Short Communication

Effect of Dose Volume on the Toxicokinetics of Acrylamide and Its Metabolites and 2-Deoxy-D-glucose

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ABSTRACT:

Acrylamide (AA) is a known mutagen and animal carcinogen. Comparison of recent studies revealed significant quantitative differences in AA-induced germ cell mutagenicity. It was hypothesized that despite the administration of AA at similar doses, the discrepancy in the observed effects was most likely due to varying AA concentrations in the administered dosing solution. To test this hypothesis, AA was administered i.p. to mice at 50 mg/kg in a dose volume of 5 or 50 ml/kg, blood was collected at various time points, and AA and its metabolites were quantitated. Changes in dose volume resulted in significant differences in the toxicokinetics of AA and its metabolites and suggested that increased C_{max} of AA led to increased metabolism. This theory, in conjunction with the fact that higher levels of AA-derived radioactivity were detected in

the testes, may explain the greater toxicity of a 50 mg/kg dose when administered in 5 versus 50 ml/kg. The impact of dose volume on the toxicokinetics of 2-deoxy-D-glucose (DG), a nonreactive, nonmetabolizable substance, was also investigated. The areas under the curve for DG were not different for the two dose volumes; however, C_{max} for the more concentrated dose was significantly higher. In conclusion, current studies show that the toxicokinetics of an administered xenobiotic and its metabolites is influenced by the concentration of the parent chemical in the dosing solution. Therefore, it is important to consider the concentration of an administered xenobiotic in the dosing solution because it may affect its toxicokinetics and metabolism and subsequently affect the biological effects of the administered chemical.

Acrylamide (AA; $CH_2 = CH-CO-NH_2$) is an important industrial chemical primarily used in the production of polymers and copolymers. Polyacrylamide polymers and copolymers are used as binders in the paper and textile industries, as flocculants in the treatment of sewage and wastewater, as soil conditioners, in ore processing, and in cosmetics (Friedman 2003). AA is found in carbohydrate-rich foods that are prepared at high temperatures, such as French fries and potato chips and consumed by humans. Consumption of these foods may result in significant human exposure to AA (Tareke et al., 2002). AA is neurotoxic (LoPachin et al., 2003), clastogenic (Ghanayem et al., 2005c), and mutagenic in somatic and germ cells (Ghanayem et al., 2005a,b) in experimental animals and was classified as a probable human carcinogen (IARC, 1994).

AA is well absorbed regardless of the route of administration, and it is metabolized by either conjugation with glutathione and/or oxidation to glycidamide (GA) (Sumner et al., 1992). Oxidation of AA to GA is mediated primarily by cytochrome P450 2E1 (CYP2E1) (Sumner et al., 1999; Ghanayem et al., 2005c). Both AA and GA are capable of binding with hemoglobin and DNA to form adducts (Friedman, 2003; Gamboa da Costa et al., 2003; Ghanayem et al., 2005c). Recent studies in this laboratory confirmed that AA metabolism to GA is a prerequisite for AA-induced somatic and germ cell mutagenicity (Ghanayem et al., 2005a,b). Furthermore, formation of GA-DNA adducts may be involved

in AA-induced toxicity, mutagenicity, and carcinogenicity (Besaratnia and Pfeifer, 2004, 2007; Ghanayem et al., 2005c; Manjanatha et al., 2006).

AA administration (i.p.) at 50 mg/kg to male mice followed by mating with untreated female mice resulted in a 60% pregnancy rate compared with 100% in vehicle-treated mice in association with increased dominant lethal mutations as a result of AA treatment (Adler et al., 2004). In a more recent study, i.p. administration of the same AA dose to male mice followed by mating with untreated female mice caused greater reduction in the pregnancy rate (4.69% compared with 62% in vehicle-treated mice) (Ghanayem et al., 2005a) in comparison to the Alder studies (2004). A close comparison of the two studies revealed that, in addition to the difference in the mouse strain used, the dose volume used in the Alder et al. (2004) studies was 10 ml/kg compared with 5 ml/kg in the Ghanayem et al. (2005a) study. In another dominant lethal mutations study, AA was administered in a dose volume of 100 ml/kg (Adler et al., 2000). Therefore, it was concluded that dose volume was one of the main differences between these studies. We therefore hypothesized that increased dose volume may affect the absorption rate and subsequent toxicokinetics of AA and its metabolites. Current studies were undertaken to compare the effect of dose volume on the toxicokinetics of AA, GA, and the AA-mercapturic acid (AAMA) in mice. In addition, to confirm the universality of this phenomenon, we selected a poorly metabolized chemical, 2-deoxy-D-glucose (DG), and compared the effect of dose volume on the toxicokinetics of this chemical.

