Role of Renal Metabolism and Excretion in 5-Nitrofuran-induced Uroepithelial Cancer in the Rat

Leslie A. Spry, Terry V. Zenser, Samuel M. Cohen, and Bernard B. Davis
Geriatric Research, Education and Clinical Center, Veterans Administration Medical Center; Departments of Medicine and Biochemistry, St. Louis University School of Medicine, St. Louis, Missouri 63125; and Department of Pathology and Laboratory Medicine, The Eppley Institute for Research in Cancer, University of Nebraska Medical Center, Omaha, Nebraska 68105

Abstract

5-Nitrofurans have been used in the study of chemical carcinogenesis. There is substantial evidence that *N*-[4-(5-nitro-2-furyl)-2-thiazolyl] formamide (FANFT) is deformylated to 2-amino-4-(5-nitro-2-furyl)thiazole (ANFT) in the process of FANFT-induced bladder cancer. Paradoxically, ANFT is less potent as a uroepithelial carcinogen than FANFT when fed to rats. Feeding aspirin with FANFT to rats decreases the incidence of bladder cancer. Isolated kidneys were perfused with 5-nitrofurans to determine renal clearances and whether aspirin acts to decrease urinary excretion of the carcinogen. In FANFT-perfused kidneys, FANFT was deformylated to ANFT and excreted (1.06±0.22 nmol/min) at a rate eightfold higher than excretion of FANFT. In kidneys perfused with equimolar ANFT, excretion of ANFT was 0.25±0.05 nmol/min, which suggests a coupling of renal deformylation of FANFT to excretion of ANFT in FANFT-perfused kidneys. Neither aspirin nor probenecid altered the urinary excretion or half-life of FANFT or ANFT. In rats fed 0.2% FANFT as part of their diet, coadministration of aspirin (0.5%) increased urinary excretion of ANFT during a 12-wk feeding study, which suggests decreased tissue binding or metabolism of ANFT. Kidney perfusion with acetylated ANFT (NFTA), a much less potent uroepithelial carcinogen, resulted in no ANFT excretion or accumulation, which indicates the specificity of renal deformylase. Renal deformylase activity was found in broken cell preparations of rat and human kidney. These data describe a unique renal metabolic/excretory coupling for these compounds that appears to explain the differential carcinogenic potential of the 5-nitrofurans tested. These results are consistent with the hypothesis that aspirin decreases activation of ANFT by inhibiting prostaglandin H synthase.

Introduction

5-Nitrofurans are a group of compounds known to cause renal and urinary tract cancer in the rat, dog, hamster, and mouse (1, 2). 5-Nitrofurans are produced commercially as human and veterinary medicinals and as food additives (1). Many exhibit cytotoxic, genotoxic, and carcinogenic activities (2). They have been useful as model compounds in studying the mechanisms behind chemical carcinogenesis because of the high incidence of cancers in animals fed the compounds and because of the effects of slight changes in chemical structure on tissue specificity (2). Both oxidizing and reducing enzyme systems are capable of activating 5-nitrofurans. NADPH-cytochrome *c* reductase and prostaglandin H synthase have been shown to activate 5-nitrofurans (3–5). Activation is followed by binding to protein and DNA (5–7). *N*-[4-(5-Nitro-2-furyl)-2-thiazolyl]formamide (FANFT)[1] (see structure, Fig. 1) is a potent urinary bladder carcinogen in rats (2). We have previously shown (8) that aspirin inhibits FANFT-induced bladder cancer when both compounds are fed to rats. The demonstration of activation by prostaglandin H synthase in vitro and the inhibition of cancer with feeding of aspirin, a known enzyme inhibitor, in vivo were offered as evidence that prostaglandin H synthase had an important role in the molecular mechanism of FANFT-induced urinary bladder cancer. An alternative explanation of the results of the feeding experiment could be that aspirin acts by decreasing excretion of the carcinogen and thus reducing concentrations of the carcinogenic compound at the target organ, the urinary bladder. Previous studies have shown that some 5-nitrofurans are transported in the kidney by an organic acid transport mechanism (9, 10). Low concentrations of aspirin inhibit organic acid transport in the kidney (11). Thus, inhibition of organic acid transport by aspirin could decrease the urinary excretion of the carcinogen.

