Metabolism and Disposition of Bladder Carcinogens in Rat and Guinea Pig: Possible Mechanism of Guinea Pig Resistance to Bladder Cancer


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ABSTRACT

The metabolism and disposition of N-[4-(5-nitro-2-furyl)-2-thiazoly]formamide (FANFT) and 2-amino-4-(5-nitro-2-furyl)thiazole (ANFT) were studied in rat and guinea pig. Rat is susceptible whereas guinea pig is resistant to FANFT-induced bladder cancer. Rats and guinea pigs were p.o. administered either 2-[14C]ANFT or 2-[14C]FANFT (100 mg/kg), and 18-h urine and feces were collected. Tissue distribution of radiolabel was determined. In both species, the highest concentrations of radioactivity expressed as nmol/g tissue were observed in the urine and intestines. Urinary metabolites were separated by high-performance liquid chromatography and radioactivity determined by radioanalytical detection. FANFT was not detected in urine from either species under any experimental condition. More ANFT was observed in urine following FANFT than ANFT administration. This deformatylation-dependent excretion of FANFT was demonstrated in both species and has been previously described as renal metabolic/excretory coupling. Less ANFT, the carcinogen more proximate than FANFT, is excreted in guinea pigs compared with rats. A unique ANFT metabolite was identified in guinea pig but not rat urine. This metabolite represented 80 and 18% of radioactivity recovered in guinea pig urine following ANFT and FANFT administration, respectively. A metabolite produced by guinea pig liver and kidney microsomes in the presence of uridine-5'-diphosphoglucuronic acid coeluted with this unique metabolite. The urinary metabolite was characterized using hydrolytic enzymes, acid hydrolysis, and mass spectrometry and identified as an ANFT-β-glucuronide. A unique UDP-glucuronosyltransferase appears to be responsible, at least in part, for the reduced amount of free ANFT excreted by guinea pigs compared with rats. Reduced levels of urinary ANFT observed in guinea pigs may partially explain the resistance of this species to FANFT-induced bladder cancer.

INTRODUCTION

Aromatic and heterocyclic amines elicit mutagenic and carcinogenic effects (1-5). These effects exhibit species and tissue specificities (6-8). Unique metabolic and disposition pathways play important roles in species and tissue specificity. Many examples of this are provided by studies with aromatic amines. The involvement of sulfotransferase in carcinogenesis is demonstrated by studies with brachymorphic mice. This strain, deficient in 3'-phosphoadenosine-5'-phosphosulfate (9), is resistant to N-hydroxy-2-acetylaminofluorene-induced liver tumors (10). In humans, aromatic amine-induced bladder cancer occurs primarily in the slow- rather than the fast-acetyulating phenotype (11, 12). The nonacetyulating dog develops aromatic amine-induced bladder cancer (6, 7), whereas rapidly acetyulating rodents develop liver rather than bladder cancer (13). In addition to acetylation, deacetylation can also contribute to increases in susceptibility to carcinogenesis. The highest rate of N-hydroxy-2-acetylaminofluorene deacetylation in rat occurs in the Zymbal's gland, an organ that is highly susceptible to carcinogenesis by 2-acetylaminofluorene and N-hydroxy-2-acetylaminofluorene (14). The heterocyclic amine, FANFT, is a model compound used to study bladder cancer in rats, mice, and hamsters (15). However, guinea pigs are resistant to FANFT-induced bladder cancer (16). The more proximate carcinogen in FANFT-induced bladder cancer is thought to be ANFT, deformatylated FANFT. Metabolism and disposition of FANFT and ANFT in guinea pig were compared to rat in an effort to provide an explanation for guinea pig resistance to FANFT-induced bladder cancer.