Materials and Methods

Chemicals. [2- 3 H]AA, specific activity 5.0 mCi/mmol, was obtained from American Radiolabeled Chemicals (St. Louis, MO). [1- 14 C]DG, specific activity

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ABBREVIATIONS: AA, acrylamide; GA, glycidamide; AAMA, AA-mercapturic acid; DG, 2-deoxy-D-glucose; HPLC, high-performance liquid chromatography; AUC, area under the time versus plasma concentration curve; MRT, mean residence time.

53 mCi/mmol, was obtained from Moravak Biochemicals (Brea, CA). Using high-performance liquid chromatography (HPLC), the radiochemical purity of both chemicals was determined to be greater than 97%. Unlabeled acrylamide was purchased from Fluka Biochemicals (Milwaukee, WI) and was greater than 99.5% pure. Unlabeled DG was purchased from Sigma-Aldrich (St. Louis, MO). Glycidamide and the AAMA were synthesized as described by Fennell et al. (2006).

Animals and Treatments. Five- to 6-month-old, male 129/SV mice were obtained from Charles River Laboratories, Inc. (Wilmington, MA). Animals were housed in a 12-h light/dark cycle facility and fed NIH-31 diet. Both food and water were available ad libitum throughout the experiments. Three mice were used for each time point per chemical. All animal care and experiments were conducted according to National Institutes of Health guidelines (Institute of Laboratory Animal Resources, 1996).

Dosing solutions were freshly prepared before administration by diluting

radiolabeled with unlabeled chemicals in saline solution. [^{14}C]AA or [^{14}C]DG were administered i.p. at 50 mg/kg b.wt. in a dose volume of 5 or 50 ml/kg.

After chemical administration, 3 mice were euthanized by CO_2 asphyxiation and blood was collected by cardiac puncture at each time point (0.25, 0.5, 1, 2, 4, and 8 h). Whole blood was sampled in triplicate, and ^{14}C content was quantitated via oxidation to $^{14}\text{CO}_2$ using a Packard Tri-Carb sample oxidizer (PerkinElmer Life and Analytical Sciences, Waltham, MA) and counted using a model LS6500 scintillation counter (Beckman Coulter, Inc., Fullerton, CA). Remaining blood was centrifuged at 2500 rpm for 30 min at 4°C to separate red blood cells and plasma.

HPLC Analysis of [^{14}C]AA-Derived Radioactivity in Plasma. The HPLC system consisted of a Waters 2695 Separations Module (Waters, Milford, MA) connected on line with a UV detector followed by a 515T Radiomatic Flow Scintillation Analyzer (PerkinElmer Life and Analytical Sciences). Plasma (10 μl) was analyzed using a 250×3.0 mm Luna C18(2) 5-micron column (Phenomenex,

FIG. 1. Effect of dose volume on the concentration of AA (A) and its metabolites, GA (B) and AAMA (C), as a function of time in the plasma of mice after i.p. administration at 50 mg/kg in a dose volume of 5 or 50 ml/kg. Values presented are the mean $\pm$ S.E. ($n = 3$).