FANFT is deformylated in vivo to 2-amino-4-(5-nitro-2-furyl)thiazole) (ANFT) (see Fig. 1). ANFT is considered the proximate carcinogen because the mutagenicity of urine from FANFT-fed animals has been correlated with urinary concentrations of ANFT (3, 12), and the susceptibility of different species to FANFT-induced uroepithelial cancer has been related to urinary excretion of ANFT rather than FANFT (13, 14). Yet, there appears to be a paradoxical relationship between FANFT and ANFT in that FANFT is the more potent urinary tract carcinogen when fed to rats (2). In contrast, when ANFT is fed to rats, it is a weaker urinary bladder and renal pelvic carcinogen and potent inducer of forestomach tumors (15). The tissue specificity of these compounds changes remarkably with minor changes in chemical structure. Acetylated ANFT (NFTA; see Fig. 1) is a weak renal pelvic carcinogen, but also a mammary gland, salivary gland, and lung carcinogen in the rat (2). Although in vivo feeding studies have shown the carcinogenic potential of FANFT, ANFT, NFTA, and other 5-nitrofurans on the kidney and urinary tract (1, 2), there has been no systematic appraisal of the renal handling of these compounds with respect to excretion or metabolism. This study used the isolated perfused kidney technique to evaluate renal handling of FANFT, ANFT, and NFTA. The data provide information on renal excretion and metabolism of

Address reprint requests to Dr. Spry, Medical Service (111-JC), VA Medical Center, St. Louis, MO 63125.

Received for publication 13 November 1984 and in revised form 1 April 1985.

The Journal of Clinical Investigation, Inc.
Volume 76, September 1985, 1025–1031

1. *Abbreviations used in this paper:* ANFT, 2-amino-4-(5-nitro-2-furyl)thiazole; ANOVA, analysis of variance; FANFT, *N*-[4-(5-nitro-2-furyl)-2-thiazolyl]formamide; Fe, fractional excretion; GFR, glomerular filtration rate; HPLC, high pressure liquid chromatography; NFTA, acetylated ANFT; V_d, volume of distribution.

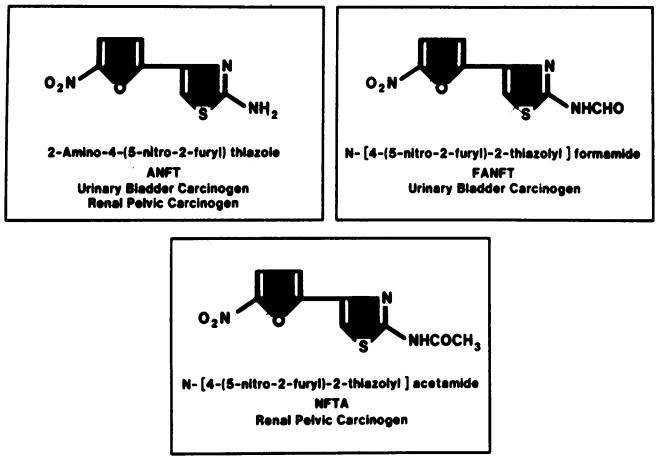

Figure 1. Structures, abbreviations, and site specificities for FANFT, ANFT, and NFTA.

FANFT, ANFT, and NFTA. In addition, this report offers an explanation for the seemingly paradoxical carcinogenicity between orally fed FANFT and ANFT.

