Environmental Health Perspectives
Vol. 99, pp. 183-186, 1993

Low-level Biological Dosimetry of Heterocyclic Amine Carcinogens Isolated from Cooked Food

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The bioavailability and the bioreactivity of the carcinogenic heterocyclic amine [2-^{14}C]2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (PhIP) have been investigated at a dose approximating that likely from the human diet by accelerator mass spectrometry (AMS). [2-^{14}C]PhIP was administered to mice at a dose equivalent to the consumption of two 100 g beef patties (41 ng/kg). The biological half-life of PhIP was 1 hr, with 90% of the dose being excreted via the urine. Peak tissue PhIP concentrations were reached within 3 hr, with the highest levels in the tissues of the gastrointestinal tract, followed by the liver, kidney, pancreas, and thymus. Since the detection limit by AMS is dependent on the natural abundance of ^{14}C, we have achieved further increases in sensitivity by producing mice that have 20% of the natural abundance of ^{14}C. Use of these ^{14}C-depleted animals allows measurements to be made near the natural level of exposure for many environmental carcinogens. PhIP–DNA adduct levels have also been measured by ^{32}P-postlabeling at doses of 1.0, 10, and 20 mg/kg. The highest adduct levels were found in the pancreas, thymus, heart, and liver and increased linearly with dose. The principal adducts are derived from guanine.

Introduction

Determining the cancer risk that chemicals pose to humans has proven to be a difficult task. Biomarkers play an integral role in this process because theoretically they indicate progress in the development of disease, but the biomarkers must be selected to validly represent the disease mechanism (*1*). Fundamentally at issue is the relationship between the delivered dose and the innate ability of an organism, both on a systemic and cellular level, to cope with the challenge. In a practical sense, translating the carcinogenic response from high-dose animal studies to low-dose human exposure scenarios is the immediate concern from the standpoint of *a*) developing valid biomarkers of exposure and effect, *b*) for understanding mechanisms, and *c*) for risk assessment.

Heterocyclic Amines in the Diet and the Need for Low-level Dosimetry

Nowhere is this risk evaluation problem more acute than in accessing the dosimetry of the genotoxic dietary heterocyclic amine carcinogens formed during the cooking of meat [discussed in detail by Felton and Knize (*2*)]. Daily human exposures for these compounds are probably on the order of a few nanograms/kilogram of body weight (assuming an average 70 kg adult consumes 200 g of meat/day), while the animal bioassays have been carried out at doses of mg/kg (*3*). Likewise, pharmacokinetic, metabolic, and macromolecular adduct measurements have all been made at doses in excess of 10^4- to 10^6-fold greater than human dietary exposure levels. To determine the feasibility of biomonitoring and for selecting appropriate biological exposure markers for use at human exposure levels, it is necessary to understand the pharmacokinetics, metabolism, and dosimetry of these carcinogens at relevant dietary levels and to reconcile these results to the high-dose studies at which bioassays have been traditionally carried out.

Our approach to this problem has been to focus on the DNA adduct as a potential biomarker for both exposure and effect, and we are using the high sensitivity offered by accelerator mass spectromery (AMS) for assessing adduct dosimetric relationships. AMS is a low-energy nuclear physics technique developed for measuring cosmogenic isotopes such as ^{14}C and is ideal for studying the low-level dosimetry of isotopically labeled xenobiotics (*4,5*). AMS isolates and counts specific nuclei as opposed to measuring atomic decay. Thus, AMS methodology results in a 10^6-fold improvement in isotope detection efficiency compared to scintillation counting for ^{14}C and has allowed us to quantitatively measure DNA adduct levels on the order of 10^{-11} to 10^{-12} adducts/nucleotide (*6*). We also have applied the ^{32}P-postlabeling assay to mechanistic studies of the effects of these carcinogens on DNA. The postlabeling assay is a highly sensitive

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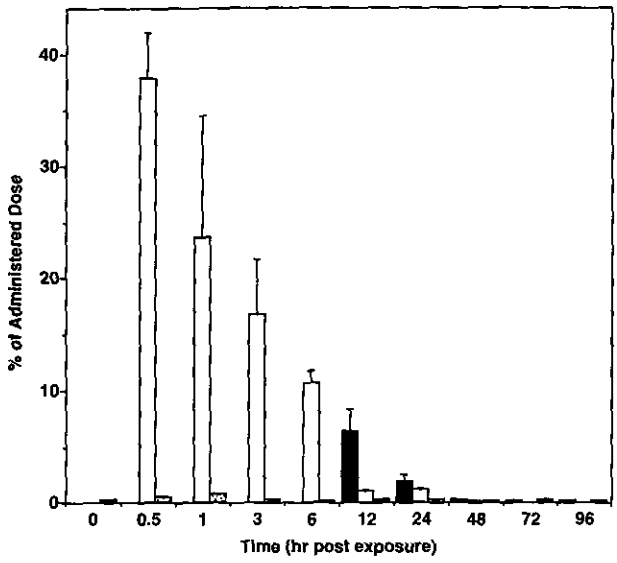

FIGURE 1. Percent of the administered dose of 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (PhIP) found in the urine (□), feces (■), and blood (▨) after administration of 1.25 ng/mouse. Means are from three animals/time point ± SE.

