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DNA–Carcinogen Adducts in Circulating Leukocytes as Indicators of Arylamine Carcinogen Exposure[1]

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DNA–carcinogen adducts in leukocytes and putative target tissues (liver and urinary bladder) of C57BL/6J mice were measured by ^{32}P-postlabeling and HPLC analysis after controlled exposure to the arylamine carcinogen 2-aminofluorene (2-AF). After an acute exposure via ip injection, adducts were detected at 3 hr in leukocytes, liver, and bladder. The disappearance of DNA–carcinogen adducts in liver and leukocytes were parallel over the 24-hr period studied. Following a 7-day continuous exposure to 2-AF via drinking water, adduct levels in leukocytes and target tissues were responsive to dose at 30, 100, and 300 ppm. Adduct levels at the highest dose reached 17,000 fmol/mg DNA in leukocytes, 1900 fmol/mg in liver, and 2300 fmol/mg in bladder. Although adduct levels after 7 days were highest in leukocytes, adducts were not detectable in leukocytes 7 days after discontinuing exposure. In contrast, liver and bladder retained approximately 50 and 75% of their respective adduct levels 7 days after exposure was stopped. The results indicate that circulating leukocytes may be useful as indicators of current exposure to arylamine carcinogens. Circulating leukocytes may also be useful as biological monitors of DNA damage in arylamine target tissues during chronic exposure to these compounds. Some important differences in persistence of DNA–carcinogen adducts between leukocytes and target tissues were observed. © 1993 Society of Toxicology.

Quantitative indicators of human exposure to environmental and occupational carcinogens would be extremely useful in risk assessment studies. Knowledge of the actual dose of carcinogens received by individuals in their workplace would provide the information necessary to set limits on carcinogen exposure and to evaluate safety measures taken to reduce such exposure. Development of such indicators has been a formidable task for a number of reasons. Humans are exposed to a wide variety of carcinogenic or potentially carcinogenic compounds most of which are unidentified. Individuals within the same environment or workplace receive different levels of exposure depending on their specific tasks and personal habits. Even individuals receiving the same dose of carcinogen will differ in their sensitivity to the chemical due to differences in metabolism and excretion based on genetics, age, sex, and nutritional and health status.

Measurement of the chemical reaction between carcinogens or their activated metabolites and macromolecules of an individual appears to be a useful way of determining carcinogen exposure. Both protein and nucleic acid modification by carcinogens have been used as indicators of carcinogen exposure (Weston *et al.*, 1989). Since DNA is the cellular macromolecule believed to be most closely involved in carcinogenesis, modification (adduct formation) of DNA by carcinogens appears to be directly relevant to potential tumor formation. White blood cells have been used to monitor occupational exposure to carcinogens as they are an easily obtainable DNA-containing human tissue. The use of extremely sensitive techniques to measure DNA–carcinogen adduct formation such as ^{32}P-postlabeling and various immunological methods have usually shown a general correlation between heavy occupational exposure to carcinogens and DNA damage as measured by DNA–carcinogen adduct formation. The use of ^{32}P-postlabeling to monitor human exposure to carcinogens has been recently reviewed by Beach and Gupta (1992) and the results of DNA adduct determinations in various environmental and occupational situations have been compiled by Schut and Shiverick (1992). However, nearly all studies of carcinogen exposure and leukocyte DNA adduct formation have been only semiquantitative in terms of exposure measurements. Several studies have classified the exposures as low, medium, or high based on industrial hygiene measurements or job categories (for example, Perera *et al.*, 1988). In addition, data relating DNA adducts in WBC to DNA damage in internal organs are lacking.

Because of several practical and ethical limitations on the use of humans in carcinogenicity studies, the use of an ani-

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mal model for testing the applicability of circulating leukocytes as indicators of carcinogen exposure and carcinogen-induced DNA damage has several advantages. The animals can be maintained under controlled conditions and exposed to known doses of specific carcinogens for selected time periods. Thus, quantitative dose–response measurements are possible. The relationship of DNA damage in the exposure indicator (the WBC)[2] to DNA damage in the carcinogen target organ can be determined, and therefore the usefulness of leukocytes as biological monitors of internal damage can be assessed. By using a model such as an inbred mouse strain, genetic differences between individuals as well as sex and age differences can be eliminated.

Human exposure to arylamine carcinogens has been and continues to be of occupational and environmental concern. Although most occupational exposure has been mainly to β-naphthylamine and to benzidine and its derivatives, arylamine carcinogens such as 4-aminobiphenyl and 2-aminofluorene are frequently used for studying the interaction of arylamines with DNA and the initiation of carcinogenesis. Much information about the metabolism and activation of these compounds to ultimate carcinogens (Miller and McQueen, 1986; Butler *et al.*, 1989; Weber, 1990), the type and structure of DNA adducts produced (Singer and Grunberger, 1983; Kadlubar and Beland, 1985), and the effect of adduction on DNA replication and function (P. K. Gupta *et al.*, 1988; Moriya *et al.*, 1988; Nairn *et al.*, 1988) has been obtained over the years.