MATERIALS AND METHODS

Radiolabeled ANFT and FANFT were synthesized from [14C]-thiourea (57.5 mCi/mmol) obtained from ICN Radiochemicals (Irvine, CA) and 2-bromoacetyl-5-nitrofuran as described by Sherman and Dickson (17). UDP-glucuronic acid, β-glucuronidase (type VII), sulfatase (type VI), and γ-glutamyltranspeptidase (type IV) were obtained from Sigma Chemical Co. (St. Louis, MO). NCS tissue solubilizer and ACS scintillation cocktail were purchased from Amersham Corp. (Arlington Heights, IL). Chlorotrimethylsilane and bis(trimethylsilyl)-trifluoroacetamide were purchased from Aldrich Chemical Co. (Milwaukee, WI). All other chemicals were reagent grade. Solvents were HPLC grade.

Animals. Male Hartley guinea pigs (100-200 g) and male Fischer 344 rats (75-130 g) were purchased from Harlan Industries (Indianapolis, IN).

In Vivo Studies. Animals were anesthetized with diethylether for approximately 20 s, dosed p.o. with either 2-[14C]ANFT or 2-[14C]-FANFT (100 mg/kg; specific activity, 80-110 μCi/mmol) dissolved in dimethyl sulfoxide, and placed in plastic metabolism cages; 18-h urine and feces were collected. Tissue distribution of radiolabel was determined. In both species, the highest concentrations of radioactivity expressed as nmol/g tissue were observed in the urine and intestines. Urinary metabolites were separated by high-performance liquid chromatography and radioactivity determined by radioanalytical detection. FANFT was not detected in urine from either species under any experimental condition. More ANFT was observed in urine following FANFT than ANFT administration. This deformatylation-dependent excretion of FANFT was demonstrated in both species and has been previously described as renal metabolic/excretory coupling. Less ANFT, the carcinogen more proximate than FANFT, is excreted in guinea pigs compared with rats. A unique ANFT metabolite was identified in guinea pig but not rat urine. This metabolite represented 80 and 18% of radioactivity recovered in guinea pig urine following ANFT and FANFT administration, respectively. A metabolite produced by guinea pig liver and kidney microsomes in the presence of uridine-5'-diphosphoglucuronic acid coeluted with this unique metabolite. The urinary metabolite was characterized using hydrolytic enzymes, acid hydrolysis, and mass spectrometry and identified as an ANFT-β-glucuronide. A unique UDP-glucuronosyltransferase appears to be responsible, at least in part, for the reduced amount of free ANFT excreted by guinea pigs compared with rats. Reduced levels of urinary ANFT observed in guinea pigs may partially explain the resistance of this species to FANFT-induced bladder cancer.

In Vitro Studies. Animals were anesthetized with diethylether for approximately 20 s, dosed p.o. with either 2-[14C]ANFT or 2-[14C]-FANFT (100 mg/kg; specific activity, 80-110 μCi/mmol) dissolved in dimethyl sulfoxide, and placed in plastic metabolism cages; 18-h urine and feces were collected. Tissue Solubilization. Tissue, heparinized blood, and fecal samples were solubilized in volumes (ml) of NCS solubilizer equal to 6 times their g weight. Samples were heated overnight at 50°C, cooled, and neutralized with glacial acetic acid. If necessary, samples were decolorized with hydrogen peroxide. Radioactivity was quantitated with a Beckman LS-6800 scintillation counter. Sample radioactivity was quench-checked.

In Vivo Studies. Animals were anesthetized as above and livers and kidneys were removed and placed in saline at 4°C. Minced tissue was homogenized 3 times for 15 s at 4°C with a Polytron homogenizer in volumes (ml) of buffer (100 mM phosphate buffer, pH 7.8, containing 20% glycerol, 1 mm diethyletriminepenta-acetic acid and 0.1 mm EDTA) and 2-bromoacetyl-5-nitrofuran as described by Sherman and Dickson (17). UDP-glucuronic acid, β-glucuronidase (type VII), sulfatase (type VI), and γ-glutamyltranspeptidase (type IV) were obtained from Sigma Chemical Co. (St. Louis, MO). NCS tissue solubilizer and ACS scintillation cocktail were purchased from Amersham Corp. (Arlington Heights, IL). Chlorotrimethylsilane and bis(trimethylsilyl)-trifluoroacetamide were purchased from Aldrich Chemical Co. (Milwaukee, WI). All other chemicals were reagent grade. Solvents were HPLC grade.