Methods

Isolated perfused kidney. Sprague-Dawley rats (300–400 g) (Eldridge Lab Animals, Barhardt, MO) were anesthetized with a 0.6-ml intramuscular injection of a mixture containing 80 mg/ml ketamine hydrochloride (Bristol Laboratories, Syracuse, NY) and 2 mg/ml acepromazine maleate (Med-Tech, Inc., Elwood, KS). The left femoral vein was exposed and 0.7 ml of 25% mannitol (Ivenex, Chagrin Falls, OH) was injected. The abdomen was opened with a midline incision and hemostasis was achieved. The superior mesenteric artery, abdominal aorta, and right renal artery were carefully dissected, and a ligature was placed superior to the renal artery around the adrenal artery and tied. A loose ligature was placed around the right renal artery and two loose ligatures were placed around the superior mesenteric artery. The perirenal fat was removed from the kidney and the ureter was exposed in its course from the kidney. The distal ureter was ligated, opened with iris scissors, and cannulated with tubing (PE 10; Clay Adams Div., Parsippany, NJ). 500 U of sodium heparin (Upjohn Co., Kalamazoo, MI) was then injected into the left femoral vein. A gravity beaker containing perfusate (described below) was placed 1 m above the operating field and its tubing was connected to the silastic perfusion tubing (Cole-Parmer Instrument Co., Chicago, IL) by means of a three-way stopcock. The distal mesenteric artery ligature was tied and a small curved forceps was used to occlude the proximal mesenteric artery. The mesenteric artery was opened with iris scissors and a 19-gauge needle bent to a 90° angle was quickly advanced into the mesenteric artery. The tubing from the gravity beaker was opened, the curved forceps was released, and the needle was quickly advanced into the renal artery. Perfusate was allowed to flush the kidney for a short time; then the ligatures were tied and the entire kidney was excised and placed into the perfusion box. Perfusion was initiated immediately. The perfusion box was modified from that used by Bekersky (16) as follows: (*a*) the perfusate was oxygenated over a membrane oxygenator adapted from the oxygenator used to perfuse human kidneys for transplantation (disposable organ preservation cassette; Waters Instruments, Inc., Rochester, MN); (*b*) the membrane was continuously gassed with an oxygen and carbon dioxide mixture at 6 liter/min and the percentage of carbon dioxide was controlled by means of a gas mixer (National Appliance Co., Portland, OR). The adjustments were based on the readings of an in-line pH meter (Fisher Scientific Co., Pittsburgh, PA). A flow meter (size 13; Gilmont Instruments Inc., Great Neck, NY) was used to monitor flow rate and perfusate pressure monitored by an aneroid sphygmomanometer. With the kidney in place, flow rate was controlled to maintain a pressure at the needle of 100±10 mmHg.

The perfusate was a modified Krebs-Henseleit buffer containing 6.5 g/dl bovine serum albumin (dialyzed, pH 7.0; Sigma Chemical Co., St. Louis, MO) and an amino acid composition as used by Epstein et al. (17). Inulin (Sigma Chemical Co.) was added to the perfusate to a final concentration of 20 mg/dl.

The kidney was allowed to equilibrate for 20 min before the initiation of the experimental protocol. The kidney was discarded whenever <200 μl of urine was made in the initial 20 min. After the equilibration period, FANFT, ANFT, or NFTA (Saber Laboratories, Morton Grove, IL) dissolved in methylsulfoxide (Aldrich Chemical Co., Inc., Milwaukee, WI) was added to a final concentration of 0.01 mM. Aspirin (Sigma Chemical Co.) was dissolved in ethanol and added to a final concentration of 0.03 mM before FANFT where noted. Probenecid (Sigma Chemical Co.) was dissolved in 0.1 N NaOH and added to a final concentration of 0.5 mM. In the case of probenecid, an equimolar quantity of 1 M sodium bicarbonate was added to offset pH changes. The concentrations of all diluents were <0.1% of the total perfusate volume.

Three 20-min experimental urine collections were obtained and urine volumes were determined gravimetrically. Perfusate samples were obtained after the equilibration period and every 10 min thereafter. Perfusate was replaced for each sample removed and urine was replaced by a solution containing 44 mM NaCl and 12 mM KCl to maintain a constant volume in the system. At the conclusion of the experiment, the kidney was weighed, urine was diluted for inulin determination, and perfusates were frozen at −20°C for further study.

Clearance determinations. Perfusate samples were diluted 1:2 and protein precipitated with an equal volume of 10% trichloroacetic acid. Urine was diluted 1:100 with distilled water. Inulin was measured in duplicate perfusate supernatants and diluted urine samples using the simplified anthrone (Sigma Chemical Co.) method of Führ et al. (18). Perfusate and urine sodium and potassium were determined on an electrode electrolyte analyzer (system E2A; Beckman Instruments, Inc., Berkeley, CA). Clearances were calculated by standard clearance formulas with the perfusate concentration of a particular substance being the mean of the three determinations for a given 20-min period. The mean clearance for the three 20-min clearance periods was used as an n of 1. The volume of distribution was held constant, so any change in perfusate concentration of a particular substance reflected either excretion or metabolism. The $t_{1/2}$ of a particular substance was calculated by cartesian or semilog plots of concentration vs. time, and data were best-fit to compartmental or noncompartmental analysis. Pharmacokinetic calculations for first-order or zero-order elimination were used to calculate the $t_{1/2}$.