TABLE 1

Effect of dose volume on the toxicokinetics of AA and its metabolites, GA and AAMA, after i.p. administration at 50 mg/kg

Dose Volume	AA			GA			AAMA		
	5 ml	50 ml	p Value	5 ml	50 ml	p Value	5 ml	50 ml	p Value
AUC (mM/h)	1.31 ± 0.07	1.62 ± 0.05	0.0006 ^a	0.308 ± 0.018	0.152 ± 0.007	<0.0001	0.268 ± 0.021	0.181 ± 0.009	0.0002
C _{max} (mM)	0.868 ± 0.032	0.664 ± 0.037	<0.0001	0.069 ± 0.005	0.032 ± 0.001	<0.0001	0.047 ± 0.004	0.026 ± 0.001	<0.0001
T _{max} (h)	0.25 ± 0.08	0.50 ± 0.04	<0.0001	2.00 ± 0.00	4.00 ± 0.00	<0.0001	1.00 ± 1.29	4.00 ± 1.36	<0.0001
MRT (h)	1.35 ± 0.04	1.91 ± 0.04	<0.0001	3.06 ± 0.12	4.22 ± 0.15	<0.0001	3.67 ± 0.07	4.11 ± 0.15	0.009

^a p Values compare 5 ml to 50 ml and are two-sided.

Torrance, CA), preceded with a C18 SecurityGuard cartridge (Phenomenex). The mobile phase consisted of a gradient of 100% trifluoroacetic acid (0.1%) at a flow rate of 0.2 ml per minute for 15 min, which was changed to 50% trifluoroacetic acid (0.1%) and 50% acetonitrile and a flow rate of 0.3 ml per minute for 7 min. This solvent system was maintained for 5 min and then changed back to 100% trifluoroacetic acid (0.1%) and a flow rate of 1 ml per minute in 1 min. This gradient and flow rate were kept for 20 min, and the flow rate was changed to 0.2 ml per minute in 1 min. UV-absorbing peaks were monitored at 210 nm. Radioactive peaks were detected using a 500- μ l flow cell with Ultima Flo-M scintillation fluid (PerkinElmer Life and Analytical Sciences) at 3 ml/min. Quantitation of AA and its metabolites was based on the serum concentration (total radioactivities) and the percentage of radioactivity in each peak (region of interest).

Statistical Analysis. The area under the time versus plasma concentration curve (AUC), C_{max}, T_{max}, and mean residence time (MRT) were calculated from the mean concentration at each time point, using a bootstrap method to estimate S.E. (Efron and Tibshirani, 1993). Using the bootstrap S.E., we applied the *t*-statistic to compare 5-ml with 50-ml groups, with the exception of T_{max}. For T_{max}, we used Fisher's exact test because there were only 2 or 3 distinct values that T_{max} assumed (i.e., it was not normally distributed).

Results and Discussion

Effect of Dose Volume on the Toxicokinetics of Acrylamide and Its Metabolites. Toxicokinetic studies using i.p. administration as the dose route are not common. Intraperitoneal, like i.v. administration, is a 100% absorption technique. Unlike i.v. administration, where systemic exposure is more or less immediate, systemic exposure after i.p. administration requires diffusion out of the peritoneal cavity. The effects of a 10-fold difference in dose volume on the toxicokinetics of i.p.-dosed AA in mice have been determined. HPLC analysis of AA-derived radioactivity in plasma revealed the presence of AA, GA, and the AAMA. Plasma concentrations of the three chemicals were modeled using non-compartmental modeling. The AUC of 50 mg/kg doses of AA was slightly but significantly higher when AA was administered in a dose volume of 50 versus 5 ml/kg (Fig. 1A; Table 1). These data are consistent with the fact that the AUC would be expected to be similar for the two dose volumes because the amount of AA administered is the same and i.p. administration is a "100% absorption" route, and eventually the whole dose will diffuse from the peritoneal cavity into the blood. Whereas the AUC is about the same for both dose volumes, the appearance of the AA concentration versus time plot shows important differences. Diffusion from the site of administration into blood is expected to be faster for the more concentrated AA solution (5 ml/kg) because the concentration gradient is greater. Indeed, C_{max} is significantly higher (Table 1), and the plasma concentration of AA is higher for the first two time points after dosing with the 5 ml/kg volume (Fig. 1A). In addition, T_{max} (time to achieve C_{max}) was shorter when AA was administered in 5 ml versus 50 ml (Table 1). In contrast, the plasma AA concentration is slightly higher from approximately 1 to 8 h after dosing with the 50 ml/kg volume. The MRT reflects the differences in the plasma concentration curves: the slower diffusion from the less concentrated dosing solution results in a higher MRT (Table 1).