Recording. Rats and guinea pigs were killed by cervical dislocation at 24 h, 48 h, and 7 days after dosing. Livers and kidneys were removed for subsequent radioactive analysis. Samples were stored at -20°C.

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4 The abbreviations used are: FANFT, N-[4-(5-nitro-2-furyl)-2-thiazoly]formamide; ANFT, 2-amino-4-(5-nitro-2-furyl)thiazole; HPLC, high-performance liquid chromatography; TSP/MS, thermospray liquid chromatography/mass spectrometry; EI, electron ionization; CI, chemical ionization; UDP-glucuronic acid, uridine 5'-diphosphoglucuronic acid, sodium salt; TMS, trimethylsilyl.
dithiothreitol) equal to 3 times the sample g weight (18). The homog-
enate was centrifuged for 15 min at 20,000 × g. The supernatant was
filtered through 2 layers of gauze and centrifuged for 90 min at 100,000
× g. The supernatant was decanted and the microsomal pellet resus-
pended by homogenization in one-third the original tissue weight of 20
mm phosphate buffer, pH 7.4. Both supernatant and microsomal frac-
tions were dispensed and stored at −70°C. Protein content was deter-
mined by the method of Lowry et al. (19).

UDP-glucoronosyltransferase activity (EC 2.4.1.17) activity was determined
using a modification of the method of Gorski and Kasper (20). The
assay mixture contained 150 mM malate-Tris buffer, pH 7.7, 20 mM
MgCl₂, 5 mM UDP-glucuronic acid, and 0.05 mM 2-[¹⁴C]ANFT (final vol-
ume, 0.25 ml) for 10 min at 37°C. Incubations were started with the
addition of protein. The incubation was stopped with the addition of 2 volumes of water-saturated ethyl acetate. Unconjugated ANFT was removed by 3 extractions with water-
saturated ethyl acetate. Radioactivity remaining in the aqueous layer
was assayed using a Beckman LS-6800 scintillation counter. Results are expressed as
nmol/min/mg protein.

UDP-glucuronosyltransferase activity for ANFT was also assessed.
Liver and kidney samples were incubated in 75 mM malate-Tris buffer,
pH 7.4, containing 10 mM MgCl₂, 5 mM UDP-glucuronic acid, and
0.05 mM 2-[¹⁴C]ANFT (final volume, 0.25 ml) for 10 min at 37°C.
Incubations were started with the addition of protein. The incubation
was stopped with the addition of 2 volumes of water-saturated ethyl acetate. Radioactivity remaining in the aqueous layer
was assayed using a Beckman LS-6800 scintillation counter. Results are expressed as
pmol/min/mg protein. Similar results were observed when reaction mixtures were assayed by HPLC.

HPLC Characterization of Urine Samples. Urine samples were de-
salted and purified on a water-equilibrated C-18 disposable extraction
column. Retained material was eluted with methanol, concentrated,
and used for subsequent analyses. Metabolites were separated by a
HPLC system consisting of a μ-Bondapak C-18 semipreparative col-
umn and C-18 guard column using gradient solvent program 1. Gra-
dient elution was controlled by a Beckman Gold Analog Interface and
a NEC computer. Solvent program 1 conditions were as follows: flow
rate, 1.5 ml/min; 5% methanol:95% ammonium formate, pH 6.0, 0–10 min; 5–35% methanol linear gradient, 10–70 min; 35–100%
methanol linear gradient, 70–100 min. Solvent program 2 consisting of isocratic conditions (45% methanol:55% water; flow rate, 2.0 ml/
imin) was used for the quantification of ANFT and FANFT. Radioac-
tivity in HPLC eluents was measured using a FLO-ONE radioanalytical
detector.

