-postlabeling assay, no adenine adducts of N-acetoxy-PhIP were detected. However, under intensification conditions, some spots were observed in the chromatogram after a 24 h exposure of the autoradiogram at −70°C (Figure 6A). These adducts were chromatographically different from those found in PhIP-adducted DNA.

Comparison of individual IQ, MelQx and PhIP DNA adducts

Figure 7 shows the percentage of individual adducts formed in vitro upon reaction of DNA with the N-acetoxy derivatives of IQ, MelQx or PhIP (panel A) and in vivo in liver of monkeys fed the respective parent compound (panel B). Following in vitro incubation of DNA with the N-acetoxy derivatives, ~65% of the total adduct levels seen with the three compounds was due to adduct 1, the C8-guanine adduct (Figure 7A). Adducts 2 and 3/4 constituted 17–21% and 10–19% respectively of the total adduct levels. In vivo, in the liver of monkeys fed IQ, MelQx or PhIP, adduct 1 (the C8-guanine adduct) was the major adduct for all three compounds comprising 68, 50 and 47% of total adduct levels respectively (Figure 7B). Total levels of the three adducts per 10⁷ nucleotides were 20.5, 1.6 and 5.0 in one IQ-, MelQx- and PhIP-treated monkey respectively. The differences in total adduct levels may be partly due to differences in the administered dosages and to differences in the in vivo metabolic activation of the distinct heterocyclic amines (30).
IQ, MeIQx and PhIP adduct levels in nucleic acids and effect of acetic anhydride

Acetic anhydride catalyzes the chemical synthesis of the N-acetoxy-derivatives of IQ, MeIQx and PhIP from the corresponding N-hydroxylamines (13-16,28). With all three compounds, N-acetoxy formation enhanced the binding of the N-hydroxylamine to nucleic acids containing guanine (Table I). Among the N-hydroxylamines, only N-hydroxy-IQ showed detectable levels of adducts in guanine- or adenine-containing polynucleotides under the conditions of our assay.

Discussion

There are similarities among the profiles of IQ-, MeIQx- and PhIP-DNA adducts observed by the 32P-postlabeling assay, though different running buffers were used for chromatography. Each compound formed three principal DNA adducts in vitro upon reaction of DNA with the corresponding N-acetoxy derivative and in vivo in hepatic DNA from monkeys fed IQ, MeIQx or PhIP (Figures 1, 3 and 5). With each compound the adducts found in vitro were chromatographically identical to those found in vivo. 32P-Postlabeling studies with authentic C8-guanine adduct standards further confirmed that adduct 1 of IQ, MeIQx and PhIP was IQ-C8-guanine, MeIQx-C8-guanine and PhIP-C8-guanine respectively. The C8-guanine adduct of each compound was the major adduct found in DNA in vitro and in vivo in liver of treated monkeys (Figure 7). Our findings obtained by 32P-postlabeling analysis are in agreement with those obtained using HPLC methods which also showed that the C8-guanine adduct of IQ, MeIQx and PhIP is the major adduct found in vitro in DNA (13-16). In addition, our results with PhIP are similar to those reported in a recent study (16) which used the 32P-postlabeling method to examine PhIP adducts formed in vivo in rats.

The results from 32P-postlabeling studies with polynucleotides reacted in vitro with N-acetoxy-IQ, N-acetoxy-MeIQx or N-acetoxy-PhIP suggest that in addition to the C8-guanine adduct, there may be two minor guanine adducts of IQ, MeIQx and PhIP in DNA. The two minor adducts of IQ, MeIQx and PhIP were found only in nucleic acids containing guanine such as poly(dG-dC), poly(dG-dC), poly(dG), poly(dC), and poly(dG), and not in poly(dC) or the adenine-containing polynucleotides. The chromatographic properties of the IQ, MeIQx and PhIP adducts in poly(dG-dC), poly(dG-dC) were identical to those observed in DNA (Figures 1, 3 and 5). The two minor DNA adducts of IQ and MeIQx were also seen in poly(dG), poly(dC) and in poly(dG) (Figures 1 and 3). Since it is expected that an adducted dinucleotide of dG-dC, arising from incomplete digestion of poly(dG-dC), poly(dG-dC), would have a different mobility from that of dG-dG, arising from incomplete digestion of poly(dG), poly(dG), it appears that the adducts of IQ and MeIQx may be unique guanine monoadducts rather than incompletely digested adducted oligonucleotides containing the same guanine adduct. Adduct 2 of PhIP also appears to be a unique guanine adduct rather than a spot arising from an incompletely digested adducted dinucleotide since this adduct spot is found in poly(dG), poly(dG), and poly(dG) as well as in poly(dG-dC), poly(dG-dC) (Figure 5). It is noteworthy that adduct 3 of PhIP was not observed in poly(dG), poly(dG) or poly(dG) (Figure 5D). Others have shown that the coformation of nucleic acids influences the formation of carcinogen adducts (37-40), and perhaps poly(dG), poly(dC) and poly(dG) do not possess the necessary conformation for the formation of PhIP adduct 3. The absence of the two minor guanine adducts of IQ, MeIQx and PhIP in dGMP reacted with the respective N-acetoxy derivatives may also be explained, in part, by the lack of secondary and tertiary structure necessary for the formation of these adducts. Intercaletion of heterocyclic amines in double-stranded DNA (41) may be necessary for the proper alignment of electrophilic sites in the carcinogen with nucleophilic sites in the DNA bases, thus facilitating the adduction. It is tempting to speculate that the two minor adducts of IQ, MeIQx and PhIP are to the guanine base and, in light of recent studies (14), that one of the unknown adducts of IQ and MeIQx is the N2-guanine adduct.