AA is metabolized to GA primarily via CYP2E1, and GA was detectable in the blood of mice treated with this AA (Ghanayem et al.,

2005a). The higher early systemic exposure to AA after dosing with the 5 ml/kg volume resulted in higher substrate concentration and subsequently greater metabolism to GA. This was substantiated by the fact that the AUC of GA was greater (0.308 versus 0.152) when AA was administered at 5 versus 50 ml/kg, and this difference was clearly statistically significant. Furthermore, C_{max} of GA more than doubled (0.069 versus 0.032) when AA was administered at the 5 ml versus 50 ml/kg, and C_{max} was achieved at an earlier time (T_{max} 2 versus 4 h) (Fig. 1B; Table 1). It is worth noting that GA plasma concentrations were higher at all time points up to 6 h after exposure to AA in the high versus low concentration dosing solution (Fig. 1B). This may explain the greater genotoxicity of 50 mg AA/kg when administered at a high versus low concentration, because GA is considered to be the ultimate genotoxic metabolite of AA (Ghanayem et al., 2005a).

Both AA and GA are subject to conjugation with glutathione, leading to the excretion of the corresponding mercapturic acids in the urine (Ghanayem et al., 2005a). The mercapturic acid derived from the AA-glutathione conjugate (AAMA) was detected and identified in the plasma. The toxicokinetics of this metabolite responded to dose volume in a manner similar to that of GA. The higher systemic exposure characteristic of the more concentrated AA dosing solution seemed to lead to greater production of AAMA with AUC = 0.268 versus 0.181, C_{max} = 0.47 versus 0.026. The differences were statistically significant (Table 1; Fig. 1C). The metabolic pathway leading to the production of AAMA from AA requires several enzymes, so the effect of AA concentration on metabolism to AAMA is not straightforward. Contrary to the metabolism of AA to GA, which is a CYP2E1-mediated epoxidation, a single metabolic step, and more directly shows the effect of concentration on rate of formation.

Administration of AA to male mice causes germ cell mutagenicity (Ghanayem et al., 2005a). The amount of AA-derived radioactivity in the testes of the animals in the current toxicokinetic study was determined as a way of correlating AA internal dose at the target organ and the toxicokinetics of this chemical. Data presented in Fig. 2 show that significantly higher concentrations of AA-derived radioactivity was detected in the testes at 15, 30, and 60 min after AA administration at 50 mg/kg administered in a dose volume of 5 ml/kg compared with the dose administered at 50 ml/kg. The higher plasma concentration of parent and reactive metabolite (GA) after dosing with the more concentrated dosing solution results in higher exposure of target tissue to reactive species.

In light of the fact that CYP2E1 is present in mouse testes (Healy et al., 1999) and it is the primary enzyme responsible for AA metabolism to GA, the proximate germ cell mutagen in mice (Ghanayem et al., 2005a), it is likely that in situ metabolism of AA to GA in the testes may occur. Greater in situ formation of GA in the testes of mice receiving AA solution at a high concentration may be attributed to the higher C_{max} and AUC of this metabolite when the same dose of AA was administered in a dose volume of 5 versus 50 ml. Higher levels of AA in the testes of mice treated with AA in 5 ml are expected to lead to the formation of higher levels of GA (higher C_{max} and AUC), leading to greater germ cell mutagenicity than in mice treated with the

FIG. 2. Effect of dose volume on the concentration of AA-derived radioactivity in the testes of mice treated with a single dose of AA at 50 mg/kg in a dose volume of 5 or 50 ml/kg. Values presented are the mean $\pm$ S.E. ($n = 3$).

same dose of AA in 50 ml/kg. In support of this scenario, calculation of the testes/plasma ratios of total AA-derived radioactivity revealed that the ratios are consistently greater than unity. At 50 mg/5 ml, the ratios were as follows: 1.23, 1.22, 1.85, 1.68, 2.43, and 3.07 at 0.25, 0.5, 1.0, 2.0, 4.0, and 8.0 h, respectively. Whereas the testicular levels of total AA-derived radioactivity were significantly lower at 50 mg/50 ml, the tissue/plasma ratios (1.39, 1.37, 1.59, 1.87, 2.41, and 3.11 at 0.25, 0.5, 1.0, 2.0, 4.0, and 8.0 h, respectively) remained comparatively similar. This clearly proved that AA-derived radioactivity had a tendency to distribute to and remain in the testicular tissue, and this function may occur to a greater extent when AA is administered at the same dose at a higher concentration. Earlier studies from this laboratory demonstrated significantly greater formation of GA-DNA adducts in the testis of wild-type versus *Cyp2e1*-null mice (Ghanayem et al., 2005c). This suggested that the majority of the radioactivity found in the testes may be attributed to the in situ GA formation via CYP2E1 enzyme and may explain the greater germ cell mutagenicity of AA when administered at the same dose at a high versus low concentration.