Our laboratory has previously reported DNA–2-AF adduct formation in liver (Levy and Weber, 1989) and in urinary bladder (Levy and Weber, 1992) of inbred mice. The present report describes the formation of DNA–2-AF adducts in circulating leukocytes and assesses the usefulness of leukocyte DNA adducts as exposure indicators and also as biological monitors of DNA damage at target organs of mice exposed to a model carcinogen.

MATERIALS AND METHODS

Animals and treatments. All mice were male C57BL/6J obtained from Jackson Laboratories, Bar Harbor, ME. The mice were kept at 25°C with controlled humidity and a 12-hr light/dark cycle and were used at 8 weeks of age. Mice were allowed Purina Mouse Chow and water *ad libitum*, except for the 7-day carcinogen exposure experiments.

Acute exposure to 2-AF (60 mg/kg) was done by intraperitoneal injection of 2-AF in DMSO (16 mg/ml). Control mice received an equal volume of DMSO. Blood was obtained from the tail vein and mice were sacrificed at 3, 6, 12, and 24 hr after injection. Liver and urinary bladder were removed at the time points and quick-frozen in liquid nitrogen. All tissues were stored at −70°C until DNA isolation.

[2] Abbreviations used: AF, aminofluorene; dG-C8-AAF, C8-(N^2-acetylaminofluorenyl)-deoxyguanosine-3′,5′-diphosphate; dG-C8-AF, C8-(N^2-aminofluorenyl)-deoxyguanosine-3′,5′-diphosphate; DMSO, dimethyl sulfoxide; EDTA, ethylenediaminetetraacetic acid; SDS, sodium dodecyl sulfate; WBC, white blood cells.

Seven-day exposure to 2-AF was done by preparing acidified water (HCl to pH 2.5) and dissolving 2-AF in the water to give 0, 30, 100, and 300 ppm concentration. Mice were randomly assigned into five groups of three mice each. Four of the groups received the 2-AF dose indicated and the fifth group received 300 ppm for 7 days followed by tap water for an additional 7 days. Initial and 7-day weights were recorded as was water consumption. After Day 7, blood was obtained from the tail vein of mice in groups 1 through 4 and the mice were sacrificed and tissues removed and stored as above. Group 5 mice were weighed and switched to tap water. Seven days later group 5 mice were again weighed, then bled, sacrificed, and tissues obtained.

Chemicals. Polynucleotide kinase, 3′-phosphatase free was from Boehringer-Mannheim Biochemicals (Indianapolis, IN). [γ-^{32}P]ATP was from Amersham (Arlington Heights, IL). Guanidine isothiocyanate was from BRL (Gaithersberg, MD). Most other chemicals and enzymes were from Sigma (St. Louis, MO).

Isolation of leukocyte DNA. Approximately 800 μl of frozen whole blood from individual mice was allowed to thaw at room temperature with an equal volume of blood lysis solution composed of 320 mM sucrose, 5 mM $MgCl_2$, and 1% Triton X-100 in 10 mM Tris–HCl, pH 7.5 (Ciulla *et al.*, 1988), while rotating at moderate speed for 30 min. Samples were then centrifuged at 1000*g* for 10 min, the supernatant was mostly removed and discarded, and the nuclear pellet was rotated with an additional 500 μl of blood lysis solution for an additional 10 to 20 min. Centrifugation was repeated and as much supernatant as possible was removed. The remaining leukocyte nuclei were lysed with 300 μl of a solution of 4 M guanidine isothiocyanate and 25 mM Na acetate (Ciulla *et al.*, 1988) by rotation for several hours at room temperature. DNA was precipitated with ethanol and Na acetate at −20°C, collected by centrifugation, and redissolved in 200 μl of 10 mM Tris–HCl, pH 7.5, containing 1 mM EDTA. DNA was further purified by digestion with RNase A and T_1, protease K digestion, chloroform/isoamyl alcohol extraction, and ethanol precipitation as described previously (Levy and Weber, 1988). DNA was finally dissolved in 100 μl of succinate buffer (20 mM Na succinate, 10 mM $CaCl_2$, pH 6.0) and the DNA concentration was obtained from the absorbance at 260 nm. Yields of DNA averaged about 12 μg/ml whole blood.

Isolation of liver DNA. DNA from liver samples was obtained either by homogenization and lysis in SDS followed by RNase and protease K digestion and organic extraction as described (Levy and Weber, 1988) or by homogenization of 100 mg of liver in the blood lysis solution described above followed by nuclear lysis with guanidine isothiocyanate and further purification essentially as described for leukocyte DNA. Liver DNA was dissolved in 250 μl of succinate buffer. Both methods yielded DNA of approximately equal quality and quantity, averaging about 1.6 μg/mg liver.