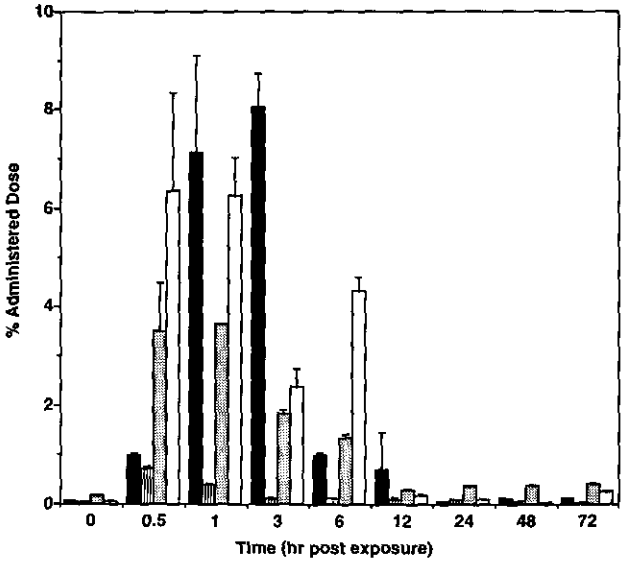

FIGURE 2. Percent of the administered dose in representative tissues. Shown are data from the intestine (■), stomach (□), liver (▨), and kidney (▥). Means are from three animals/time point ± SE.

assay for DNA adduct detection that includes chromatographic separation of specific adducts and thus is quite useful for studying mechanisms of adduct formason and repair. Here we describe the fate and distribution of 2-amino-1-methyl-6-phenylimidazo[4,5-bipyridine (PhIP) at a dose equivalent to the consumption of 200 g cooked meat and on the formation of PhIP adducts at higher doses.

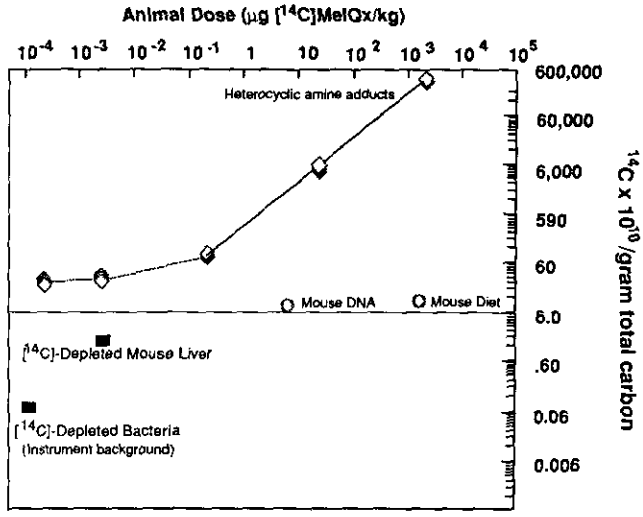

FIGURE 3. The sensitivity of accelerator mass spectrometry is limited by the natural abundance of ^{14}C in biological material as shown by [^{14}C]2-amino-3,8-dimethylimidazo[4,5-*f*]quinoxaline-DNA adduct data (*6*) mice raised on a diet depleted in ^{14}C (■) show an 80% reduction in ^{14}C. This will allow an additional 5-fold increase in the detection of macromolecular damage. Also shown is the instrument background for ^{14}C. Data are reported as the ratio of ^{14}C atoms to total carbon atoms isolated from the samples.

Methods

C57BL/6 male mice were obtained from Simonsen Laboratory (Gilroy, CA) at 6–8 weeks of age and allowed to acclimatize for 1 week. For determination of the distribution and fate of PhIP, the mice were administered [2-^{14}C]-labeled PhIP (50 mCi/mmole) by stomach intubation at a dose of 41 ng/kg body weight. Animals were sacrificed at time 0, 0.5, 1, 3, 6, 12, 24, 48, and 96 hr following exposure, and tissues were immediately removed. Urine was collected over ice at the same time points and pooled with urine removed from the bladder. Feces were likewise collected and homogenized in water/methanol (1:1). All samples were stored at -20°C until use. Three independent replications of the experiment were carried out. Sample preparation for AMS and ^{14}C determinations were carried out using our previously described methods (*7,8*).