Effect of Dose Volume on DG Kinetics. To further assess the effect of dose volume, we investigated the effect of dose volume on the toxicokinetics of DG in mice. DG was selected because it is poorly metabolized and has negligible pharmacological or toxicological effects. The appearance of the concentration versus time plots for DG

FIG. 3. Effect of dose volume on the concentration of DG as a function of time in the plasma of mice after i.p. administration at 50 mg/kg in a dose volume of 5 or 50 ml/kg. Values presented are the mean $\pm$ S.E. ($n = 3$).

TABLE 2

Effect of dose volume on the toxicokinetics of DG after i.p. administration at 50 mg/kg

	2-Deoxy-D-Glucose		
	5 ml	50 ml	<i>p</i> Value
AUC (mM/h)	0.299 $\pm$ 0.020	0.342 $\pm$ 0.015	0.075 ^a
<i>C</i> _{max} (mM)	0.386 $\pm$ 0.027	0.258 $\pm$ 0.010	<0.0001
<i>T</i> _{max} (h)	0.25 $\pm$ 0.00	0.25 $\pm$ 0.00	1.00
MRT (h)	1.18 $\pm$ 0.06	1.31 $\pm$ 0.04	0.054

^a *p* Values compare 5 ml to 50 ml and are two-sided.

was similar to those for AA, consistent with a higher rate of diffusion from the peritoneal cavity from the more concentrated dosing solution. Regardless of the dose volume, *T*_{max} for DG in plasma occurred 15 min after i.p. administration (Fig. 3; Table 2). *C*_{max} and MRT were higher after administration of DG in 5 versus 50 ml/kg (Fig. 3; Table 2). The AUC was slightly decreased after the administration of 50 mg DG/kg in 5 versus 50 ml/kg (Table 2), but in contrast to AA, the difference was not significant. AA is both reactive and metabolized, and this may explain the difference in AUCs. Unlike DG, AA could react with nucleophiles in the peritoneal cavity or undergo biotransformation in a concentration-dependant manner elsewhere.

In conclusion, current data demonstrated that the diffusion rate from the peritoneal cavity is dependent on the concentration of the administered xenobiotic in the dosing solution. The greater the concentration of the administered xenobiotic in the dosing solution, the greater the absorption rate and subsequently the greater *C*_{max}. It is logical to suggest that changes in the toxicokinetics of an administered xenobiotic will affect the pharmacological and/or toxicological effects of such chemicals. Current results also showed that the toxicokinetics of the metabolites of an administered xenobiotic also changes as a function of the concentration of the administered parent compound. Increased concentration of parent chemical in the dosing solution is associated with increased *C*_{max} as well as decreased *T*_{max} of that chemical. The increase in the substrate concentration may subsequently lead to increased metabolism. It is therefore concluded that an increase in AA diffusion rate and *C*_{max} associated with high concentration in the dosing solution leads to increased rate of AA metabolism (due to increased plasma substrate concentration) to GA. Because metabolism to GA is required for AA-induced somatic and germ cell mutagenicities (Ghanayem et al., 2005a,b,c), current studies demonstrated that changes in the concentration of AA in the dosing solution leads to significant changes in the toxicokinetics of its metabolites and as a result causes a significant quantitative impact on the toxicological effects of this chemical. In the current study, the impact of dose volume was investigated after i.p. administration; however, there is no reason that dose volume will not have a similar impact on the toxicokinetics of xenobiotics after gavage or s.c. administration.

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References

- Adler ID, Baumgartner A, Gonda H, Friedman MA, and Skerhut M (2000) 1-Aminobenzotriazole inhibits acrylamide-induced dominant lethal effects in spermatids of male mice. *Mutagenesis* 15:133–136.