Isolation of bladder DNA. DNA from individual mouse bladders was obtained either by homogenization followed by lysis in SDS and purified as described previously (Levy and Weber, 1992) or by homogenization of minced bladder in blood lysis solution followed by lysis of the nuclear pellet with guanidine isothiocyanate as for liver and leukocytes. Further purification of bladder DNA was by digestion with RNase and protease K, solvent extraction, and ethanol precipitation. DNA was dissolved in 100 μl succinate. As with liver, both methods gave similar yields and purity of DNA. On average, about 16 μg of DNA was obtained per bladder.

^{32}P-postlabeling of DNA adducts. DNA (2–5 μg) was hydrolyzed with a mixture of micrococcal nuclease and spleen phosphodiesterase II for $3\frac{1}{2}$ hr at 37°C followed by enrichment of adducts by butanol extraction in the presence of tetrabutylammonium chloride (Gupta, 1985). The dried, enriched adducts were dissolved in 15 μl water and labeled at pH 7.6 by adding 10 μl of a mixture containing 50 μCi of [γ-^{32}P]ATP (5000–6000 Ci/mmol), 8 U polynucleotide kinase, 3′-phosphatase-free, 3 μl buffer (0.5 M Tris–HCl, 0.1 M $MgCl_2$, 50 mM dithiothreitol, 1 mM spermidine, 1 mM EDTA, pH 7.6), and 2 μl water. After incubation at 37°C for 40 min, 100

mU apyrase in 4 μl water was added and incubation continued an additional 20 min.

HPLC. HPLC analysis of adducted, postlabeled nucleotides was carried out by reverse-phase ion pairing using an Ultrasphere ion pair C18 4.6 mm × 25-cm column with a 70-mm guard column of ODS on a Varian 5060 ternary liquid chromatograph as described previously (Levy and Weber, 1989). Ten microliters of the 29 μl labeling reaction was injected with an unlabeled adduct standard of dG-C8-AAF [Levy and Weber, 1988] and sufficient 30 mM K-PO_4, pH 6.0, 10% CH_3CN to make 40 μl. The elution program was 30 mM K-PO_4, pH 6.0, 10% CH_3CN for 10 min followed by a linear gradient of 90% solvent B (30 mM K-PO_4, 5 mM tetrabutylammonium phosphate, pH 6.0), and 10% CH_3CN changing to 50% solvent B and 50% CH_3CN at 63 min. The flow rate was 1.5 ml/min. Under these conditions, with the columns used, the internal standard eluted at 35.5 ± 0.5 min.

Quantitation of adducts. One-minute (1.5 ml) fractions of the chromatogram were counted without scintillation fluid (Cerenkov counting) in an LKB 1218 scintillation spectrometer. Determination of adducts was made by dividing the radioactivity in the adduct peak (after correcting for overall efficiency and recovery) by the specific activity of the ATP. The amount of adducts per microgram of DNA was the amount of adducts found divided by the amount (in μg) of DNA initially hydrolyzed, corrected for aliquoting during the procedure (Levy and Weber, 1989).

RESULTS

Acute exposure. Exposure of mice to 2-AF at 60 mg/kg resulted in formation of measurable WBC DNA-AF adducts at the 3-hr time point. The chromatogram of ^{32}P-postlabeled, adducted nucleotides is shown in Fig. 1. The overall pattern of the chromatogram is very similar to that seen from liver (Levy and Weber, 1989) and bladder (Levy and Weber, 1992). Fraction 2 (not shown) contains the greatest amount of radioactivity (~30 × 10^6 dpm) followed by decreasing amounts in succeeding fractions until a baseline is reached sometime after fraction 10. These early fractions contain unreacted ATP, ATP hydrolysis products (including ADP, AMP, P_i), and postlabeled normal nucleotides (Levy and Weber, 1988). Small unidentified peaks of ^{32}P-containing material are seen in chromatograms of DNA from both control and treated animals. Some of these may correspond to "I-compounds" or "test tube spots" discussed by Beach and Gupta (1992). Next, a small peak is seen at the position of the internal standard dG-C8-AAF (fractions 35–36) and the main adduct peak occurs two fractions later. This latter peak is dG-C8-AF, although other adducts, formed in minor amounts, may also be present (Levy and Weber, 1988). To verify the identity of the major adduct peak, dG-C8-AAF was subjected to alkaline hydrolysis in the presence of β-mercaptoethanol as described by Shibutani *et al.* (1991) to yield dG-C8-AF. The elution of dG-C8-AF coincided with the peak of adduct radioactivity, indicating that the radioactive peak is dG-C8-AF.