High pressure liquid chromatography (HPLC) assay of ANFT, FANFT, and NFTA. Perfusates were extracted with two parts acetonitrile (HPLC grade; Fisher Scientific Co.) and centrifuged at 3,500 rpm for 30 min to precipitate protein, and 20–50 μl of supernatant was injected for HPLC analysis. Extraction recoveries for FANFT, ANFT, and NFTA from perfusate were 95±1, 98±1, and 96±2%, respectively. Urine was injected directly in samples of 20–50 μl. Recoveries of FANFT, ANFT, and NFTA from urine were not measurably different from 100% in each case. A high pressure liquid chromatograph (model 332; Beckman Instruments, Inc.) was used. The mobile phase consisted of 40% methanol (Fisher Scientific Co.) in 0.1 M ammonium acetate adjusted to a pH of 6.0, and was pumped at a rate of 1 ml/min through a 5-μm reverse-phase column (Bioanalytical Systems Inc., W. Lafayette, IN). Repetitive programmed injections were accomplished by a Waters intelligent sample processor (Waters Associates, Milford, MA). Simultaneous electrochemical and spectrophotometric detection was done on the HPLC eluate. The variable-wavelength spectrophotometer (model 155; Beckman Instruments, Inc.) was monitored at 400 nm at a sensitivity of 0.01 absorbance units full scale (AUFS) and detected NFTA, FANFT, and ANFT. The electrochemical detector (Bioanalytical Systems Inc.) was held at +750 mV vs. Ag/AgCl and monitored at 50 nA full-scale deflection, and detected only ANFT. The retention times for NFTA, FANFT, and ANFT were 24, 18, and 13 min, respectively. Quantification of NFTA, FANFT, and ANFT was accomplished by integration of sample

Table I. Isolated Perfused Kidney Clearance Data for Control, FANFT, and FANFT Plus Aspirin

Condition	GFR	Fe Na	FANFT excretion	ANFT excretion	$t_{1/2}$	r
	cm³/min	*%*	*nmol/min*	*nmol/min*	*min*	
Control	0.45±0.06	8.0±1.5				
FANFT	0.70±0.18*	6.2±1.1	0.15±0.06	1.06±0.22	41±3	0.984±0.001
FANFT plus aspirin	0.47±0.12	9.6±1.2	0.14±0.04	1.23±0.32	47±11	0.947±0.016

Isolated kidneys were perfused for 1 h under the conditions noted (n = 5). Data are expressed as mean±SEM. FANFT and ANFT excretions were calculated by multiplying urinary concentrations by urine flow rate. The $t_{1/2}$ was calculated by creation of a linear regression for the $\log_e$ of the FANFT concentration over time followed by standard calculations of the elimination constant: $t_{1/2}$ = (0.693)/the elimination constant (K). The r was derived from the linear regression. The original FANFT concentrations were ~10 μM and aspirin concentrations were 30 μM. * $P < 0.05$ vs. control GFR.

peaks on an integrator (model HP 3392 A; Hewlett-Packard Co., Palo Alto, CA) and comparison with peak areas for NFTA, FANFT, and ANFT standard injections. Standards were reinjected every 10 samples. Peak areas were converted into concentration terms by the integrator, then corrected for volume, and these concentrations were used for calculation of clearance terms and $t_{1/2}$ described above. This assay was found to be linear with respect to volume of injection and for the concentration of 5-nitrofurans used in these experiments.

Feeding experiment. Fischer 344 rats (Charles River Breeding Laboratories, Inc., Wilmington, MA) were fed standard rat chow (Purina Mills, Ralston Purina Co., Inc., St. Louis, MO) supplemented with 0.2% FANFT with or without 0.5% aspirin for 12 wk. Control rats were fed rat chow only. 24-h urine collections were obtained in metabolic cages at weeks 1, 3, 6, 9, and 12. Urine was then frozen at −70°C until HPLC analysis. Urine creatinine was determined by the alkaline picrate reaction (19). After HPLC assay of urine, excretion of 5-nitrofuran was expressed as nanomoles per milligram of creatinine.

Statistical analysis. Data were analyzed using a t test for comparison of unpaired means of two sets of data. An analysis of variance (ANOVA), followed by a Tukey test for significance, was used when comparisons were made among more than two sets of data. The number of determinations (n) is noted in the tables. A P value of <0.05 was used to indicate a significant difference between sets of data. Results are presented as the mean±SEM.