For assessing PhIP DNA adduct dosimetric relationships, PhIP was administered to (6–8-week-old C57BL/6 male mice by gavage at doses of 0, 1.0, 10.0, and 20.0 mg/kg. Tissues were removed 24 hr later and stored at -20°C until use. In addition, 2′-deoxyribobonucleoside-3′-monophosphates were incubated with *N*-hydroxy-PhIP in the presence of mouse hepatic cytosol as described in Buonarati et al. (*9*). DNA from the tissues and *in vitro* incubations were isolated and ^{32}P-postlabeled as described previously (*9*).

Results

PhIP was rapidly absorbed at the 41 ng/kg dose and maximal blood levels were reached within 0.5 hr (Fig. 1). Excretion was equally rapid. Ninety percent of the administered dose was eliminated via the urine and 10% via the feces over the 96-hr period. The systemic half-life of the compound was 1 hr.

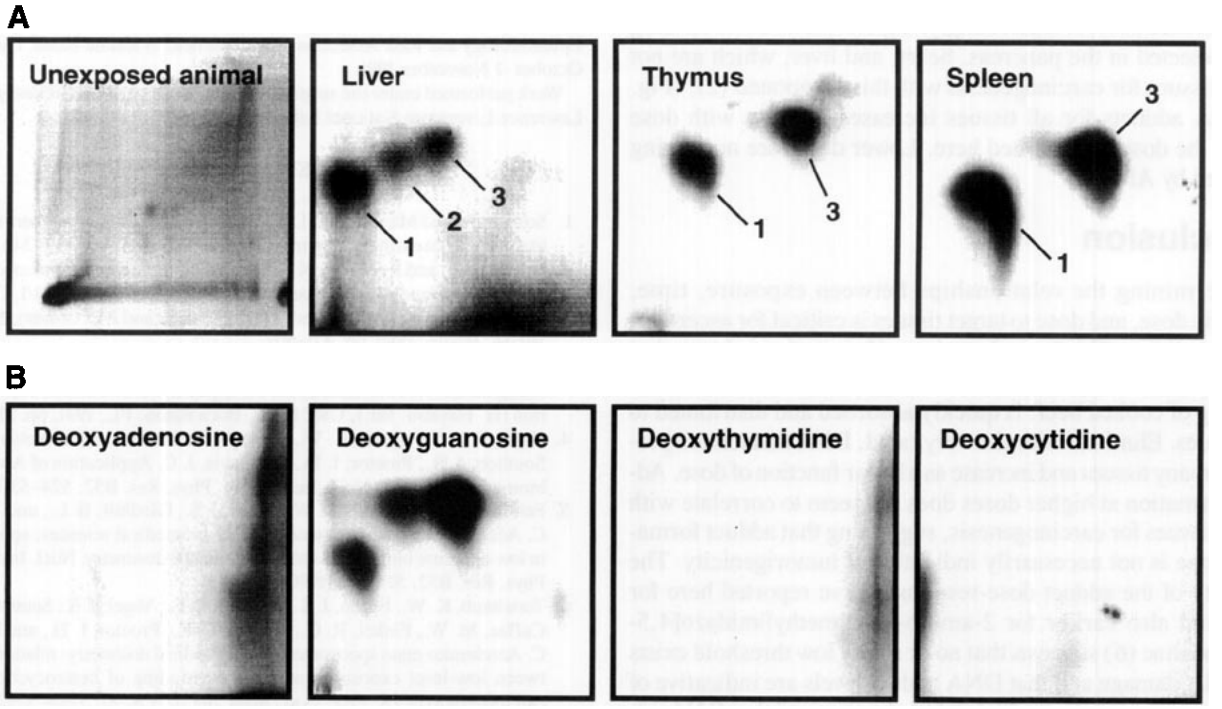

FIGURE 4. ^{32}P-Postlabeling maps from mice given PhIP and *in vitro* reactions of deoxynucleosides with DNA binding (PhIP) intermediates. (*A*) Adduct maps of DNA isolated from representative mouse tissues 24 hr after exposure to PhIP. DNA from an unexposed animal is shown for reference. Three PhIP-related adduct spots are present in the liver, whereas only two PhIP-related adduct spots are present in other tissues. These correspond to liver adducts labeled 1 and 3. (*B*) Adduct maps from a separate experiment showing DNA adducts formed from incubations of reactive PhIP intermediates with the 2′-deoxynucleotide monophosphates. PhIP can be seen to bind only to deoxyguanosine-3′-monophosphate. These adduct spots co-migrate with adducts isolated from mouse tissues.