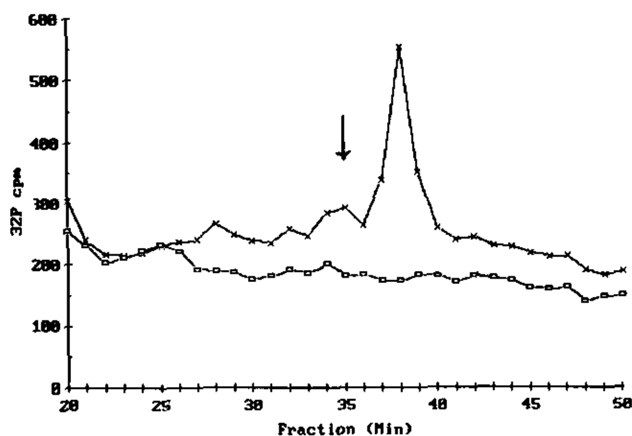

FIG. 1. Chromatogram of ^{32}P-postlabeled nucleotide adducts from mouse leukocytes 3 hr after ip injection of 2-AF (60 mg/kg). Fractions 20–50 are shown. Arrow indicates elution of a standard of dG-C8-AAF. □, control; ×, treated.

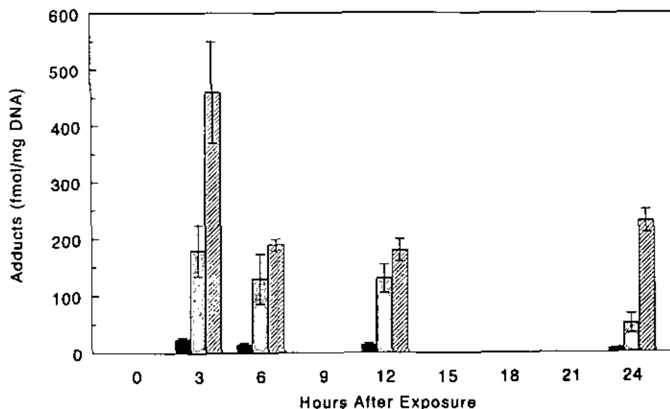

FIG. 2. DNA adduct levels in leukocytes and target tissues of C57BL/6J mice 3 to 24 hr after ip dose of 2-AF (60 mg/kg). Nine mice were used for the 3-hr observation and 3 mice for each of the other time points. Means ± SE are shown. ■, leukocytes; ▨, liver; □, bladder.

The persistence and disappearance of DNA–AF adducts in WBC, liver, and bladder were followed for 24 hr after the acute 60 mg/kg exposure. Nine mice were used for the 3-hr observation and three mice for each of the subsequent time points. Figure 2 shows that at each time point urinary bladder DNA was the most highly adducted followed by hepatic DNA. WBC DNA had the least adduct formation of the three tissues examined. Throughout the 24-hr period WBC adduct levels were 10–15% of the liver adduct levels. The bladder DNA adducts decreased in an irregular manner with the 24-hr time point showing increased adduct levels compared to 6 or 12 hr, but were less than the earliest time examined, 3 hr.

Seven-day exposure. Seven-day continuous exposure to 2-AF was performed in 2 separate, identical experiments carried out 10 months apart. Mouse weight, water consumption, and dose of 2-AF consumed are tabulated in Table 1. The mice tended to lose a small and not significant

TABLE 1
Weights of Mice, Concentrations of 2-AF, and Consumption of Water and 2-AF

Group[a]	Conc. of 2-AF (mg/ml)	Mouse weight (g)			Water consumed (ml)[b]		Dose consumed (mg/kg)
		Day 0	Day 7	Day 14	Days 0–7	Days 8–14	
				Experiment 1			
1	0.000	21.8 ± 0.4[c]	22.6 ± 0.2		24.0		0
2	0.030	22.9 ± 0.7	22.6 ± 1.3		23.5		31
3	0.102	22.9 ± 0.2	22.2 ± 0.6		21.2		96
4	0.300	21.2 ± 0.3	20.9 ± 0.3		21.4		305
5	0.300	22.0 ± 0.8	22.2 ± 0.9	24.3 ± 0.6	18.8	32.0	255
				Experiment 2			
1	0.000	21.4 ± 1.0	22.0 ± 0.9		23.8		0
2	0.030	21.2 ± 0.6	21.9 ± 0.8		23.1		32
3	0.102	22.2 ± 1.2	22.1 ± 1.2		22.4		103
4	0.300	21.4 ± 0.6	22.0 ± 0.6		20.3		280
5	0.300	22.2 ± 1.2	21.9 ± 1.0	24.1 ± 1.4	19.6	36.4	267

[a] Three mice per group.
[b] Average amount of water consumed per mouse for the period.
[c] Means ± SE.

amount of weight during exposure, but those mice that were continued beyond 7 days with a change to tap water regained the weight and increased their weight to more than the starting values. The changes in weight appear to be due to changes in water consumption as the groups exposed to higher concentrations of 2-AF decreased their water consumption, while the group that was changed to tap water had a much increased consumption of water after the change. There were no mortalities and no signs of morbidity in any group during either experiment.