Pooled urine from guinea pigs treated with ANFT was subjected to
specific hydrolytic conditions for metabolite characterization. Samples
were digested for 18 h at 37°C with either 5 units of γ-glutamyltrans-
peptidase in 0.1 M Tris-phosphate buffer, pH 7.1; 50 units of β-
glucuronidase in 0.25 M acetae buffer, pH 5.2; 0.1 units of sulfatase in
0.1 M Tris-phosphate buffer, pH 7.1; or 0.02 M citric acid, pH 1.4.
Enzymes were precipitated with 2 volumes of cold ethanol. Supernat-
a were analyzed by HPLC using solvent program 1.

Mass Spectrometry. TSP/MS experiments were performed on a Ves-
tec quadrupole system (Houston, TX) equipped with a Technivent data
system (St. Louis, MO). Ten-μl samples were flow-injected into the
TSP/MS system using the column bypass mode at a flow rate of 1.5
ml/min with aqueous 0.1 M ammonium acetate:methanol (70:30 v/v)
as the thermospray buffer solution. TSP/MS analyses were also carried
out using a Shimazu (Tokyo, Japan) LC-6A liquid chromatograph
equipped with a C-18 reverse-phase semi preparative column and oper-
ated isocratically (30% methanol in 0.1 M ammonium formate) at a
flow rate of 1.5 ml/min. At this flow rate, the ionizer pressure was
typically 1 × 10⁻⁵ Torr and the vaporizer (tip) and jet temperatures
were 188°C and 240°C, respectively.

Both EI and CI spectra were acquired with a Hewlett Packard 5988
(Palo Alto, CA) gas chromatography/mass spectrometry system with
extended mass range. Source temperature in EI and CI were 200°C
and 100°C, respectively. Samples were introduced by direct insertion
probe. The per-O-TMS derivative was prepared by adding bis(trimethylsilyl)trifluoroacetamide (with 1% chlorotrimethylsilane)
and heating the mixture at 60°C overnight. Fast atom bombardment
high-resolution measurements were performed using a VG ZAB-SE
double-focusing mass spectrometer equipped with a VG-11 data system.
High-resolution measurements were made by manual peak matching
against appropriate ions in the spectrum of glycerol.

Statistical Analysis. Results are presented as the mean ± SE. Data
were analyzed using a Student’s t test for comparison of unpaired
means of 2 sets of data. The number of determinations is noted in the
tables. Significant difference between sets of data is indicated by P <
0.01.

RESULTS

The distribution of radioactivity in rat and guinea pig after
p.o. dosing of 2-[¹⁴C]ANFT or 2-[¹⁴C]FANFT was determined (Table 1). In both guinea pig and rat, the major portion of
recovered radioactivity from either compound accumulated in
the urine, intestines, and carcass. In rat, the ANFT-dosed
animals had concentration values (nmol/g tissue) significantly
higher in spleen and carcass than FANFT-dosed animals. Cor-
responding values were also higher for ANFT compared with
FANFT in kidney, liver, stomach, small intestine, heart, and

<table>
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<tr>
<th>Table 1 Distribution of p.o. administered 2-[¹⁴C]ANFT and 2-[¹⁴C]FANFT in guinea pig and rat</th>
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<tr>
<td>Animals (n = 3–6) were sacrificed 18 h after a p.o. dose of 2-[¹⁴C]ANFT or 2-[¹⁴C]FANFT (100 mg/kg) in dimethyl sulfoxide. Radioactivity measurements were quench-corrected. Results are expressed as the mean ± SEM of nmol/g tissue (wet weight). See “Materials and Methods” for details.</td>
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<td>Urine</td>
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<td>Bladder</td>
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<td>Small intestine</td>
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<td>Large intestine</td>
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<td>Feces</td>
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<td>Carcass</td>
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<td>Plasma (% body wt)</td>
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<tr>
<td>% dose recovered</td>
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* Values significantly different from corresponding FANFT-dosed guinea pig values.
* Values significantly different from corresponding ANFT-dosed guinea pig values.
* Values significantly different from corresponding ANFT-dosed rat values.
lung. In guinea pig, no significant differences were observed between ANFT- compared with FANFT-treated animals. Significant differences were observed between rat and guinea pig dosed with FANFT. Higher concentration values (nmol/g tissue) were reported in rat compared with guinea pig with respect to bladder, kidney, liver, stomach, heart, carcass, and plasma. Significant differences were also reported for rat and guinea pig dosed with ANFT. The nmol/g tissue values of rat dosed with ANFT were significantly higher in the kidney, liver, lung, spleen, and carcass than guinea pig. Thus, in general the susceptible species, rat, accumulated more radioactivity from these carcinogens than the correspondingly dosed resistant species, guinea pig.