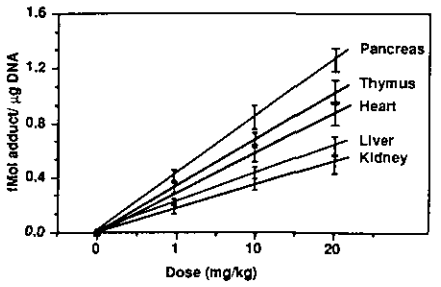

FIGURE 5. DNA adduct levels in representative tissues of the mouse 24 hr after administration of PhIP. Adduct levels were determined by ^{32}P-postlabeling. Means of three animals/dose ± SE.

Maximal concentrations of PhIP were attained in the tissues between 0.5 and 3 hr after exposure, shown in Figure 2. The highest tissue levels of PhIP or its metabolites were found in the stomach and intestine, followed by the liver, kidney, and pancreas. Some of the PhIP found associated with the stomach may have been due to adsorption of the unabsorbed compound from the lumen surface of the tissue. PhIP levels in the tissues 96 hr after exposure were 0.5–2% of the administered dose. The residual levels of PhIP in the tissues were due possibly to covalent binding of active metabolites to DNA and protein and are currently under study.

Sensitivity of AMS

The absolute sensitivity limit for biological specimens is imposed by the natural ^{14}C abundance present in all biological material (Fig. 3). The absolute detection limit of the instrument for ^{14}C is 1 part ^{14}C/10^{15} total carbon. The natural abundance of ^{14}C in biological matter is 1 part ^{14}C/10^{12} total carbon, which is two orders of magnitude above what can be routinely measured by AMS. In an effort to further increase our ability to measure macromolecular damage at low dose, we have attempted to reduce the amount of ^{14}C present naturally in our animal models. Mice fed on a diet containing yeast protein obtained from organisms grown on petroleum-derived methanol have 20% of the ^{14}C present in contemporary biological matter. This decrease has been achieved within one generation of feeding the special diet and will result in a 5-fold increase in the sensitivity of biological AMS measurements. Low-dose DNA binding analysis with heterocyclic amines is currently underway using these animals.

PhIP Adduct Levels

PhIP–DNA adduct levels have been measured in the mouse by ^{32}P-postabeling. Three adduct spots are evident in liver tissue at all doses tested, and two adduct spots were found in all other tissues (Fig. 4*A*). The structural nature of these PhIP adducts is as yet undetermined. Incubation of reactive PhIP metabolites with 2′-deoxynucleotide-3′-monophosphates indicate that all three adduct spots are derived from deoxyguanosine (Fig. 4*B*).

The deoxyguanosine adducts co-chromatograph with the adduct spots formed *in vivo*. Interestingly, high levels of DNA binding were detected in the pancreas, heart, and liver, which are not target tissues for carcinogenesis with this compound (*2*), (Fig. 5) DNA adducts for all tissues increased linearly with dose within the dose range tested here. Lower doses are now being assessed by AMS.

Conclusion

Determining the relationships between exposure, time, systemic dose, and dose to target tissues is critical for ascertaining the validity of biological markers. Here we have shown that the dietary carcinogen PhIP, at a dose equivalent to consumption of 200 g of cooked beef, is quickly absorbed and distributed to the tissues. Elimination is also very rapid. DNA adducts are present in many tissues and increase as a linear function of dose. Adduct formation at higher doses does not seem to correlate with target tissues for carcinogenesis, suggesting that adduct formation alone is not necessarily indicative of tumorigenicity. The linearity of the adduct dose-response curve reported here for PhIP and also earlier for 2-amino-3,8-dimethylimidazo[4,5-*f*]quinoxaline (*6*) suggests that no or a very low threshold exists for DNA damage and that DNA adduct levels are indicative of exposure. The levels at which DNA damage and that DNA adduct levels are indictive of exposure. The levels at which DNA damage translates into a significant biological effect need to be determined before adducts can be validated as markers for effect or risk. Further studies are in process to define the relationship between exposure, macromolecular damage, and biological effect at low doses.

This manuscript was presented as a poster at the Conference on Biomonitoring and Susceptibility Markers in Human Cancer: Applications in Molecular Epidemiology and Risk Assessment that was held in Kailua-Kona, Hawaii, 26 October–1 November 1991.

Work performed under the auspices of the U.S. Department of Energy by the Lawrence Livermore National Laboratory (W-7405-ENG-4S).

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