Formation of DNA–AF adducts in WBC, liver, and bladder was proportional to dose after 7 days of continuous exposure. Figure 3 shows combined chromatograms for liver DNA from an individual from each of the five groups. In the 7-day experiment only the one adduct-containing peak corresponding to dG-C8-AF is found. Increasing concentrations of 2-AF led to increased radioactivity in the adduct peak. A 7-day washout period after the 7-day 300 ppm exposure led to a reduction in the size of the adduct peak. Chromatograms of WBC and bladder DNA showed similar results.

DNA adduct formation in response to increasing dose of 2-AF was approximately linear in the indicator tissue. Experiments 1 and 2 gave very similar results for leukocyte DNA adduct formation (Fig. 4). Mice that received tap water for the 7-day period after a 7-day exposure to 300 ppm 2-AF had no detectable carcinogen adducts in their leukocyte DNA. Figures 5A and 5B show the corresponding adduct formation versus dose data for the two target tissues, liver and bladder, respectively. Both tissues show similar, approximately linear dose responses, and both show the reproducibility between the two experiments. Variation at the high-dose level is fairly large, particularly for bladder (>20% of mean value), which may be a reflection of individual differences in drinking and urination. In contrast to the results in leukocytes, both liver and bladder showed DNA adducts in the group 5 mice. In liver, approximately 50% of the DNA adduct level seen after 7 days at 300 ppm 2-AF was found after the additional 7 days without 2-AF (Fig. 5A). The corresponding result for bladder

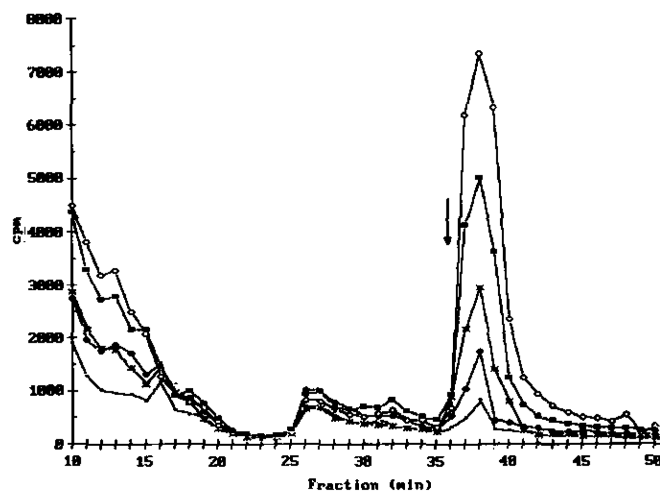

FIG. 3. Composite chromatogram of ^{32}P-postlabeled nucleotide adducts from mouse liver after 7 days continuous dosing with 2-AF. Fractions 10–50 are shown. Arrow indicates elution of a standard of dG-C8-AAF. ·, 0 ppm; ●, 30 ppm; +, 100 ppm; O, 300 ppm; ■, 300 ppm for 7 days followed by 0 ppm for 7 days.

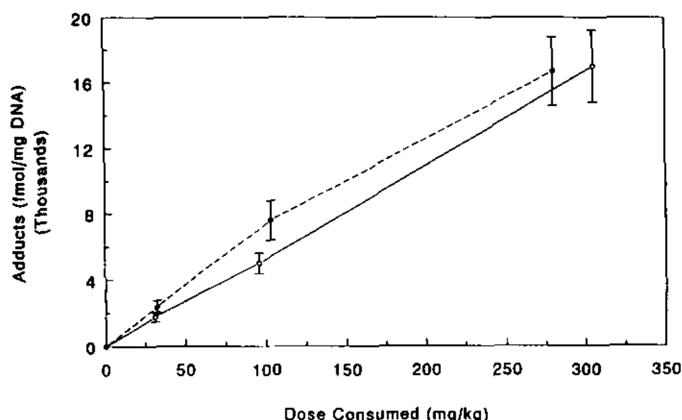

FIG. 4. DNA adduct levels in mouse leukocytes after 7 day continuous dosing with 2-AF. Means ± SE are shown. ○, Experiment 1; ●, Experiment 2.

showed about 75% of the DNA adducts remained after 7 days without 2-AF (Fig. 5B).