Urinary profiles from ANFT-treated rats and guinea pigs are shown in Fig. 1. In ANFT-treated rats, ANFT is metabolized to a large number of compounds (Fig. 1A). In ANFT-treated guinea pigs, 70–90% of the recovered radioactivity elutes at 58 min (Fig. 1B).

Urinary profiles from FANFT-treated rats and guinea pigs are shown in Fig. 2. In both species, FANFT is metabolized to a large number of compounds. The 58-min metabolite seen in ANFT-treated guinea pig urine is a major metabolite of the FANFT-treated guinea pig urine. This metabolite is not observed in rat urine.

The relationships between dosing with either ANFT or FANFT and the amount of ANFT and the new metabolite present in urine from rat and guinea pig were examined (Table 2). In both species, more ANFT was detected when FANFT was administered compared with ANFT administration. When ANFT was administered, ANFT was not recovered from guinea pig urine. After treatment with ANFT, only guinea pigs excreted the new metabolite. Total radioactivity observed in the new metabolite plus ANFT was greatest in the ANFT-treated guinea pigs. The new metabolite from ANFT-treated guinea pig was further characterized. This compound was resistant to hydrolysis by β-glucuronidase, sulfatase, and γ-glutamyltranspeptidase. The metabolite was hydrolyzed under highly (pH 1.4) but not mildly (pH 5.0) acidic conditions, with ANFT being recovered. Absorbance maxima of the new metabolite were at 385 and 280 nm. These results are consistent with the new metabolite being an N-glucuronide.

ANFT UDP-glucuronosyltransferase activity was determined in kidney and liver from rat and guinea pig (Table 3). The enzymatic activity was primarily localized in the microsomal
fraction of liver and kidney in guinea pig. In guinea pig, activity was significantly higher in liver compared with kidney microsomes. The microsomal product coeluted with the new metabolite peak observed in guinea pig urine. Acid hydrolysis of the microsomal product yielded ANFT. No ANFT transferase activity was observed in rat preparations.

*p-Nitrophenol UDP-glucuronosyltransferase activity was determined in kidney and liver microsomal preparations from rat and guinea pig (Table 4). Enzyme activity in guinea pig liver and kidney microsomes was not significantly different. The activity observed in rat kidney was significantly higher than that in liver microsomes. Transfase activity of guinea pig liver and kidney microsomes was significantly higher than that of corresponding tissues in rat.

Mass spectral analyses were performed to identify the new urinary metabolite from ANFT-treated guinea pigs. Fig. 3 shows the TSP/MS of the metabolite with the [MH]+ ion at m/z 388, base peak at m/z 254, a major ion at m/z 212, and 2 important ions at m/z 284 and m/z 182. The EI mass spectrum of the per-O-TMS derivative showed molecular ion at m/z 675 (387 + [4 × 72]) and ions at m/z 375, 305, 285, 217, and 204, characteristic of tetrasilylglucuronic acid (21, 22). The methane CI mass spectrum of the per-O-TMS derivative gave a [MH]+ ion at m/z 676 (20%), a [M + C3H5]+ ion at m/z 704 (10%), a [M + C3H5]+ ion at m/z 716 (5%), and the base peak at m/z 212. Fast atom bombardment high-resolution measurement of the [MH]+ ion was as follows:

| Table 2 Relationships between dosing with either ANFT or FANFT and the amount of ANFT and the new metabolite present in urine from guinea pig and rat |
|-----------------------------|------------------|------------------|
| Animals were dosed p.o. with 2-[^14C]ANFT or 2-[^14C]FANFT (100 mg/kg) and 18-h urine was collected. Urine samples from individual animals were separated by HPLC, and radioactivity was determined by radioanalytical detection. ANFT was quantitated using solvent program 2 and the new metabolite was quantitated using a modification of solvent program 1. Total represents the sum of the % of dose administered present in urine as ANFT and New metabolite. See “Materials and Methods” for details. |