N-Acetoxy-IQ and N-acetoxy-MeIQx, and to a much lesser extent N-acetoxy-PhIP, formed adducts with adenine-containing polynucleotides (Figures 2, 4 and 6). To our knowledge, this is the first demonstration of the reactivity of N-acetoxy derivatives of IQ, MeIQx and PhIP with adenine by the 32P-postlabeling method. N-Hydroxy-IQ also showed some reactivity with polynucleotides containing adenine, though the other two N-hydroxylamines did not. This finding is consistent with the higher reactivity of N-hydroxy-IQ in comparison to N-hydroxy-MeIQx and N-hydroxy-PhIP (Table I). N-Acetoxy-PhIP showed a much lower capacity to react with adenine than did N-acetoxy-IQ or N-acetoxy-MeIQx, and this reaction was detectable only under intensification conditions (minimum level of detection 1-4 adducts/10^6 nucleotides). However, the adenine adducts of IQ, MeIQx and PhIP were chromatographically distinct from the three principal adducts found in DNA in vitro or in vivo. Thus, if the adenine adducts form in DNA they must be present at substantially lower levels than the guanine adducts, and it is likely that such adducts would not be detectable by the 32P-postlabeling method. The possibility and significance of adenine adduct formation in vivo requires further study.

Since DNA isolated from the liver of monkeys fed IQ, MeIQx or PhIP showed an adduct pattern identical to that seen in DNA reacted in vitro with the corresponding N-acetoxy derivative (Figures 1, 3 and 5), the same three principal guanine adducts of IQ, MeIQx and PhIP appear to be formed in vivo in these monkeys. In addition, adduct 1, the C8-guanine adduct, was the major adduct found in monkeys fed IQ, MeIQx or PhIP. The similarity between the adducts found in vivo to those found in vitro suggests that the adducts of IQ, MeIQx and PhIP found in vivo are derived from the corresponding N-hydroxylamines or reactive esters. Acetic anhydride catalyzes the in situ chemical formation of the N-acetoxy derivatives, causing a dramatic increase in the adduct levels in guanine-containing polynucleotides for all three compounds (Table I) and suggests that O-acetylation, or a comparable esterification pathway, may participate in the activation of N-hydroxylamines in vivo in monkeys. The lack of reactivity of N-hydroxy-MeIQx and N-hydroxy-PhIP with DNA (at physiological pH) suggests that esterification is required for MeIQx and PhIP-DNA adduct formation in vivo. However, as noted previously (9,12,13) and in the current study, N-hydroxy-IQ is able to react with DNA without esterification, albeit at a much lower level than N-acetoxy-IQ. Studies with N-hydroxy-MeIQx and N-hydroxy-PhIP have also indicated that under anaerobic conditions at pH 7.0, and to a greater extent at pH 5.0, some reaction of these N-hydroxyamines with DNA occurs (12,13).

The carcinogenicity of IQ, MeIQx and PhIP is currently being evaluated in cynomolgus monkeys. To date, IQ has been shown to be a potent hepatocarcinogen in these animals (6,7). DNA from the liver of IQ-fed monkeys that developed hepatocellular carcinoma generally shows a high level of the C8-guanine-IQ.
adduct (68 ± 8% of total adduct levels, mean ± standard error, n = 3 monkeys; Figure 7B). The role that the individual guanine adducts play in the initiation of cancer, however, requires further study. It is interesting that despite the similarities among IQ, MeIQx and PhIP in profiles of adducts formed in DNA, specifically in their formation of guanine adducts and the predominance of the C8-guanine adduct, a number of the target organs for IQ and MeIQx carcinogenicity in rodents have been shown to be different from those observed for PhIP (2,3). It is not known if there are sequence-selective differences in adduct formation to guanine nucleotides or perhaps to adenine nucleotides among these heterocyclic amines. Further studies examining the adduct distribution, repair and mutagenicity may help to clarify, in part, the role that distinct heterocyclic amine adducts play in the initiation of cancer.

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References


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