DISCUSSION

Previous studies have attempted to use DNA adducts in human WBC or other tissues as indicators of carcinogen exposure to relate adduct formation to intensity of exposure (reviewed in Schut and Shiverick, 1992). However, controlled studies relating a known exposure to a defined carcinogen to adduct formation in WBC cannot be done in humans. Few studies have been reported using animals to examine the dose response of WBC to carcinogens. One such study (Walker *et al.,* 1990) examined DNA adduct formation in WBC and several other tissues of rats repetitively exposed to ethylene oxide, a direct-acting carcinogen. Schut and Herzog, (1992) measured DNA adducts of 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (PhIP) in WBC and tissues of Fischer-344 rats after a single oral dose. Adduct formation at 24 hr after exposure was related to dose and was greatest in WBC and the target organ (colon) of this carcinogen. In the present report, an animal model is used to assess leukocyte DNA adduct formation after both a single, acute exposure and a more chronic 7-day continuous exposure to the model carcinogen 2-AF. In addition to investigating leukocytes as carcinogen exposure indicators, comparisons of DNA adduct formation were also made to the arylamine target tissues to determine the usefulness of leukocytes as biological monitors of DNA damage in internal, hard-to-sample tissues.

Leukocyte DNA–2-AF adducts were observed within 3 hr after a single 60 mg/kg dose of carcinogen. Similarly, adducts were detected in the DNA of liver and bladder. Following the adduct levels for 24 hr indicated a fairly constant relationship between leukocyte and liver adducts. The decline in WBC adducts was parallel to the decline in liver adduct levels; leukocyte DNA adduct levels were between 11 and 14% of liver adduct levels for the entire period (Fig. 2). A similar relationship to bladder DNA adducts was not seen mainly due to the increase in bladder adducts at 24 hr compared to 12 hr. It is possible that the increase in bladder adducts at 24 hr is caused by movement of 2-AF metabolites (such as glucuronides) from liver or other tissues to the bladder followed by acid-catalyzed hydrolysis and reactivation to an ultimate carcinogen (Lang and Kadlubar, 1991).

The 7-day continuous exposure experiments demonstrated a clear dose response between the amount of 2-AF consumed and DNA adduct formation in both leukocytes and the target tissues. As shown in Figs. 4 and 5, increasing the concentration of 2-AF in the drinking water produced an almost linear increase in DNA adduct levels. Of the three tissues, leukocytes had the highest adduct levels per

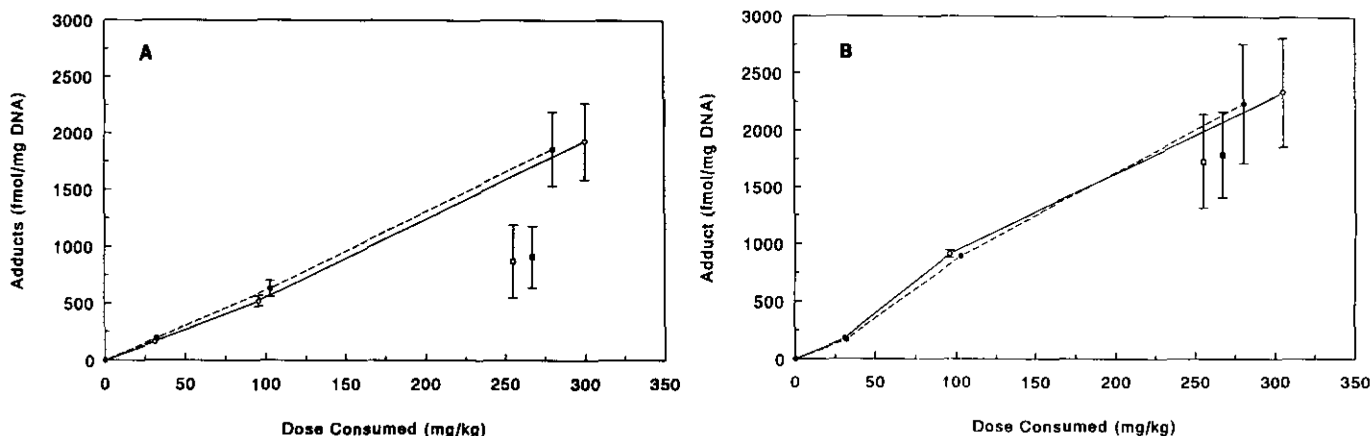

FIG. 5. DNA adduct levels in mouse arylamine carcinogen target tissues after 7-day dosing with 2-AF. Means ± SE are shown. Where error bars are not shown, SE was smaller than size of symbol. ○, Experiment 1 after 7-day exposure; ●, Experiment 2 after 7-day exposure; □, Experiment 1 after 7-day exposure at 300 ppm followed by 7 days at 0 ppm; ■, Experiment 2 after 7-day exposure at 300 ppm followed by 7 days at 0 ppm. (A) Liver; (B) bladder.

mg of DNA after the 7-day exposure. Liver and bladder DNA adducts were nearly 10-fold lower than leukocyte levels, with bladder adducts slightly greater than liver adducts. This result after the 7-day exposure is in contrast to the result of the acute exposure where leukocyte adducts were only 3–7% of bladder adducts and 11–14% of liver adduct levels (Fig. 2).