<table>
<thead>
<tr>
<th>% of dose administered</th>
<th>ANFT</th>
<th>New metabolite</th>
<th>Total</th>
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</thead>
<tbody>
<tr>
<td>Guinea pig</td>
<td>ANFT</td>
<td>0</td>
<td>24 ± 0.3</td>
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<td></td>
<td>FANFT</td>
<td>0.2 ± 0.3</td>
<td>3.2 ± 0.9</td>
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<tr>
<td>Rat</td>
<td>ANFT</td>
<td>0.2 ± 0.1</td>
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<td></td>
<td>FANFT</td>
<td>0.9 ± 0.2</td>
<td>0</td>
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| Table 3 ANFT UDP-glucuronosyltransferase activity in the liver and kidney of guinea pig and rat |
|---------------------------------------------|------|-----------------|
| UDP-glucuronosyltransferase activity was assayed by incubating enzyme preparations in the presence of 5 mm UDP-glucuronic acid and 0.05 mm ANFT. See “Materials and Methods” for details. |

<table>
<thead>
<tr>
<th>Guinea pig (pmol/min/mg protein)</th>
<th>Rat (pmol/min/mg protein)</th>
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<tbody>
<tr>
<td>Liver</td>
<td>Kidney</td>
</tr>
<tr>
<td>100,000 × g supernatant</td>
<td>5.8 ± 1.1</td>
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<td>Microsomes</td>
<td>90 ± 10</td>
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<th>Table 4 p-Nitrophenol UDP-glucuronosyltransferase activity in liver and kidney microsomal preparations from rat and guinea pig</th>
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<td>UDP-glucuronosyltransferase activity was assayed by incubating enzyme preparations in the presence of 5 mm UDP-glucuronic acid and 0.02 mm p-nitrophenol. See “Materials and Methods” for details.</td>
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<th>nmol/min/mg protein</th>
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<tr>
<td>Liver</td>
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<td>Guinea pig</td>
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<tr>
<td>Rat</td>
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DISCUSSION

This is the first study to assess differences in the metabolism and disposition of FANFT and ANFT in rat and guinea pig, species susceptible and resistant to FANFT-induced bladder cancer, respectively. The major portion of the recovered radioactivity from either species was observed in the carcass, intestines, or urine. Significantly higher nmol/g tissue values were observed in most organs from ANFT-dosed rats compared with ANFT-dosed guinea pigs. A similar phenomenon was observed with FANFT-treated rats, with the bladder being one of several tissues having a higher concentration of radioactivity. It is important to note that the total recovery of radioactivity was greater in rat compared with guinea pig. This may have contributed to higher levels of radioactivity reported in rat compared with guinea pig. The higher levels of radioactivity retained in rat relative to guinea pig is consistent with FANFT/ANFT being more carcinogenic in rat compared with guinea pig. Although the concentration of radioactivity in urine was not significantly different between or within the various groups, profiles of metabolites were distinct (Figs. 1 and 2). Significantly more ANFT was recovered in urine from animals dosed with FANFT than ANFT (Table 2). In rat, this is consistent with the reported renal metabolic/excretory coupling of FANFT/ANFT (23). According to this phenomenon, FANFT is more rapidly excreted than ANFT due to increased uptake with subsequent renal deformylation-dependent excretion. The plasma half-time for ANFT is much longer than that for FANFT (23 ± 3 versus <5 min, respectively). Thus, ANFT is more available for metabolism than FANFT. A similar pharmacodynamic study has not been performed in guinea pig to determine if metabolic/excretory coupling can be demonstrated. The formation of an additional metabolite appears to have significantly contributed to the decreased amount of urinary ANFT in ANFT- compared with FANFT-treated guinea pigs. Approximately 70–90% of the recovered radioactivity elutes...
with this metabolite (58 min) in ANFT-treated guinea pigs. This metabolite represents approximately 24 and 4%, respectively, of the dose of ANFT or FANFT administered to guinea pigs. This metabolite was not observed in rat urine. The formation of this new metabolite in guinea pig but not rat could also explain the decreased amount of ANFT recovered in FANFT-treated guinea pigs compared with FANFT-treated rats. ANFT is a more proximate carcinogen than ANFT (24). The amount of ANFT in urine has been correlated with species susceptibility to FANFT-induced bladder cancer (25). The current study confirms the reported lower level of urinary ANFT from guinea pigs compared with rats dosed with FANFT. Thus, formation of this metabolite could be a mechanism for FANFT/ANFT detoxification in guinea pig.