- Adler ID, Gonda H, Hrabé de Angelis M, Jentsch I, Otten IS, and Speicher MR (2004) Heritable translocations induced by dermal exposure of male mice to acrylamide. *Cytogenet Genome Res* **104**:271–276.
- Besaratinia A and Pfeifer GP (2004) Genotoxicity of acrylamide and glycidamide. *J Natl Cancer Inst* **96**:1023–1029.
- Besaratinia A and Pfeifer GP (2007) A review of mechanisms of acrylamide carcinogenicity. *Carcinogenesis* **28**:519–528.
- Efron B and Tibshirani R (1993). *An Introduction to the Bootstrap*. Chapman and Hall/CRC, Boca Raton, Florida.
- Fennell TR, Sumner SC, Snyder RW, Burgess J, and Friedman MA (2006) Kinetics of elimination of urinary metabolites of acrylamide in humans. *Toxicol Sci* **93**:256–267.
- Friedman M (2003) Chemistry, biochemistry, and safety of acrylamide. A review. *J Agric Food Chem* **51**:4504–4526.
- Gamboa da Costa G, Churchwell MI, Hamilton LP, Von Tungeln LS, Beland FA, Marques MM, and Doerge DR (2003) DNA adduct formation from acrylamide via conversion to glycidamide in adult and neonatal mice. *Chem Res Toxicol* **16**:1328–1337.
- Ghanayem BI, Witt KL, El-Hadri L, Hoffler U, Kissling GE, Shelby MD, and Bishop JB (2005a) Comparison of germ cell mutagenicity in male CYP2E1-null and wild-type mice treated with acrylamide: evidence supporting a glycidamide-mediated effect. *Biol Reprod* **72**:157–163.
- Ghanayem BI, Witt KL, Kissling GE, Tice RR, and Recio L (2005b) Absence of acrylamide-induced genotoxicity in CYP2E1-null mice: evidence consistent with a glycidamide-mediated effect. *Mutat Res* **578**:284–297.
- Ghanayem BI, McDaniel LP, Churchwell MI, Twaddle NC, Snyder R, Fennell TR, and Doerge DR (2005c) Role of CYP2E1 in the epoxidation of acrylamide to glycidamide and formation of DNA and hemoglobin adducts. *Toxicol Sci* **88**:311–318.
- Healy LN, Pluta LJ, and Recio L (1999) Expression and distribution of cytochrome P450 2E1 in B6C3F1 mouse liver and testes. *Chem Biol Interact* **121**:199–207.
- IARC (1994) Acrylamide. *IARC Monogr Eval Carcinog Risks Hum* **60**:389–4334.
- Institute of Laboratory Animal Resources (1996) *Guide for the Care and Use of Laboratory Animals* 7th ed. Institute of Laboratory Animal Resources, Commission on Life Sciences, National Research Council, Washington DC.
- LoPachin RM, Balaban CD, and Ross JF (2003) Acrylamide axonopathy revisited. *Toxicol Appl Pharmacol* **188**:135–153.
- Manjanatha MG, Aidoo A, Shelton SD, Bishop ME, McDaniel LP, Lyn-Cook LE, and Doerge DR (2006) Genotoxicity of acrylamide and its metabolite glycidamide administered in drinking water to male and female Big Blue mice. *Environ Mol Mutagen* **47**:6–17.
- Sumner SC, Fennell TR, Moore TA, Chanas B, Gonzalez F, and Ghanayem BI (1999) Role of cytochrome P450 2E1 in the metabolism of acrylamide and acrylonitrile in mice. *Chem Res Toxicol* **12**:1110–1116.
- Sumner SC, MacNeela JP, and Fennell TR (1992) Characterization and quantitation of urinary metabolites of [1,2,3-¹³C]acrylamide in rats and mice using ¹³C nuclear magnetic resonance spectroscopy. *Chem Res Toxicol* **5**:81–89.
- Tareke E, Rydberg P, Karlsson P, Eriksson S, and Törnqvist M (2002) Analysis of acrylamide, a carcinogen formed in heated foodstuffs. *J Agric Food Chem* **50**:4998–5006.

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