The reason for the reversal in the order of tissue DNA damage is not apparent from the experiments reported. It should be noted that the routes of administration differed between the single and continuous exposure experiments. The single dose of 2-AF was given as an intraperitoneal injection in DMSO while the continuous dosing was given orally via drinking water. It is likely that nearly all of the intraperitoneally administered dose is rapidly absorbed and travels via the portal vein to be metabolized by the liver. It would be expected that high levels of DNA adducts are formed. In addition, conjugates of 2-AF metabolites, such as glucuronides, could then travel through the circulation to the kidneys and enter the bladder in urine where hydrolysis may occur, releasing active carcinogen to react with bladder epithelial cell DNA (Lang and Kadlubar, 1991).

On the other hand, continuous dosing of 2-AF via the oral route may allow more exposure of the carcinogen to blood cells able to initiate activation to DNA-binding metabolites because more of the carcinogen may escape first-pass hepatic metabolism. This could happen if the lipophillic 2-AF enters the general circulation via the lymphatics. Thus, the area under the plasma concentration vs time curve could be significantly higher for continuously administered 2-AF leading to WBC being exposed to the compound at what is effectively a dose considerably higher than that seen by the internal organs.

The toxicokinetic hypothesis just suggested implies that leukocytes are able to activate 2-AF to a DNA-reactive species. Such a possibility was not investigated in the current work, but is presently under investigation in our laboratory. Several reports in the literature have shown that human WBC can activate carcinogens to form DNA adducts. Shen *et al.* (1990) found that 2-AF incubated with various components of human blood, together with calf thymus DNA and hydrogen peroxide formed the dG-C8-AF adduct. Formation of adducts to the exogenous DNA was greatest with the neutrophil fraction, lower with the mononuclear cell fraction, and absent with erythrocytes. The activation of 2-AF was concluded to be by peroxidative pathways. Shen *et al.* (1990), however, did not examine adduct formation in the granulocyte or mononuclear cell nuclei themselves.

Human lymphocytes cultured for 18 hr with 2-AF showed formation of the dG-C8-AF adduct in their DNA (R. C. Gupta *et al.*, 1988). There was a large interindividual variation (0–550 fmol/mg DNA) in the adduct levels produced. Using a different measure of DNA damage, nucleoid sedimentation, Gao *et al.* (1991) found that activated metabolites of 2-AF, but not 2-AF itself, caused lymphocyte DNA damage in rats *in vitro*. The ability of mouse leukocytes to activate 2-AF is presently unknown. However, mouse blood contains significant levels of 2-AF *N*-acetyltransferase activity (Mattano and Weber, 1987) and mouse WBC have high levels of 2-AF *N*-acetyltransferase activity (Chung *et al.*, 1993). Thus, it appears likely that mouse leukocytes can and do activate 2-AF to a DNA adducting metabolite.

In addition to the possibilities of oxidative and peroxidative activations of 2-AF by mouse WBC, an alternative mechanism may need consideration. Adduct and tumor formation at sites distant from the site of direct exposure prompted Ginsberg and Atherholt (1989) to investigate if electrophilic metabolites of benzo[*a*]pyrene could be detected in mouse serum several hours after dosing. They concluded that for benzo[*a*]pyrene, at least, the active metabolite could be sequestered, probably by protein, protected from hydrolysis, and transported to tissues to form DNA adducts. This type of transport has not been demonstrated for 2-AF, although transport of conjugates, such as glucuronides of *N*-hydroxyaminofluorene, through blood or urine followed by hydrolysis to *N*-hydroxyaminofluorene at a distant site has been suggested (Lang and Kadlubar, 1991).

The activation of 2-AF by leukocytes may differ between the various leukocyte cell types. As mentioned, the peroxidative (via myeloperoxidase) pathway for 2-AF activation occurs mainly in granulocytes and only at low levels in the mononuclear fraction (Shen *et al.*, 1990). Differences in the ability of human leukocytes to activate polycyclic aromatic hydrocarbons are implied by the finding that lymphocyte DNA adduct levels correlate with cigarette smoking and the plasma cotinine concentration, but granulocyte DNA adducts did not show a relationship to smoking (Savela and Hemminki, 1991). Further fractionation of the mononuclear leukocytes into lymphocytes and monocytes showed that monocytes, but not unstimulated lymphocytes, are able to activate benzo[*a*]pyrene to form DNA adducts (Holz *et al.*, 1991). It was also demonstrated that lymphocyte DNA–benzo[*a*]pyrene adducts are formed from carcinogen activated by monocytes.