Distribution of radiolabel in tissue, following FANFT dosing of rats in the current study, was similar to that observed after a p.o. dose of 2-[14C]FANFT (26). However, a smaller percentage of radioactivity was recovered in urine in the current study (44 ± 8 versus 78 ± 3%). Total recovery of radioactivity and the fraction excreted in urine in the current study were comparable with results obtained following i.v. dosing (23). More radioactivity was observed in the intestines following p.o. dosing of either ANFT or FANFT in the current study than with i.v. administration.

The unique ANFT metabolite observed in guinea pig urine was characterized. The 58-min metabolite was labile in acidic solutions, but resistant to β-glucuronidase, sulfatase, or γ-glutamyltranspeptidase. This suggests that the metabolite was not an O-glucuronide, sulfate, or glutathione conjugate. ANFT was identified as the only radiolabeled acid hydrolysis product by HPLC. These results suggest that the metabolite is an N-glucuronide. A small peak in rat urine having a retention time close to the ANFT-N-glucuronide was resistant to acid hydrolysis. Thus, the metabolite observed in guinea pig urine was not detected in rat.

The identity of the unknown metabolite was further established from its characteristic mass spectral fragmentation patterns, high-resolution measurements, and UV-Visible absorption spectra. The metabolite showed absorptions at 385 nm and 280 nm, indicative of nitrofuran and thiazole moieties (27). High-resolution measurements showed the presence of 3 nitrogen atoms in the molecule and a composition of C, H, N, O. TSP/MS (positive ion mode) of the acid hydrolysis products showed molecular ions corresponding to ANFT at m/z 212 and glucuronic acid at m/z 195, and an ammonia adduct ion of glucuronic acid at m/z 212. TSP/MS of the metabolite in the positive ion mode (Fig. 3) shows fragment ions arising from the loss of water (m/z 212), and the base peak at m/z 212 (ANFT + H*), and loss of –NO from this ion to give m/z 182, a fragmentation pattern previously reported for ANFT (28). Ions m/z 284 and m/z 254 arise from the N-C bond cleavage of glucuronide and aglycone ions resulting from ring cleavage of glucuronic acid, respectively (29). These results are consistent with this metabolite being an N-glucuronide.

UDP-glucuronosyltransferases represent a family of enzymes with different substrate specificities (33). Five isozymes were isolated from rat liver microsomes with specificity for p-nitrophenol, 17β-hydroxysteroid, 3α-hydroxysteroid, morphine, and digitoxigenin monodigitoxoside. N-Glucuronidation of α-naphthylamine and β-naphthylamine was catalyzed by both the hydroxysteroid and the p-nitrophenol UDP-glucuronosyltransferases. These aromatic amines, along with 4-aminobiphenyl, were not substrates for the other isozymes. Human liver microsomal preparations contain at least 2 forms of transferase with differing isoelectric points (34). A UDP-glucuronosyltransferase from guinea pig liver has a similar molecular weight (M, 55,000) (35) to that reported for enzymes in other species (34, 36).

Levels of p-nitrophenol UDP-glucuronosyltransferase were assessed in rat and guinea pig in the current study. Activities in both species were comparable with those in previous reports (35–38). Guinea pig liver and kidney microsomes exhibited comparable activities that were significantly more than corresponding values in rat. Rat kidney microsomes were twice as active as rat liver microsomes. Guinea pig liver microsomes produced significantly more ANFT-β-glucuronide than kidney. ANFT UDP-glucuronosyltransferase activity was not detected in rat.

Additional enzymes involved in metabolism of carcinogenic aromatic amines have been investigated in guinea pig relative to other species. Guinea pig liver has higher decaylase activity toward N-hydroxy-2-acetylaminofluorene and N-hydroxy-2-formylaminofluorene than hamster, rabbit, mouse, dog, or rat (39). In particular, guinea pig activity is approximately 30 times higher than that of rat. Guinea pig liver microsomes contain 2 deacylases, both of which are inhibited by paraxoxon. A different decaylase enzyme may affect FANFT metabolism. Guinea pig exhibits FANFT formamidase activity in various organs (40). Whereas guinea pig liver formamidase activity is approximately 2-fold greater than that of rat, formamidase activity in kidneys from both species is similar. Species differences in formamidase are considerably less than reported for N-hydroxy-2-acetylamino fluorene and N-hydroxy-2-formylaminofluorene deacylase (39), suggesting that different enzymes may be involved. FANFT deacylase activity is not effective in hydrolyzing acetylated analogues of ANFT and is not inhibited by paraxoxon (41, 42). This further suggests the deacylase activities for N-hydroxy-2-acetylaminofluorene and N-hydroxy-2-formylaminofluorene may be separate from FANFT deacylase. Whereas this discussion is important to the overall mechanism of FANFT disposition and metabolism in guinea pig, it does not directly relate to the lack of ANFT-N-glucuronide formation in rat. ANFT-N-glucuronide is not formed in rat because rat ANFT specific UDP-glucuronosyltransferase activity is not detectable or absent. The ANFT-N-glucuronide is not susceptible to hydrolysis at pH 5.0 for 18 h (data not shown). This makes the hydrolysis of ANFT-N-glucuronide to ANFT unlikely in acidic urine. The latter has been proposed as a mechanism for activation of N-hydroxy-glucuronides of arylamines (43).

These results may partially explain the resistance of guinea pig to FANFT/ANFT-induced bladder cancer. ANFT-N-glucuronide formation may contribute to the reduced amount of free ANFT excreted in guinea pig compared with rat and also to lower nmol/g tissue values observed in guinea pig compared with rat. The reduced levels of urinary ANFT and reduced binding observed in guinea pig may partially explain the resist-
ance of guinea pig to FANFT/ANFT-induced bladder cancer. Thus, ANFT UDP-glucuronosyltransferase activity of guinea pig may be an important but not the only determinant in FANFT/ANFT-induced bladder cancer. Urinary levels of ANFT in FANFT-treated guinea pigs and ANFT-treated rats are similar (Table 2). Since ANFT can induce a low level of bladder cancer in rats (44), but FANFT cannot induce bladder cancer in guinea pig (15), the level of excretion of ANFT must not be the only determinant for guinea pig resistance. Guinea pig resistance to acetylaminofluorene-induced tumors (45) has been attributed to undetectable levels of urinary N-hydroxy-acetylaminofluorene, suggesting alterations in the oxidative pathway. 2-Acetylaminofluorene tumorigenicity is thought to involve oxidation to N-hydroxy-2-acetylaminofluorene (46) and formation of a sulfuric acid ester, N-sulfonoxy-2-acetylamino-
fluorene (47), which appears to be the ultimate carcinogen. In addition to possible alterations in oxidative activation, FANFT/ANFT can also undergo reduction (26, 48, 49). Oxidative and/or reductive pathways for activation have not been explored for FANFT/ANFT in guinea pig and may also be involved in resistance. Thus, whereas ANFT UDP-glucuronosyltransferase activity would appear to be an important determinant in guinea pig resistance, other metabolic factors may also contribute.

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REFERENCES

Metabolism and Disposition of Bladder Carcinogens in Rat and Guinea Pig: Possible Mechanism of Guinea Pig Resistance to Bladder Cancer


Cancer Res 1991;51:514-520.

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