A striking difference between leukocytes and the target tissues is the lack of detectable adducts in leukocyte DNA 7 days after removal of 2-AF. Under identical conditions, bladder retained approximately 75% and liver retained approximately 50% of the adduct level observed at completion of dosing. A loss of leukocyte DNA adducts has previously been reported during vacation periods in foundry workers occupationally exposed to polycyclic aromatic hydrocarbons (Perera *et al.*, 1988). A rapid loss of polycyclic aromatic hydrocarbon–DNA adducts from leukocytes was also observed in humans a few days after completing a 7-day diet high in charcoal-broiled beef (Rothman *et al.*,

1990). In their study of ethylene oxide DNA adducts in rat tissues, Walker *et al.* (1990) found that WBC adducts decreased rapidly in the first 5 days after cessation of a 28-day exposure whereas the decrease in DNA adducts in other tissues was more prolonged. The loss of adducts from leukocytes could be due to DNA repair, cytotoxicity, or removal of the cells from circulation. While repair and cytotoxicity seem plausible for the decrease in adducts in liver and bladder, the decrease in leukocyte DNA adducts from much higher levels to nondetectable levels probably involves different or additional mechanisms. Obviously, to use leukocytes as indicators of exposure, the cells sampled for monitoring need to be the cells that were exposed to the carcinogen. If the leukocytes sampled at 7 days of exposure to 2-AF and found to contain very high levels of DNA–carcinogen adducts are removed from the circulation in the following 7 days, they will be unavailable for sampling and new, unexposed cells will be analyzed. It is possible that much of the loss of WBC adducts after ending carcinogen exposure is due to normal turnover of the circulating WBC population; removal of cells that were present during carcinogen exposure and replacement with unexposed cells. The time spent by various white cells in the circulation of the mouse has not been studied, but there are data for normal human leukocytes. Human neutrophils have a half-life of about 6 hr in the circulation. Human monocytes have a circulatory half-life of about 3 days (Golde, 1983). The time spent in the circulation for lymphocytes is more variable. From 65 to 85% of lymphocytes are long-lived T-cells with half-lives of a few months up to several years. The remaining 15–35% of lymphocytes are T-cells, B-cells, and null cells that have half-lives of a few hours to about 5 days (Carson, 1983). Leukocytes with a high degree of DNA damage (such as 17×10^3 fmol adduct/mg DNA) are likely to be recognized and removed from the circulation at increased rates. It is also unclear if the persistence of high DNA adduct levels in liver and bladder is a marker characteristic of tissues in which tumorigenesis will occur.

The composition of the mouse and human leukocyte fractions differs significantly. While human and mouse total leukocyte counts are comparable, being around $6–8 \times 10^3/\mu l$, the differential counts are quite different between the two species. In humans a normal differential WBC count shows about 60% granulocytes, 35% lymphocytes, and 5% monocytes (Golde, 1983). The corresponding values for C57BL/6J mice are 23% granulocytes, 66% lymphocytes, and 11% monocytes (Bannerman, 1983). Exactly how the differences in composition and/or circulatory life of the leukocyte fraction between humans and mice affect the use of leukocytes as carcinogen exposure indicators is unclear.

In summary, results of experiments reported here show that leukocytes can be used as indicators of arylamine carcinogen exposure. Acute exposure to 2-AF produced measurable leukocyte DNA adducts. A 7-day continuous exposure to 2-AF resulted in leukocyte DNA adduct production which was shown to be responsive to the dose of carcinogen. The major problem in the use of leukocytes as carcinogen-exposure monitors revealed by these experiments is that leukocyte DNA adducts were no longer measurable 7 days postexposure. Thus, it appears that the leukocyte is not useful as an exposure indicator except for ongoing exposure to carcinogens. Further experiments with longer exposure periods and more frequent postexposure sampling are needed to confirm this finding.

Use of leukocyte DNA adducts as biological monitors of DNA damage to target organs appears promising, although this may also be limited to continuing carcinogen exposure. DNA adduct formation in leukocytes was similar to that found in liver and bladder. All three tissues showed a linear increase in adduct formation with increase in dose. It is most likely that leukocytes are more useful in monitoring carcinogen-induced DNA damage in liver than in bladder. The results presented in Fig. 2 indicate that disappearance of DNA adducts in liver and bladder may differ and leukocyte DNA adducts follow more closely the liver time course. An additional consideration in the case of bladder DNA adducts (and possibly DNA from other extrahepatic organs) is the effect of additional arylamine activation pathways that may become important with age or other changes in the individual. For mice, a large increase in bladder DNA–2-AF adducts was found when using 23-week-old individuals in place of 8-week-olds (Levy and Weber, 1992). The increase, which did not occur in liver DNA adducts, could be due to prostaglandin synthase catalyzed activation of 2-AF (Levy and Weber, 1992). It is not known if the contribution by this pathway to arylamine carcinogen activation in humans can be monitored by leukocytes.

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