Results

Table I depicts the clearance data derived from the isolated perfused kidney for control, FANFT-, and FANFT plus aspirin–perfused kidneys. There was a statistically significant increase in glomerular filtration rate (GFR) when FANFT was perfused ($P < 0.05$). The fractional excretion (Fe) of sodium was not different among any of the groups. Urinary FANFT excretion was not altered by aspirin treatment (0.15±0.06 nmol/min for FANFT perfusion vs. 0.14±0.04 nmol/min for FANFT plus aspirin).

A surprising finding was the degree to which FANFT was deformylated to ANFT by the kidney. The first urine and perfusate samples both revealed significant levels of ANFT. The absolute excretion of ANFT was 1.06±0.22 nmol/min for the FANFT-perfused kidneys vs. 1.23±0.32 nmol/min for the kidneys perfused with FANFT plus aspirin. Note that ANFT excretion was seven to eight times the urinary excretion of FANFT in FANFT-perfused kidneys. In addition to urinary excretion of the deformylated product, ANFT, there was a progressive rise in perfusate ANFT. Fig. 2 depicts the progressive rise in perfusate ANFT concentration measured over the 60-min perfusion period. Aspirin had no measurable effect on the rate of rise of perfusate ANFT during the course of the experiment (slope FANFT vs. slope FANFT plus aspirin). The initial concentrations for FANFT determined by HPLC assay were not measurably different between the two conditions (11.9±1.5 μM for FANFT and 11.2±0.8 μM for FANFT plus aspirin). FANFT was deformylated by the kidney and either excreted or returned to the perfusate (see Table I and Fig. 2), so that by the end of the 60-min perfusion 62±2% of the FANFT was removed. Of this FANFT removed, 49±10% was recovered as ANFT in perfusate, 8±2% was excreted as ANFT in urine, and only 1.1±0.3% was excreted unchanged in the urine. This deformylation appeared to be enzymatic. Perfusion of FANFT in the perfusion

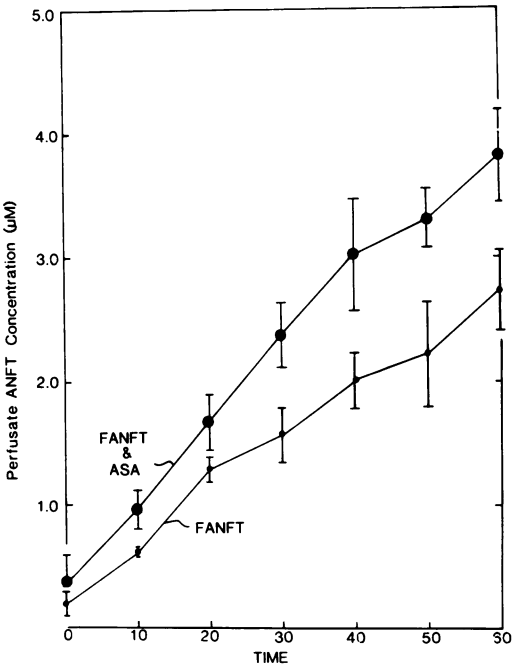

Figure 2. Perfusate ANFT accumulation over the experimental perfusion period. Kidneys were perfused with ~10 μM FANFT with and without 30 μM aspirin for 60 min under the conditions described in Methods. Perfusate was sampled at the time points shown and ANFT measured by HPLC assay. Initial FANFT concentrations by HPLC assay were 11.9±1.5 μM for FANFT and 11.2±0.8 μM for FANFT plus aspirin (ASA). Data plotted as mean±SEM (n = 5). Slopes of FANFT vs. FANFT plus aspirin are not significantly different.

Table II. FANFT and ANFT Urinary Excretion from Rats Fed 0.2% FANFT±0.5% Aspirin in the Diet

Group	Week 1	Week 3	Week 6	Week 9	Week 12	Compound analyzed*
Control	ND	ND	ND	ND	ND	ANFT
	ND	ND	ND	ND	ND	FANFT
FANFT	89±14	50±6.1	73±6.9	51±12.3	66±6.9	ANFT
	ND	ND	ND	ND	ND	FANFT
FANFT plus aspirin	119±14.4	61±8.1‡	119±16.5§	128±11.0‖	97±11.0	ANFT
	ND	ND	ND	ND	ND	FANFT

Urine was assayed without extraction by HPLC, as described in Methods. ND, none detected. * Data are expressed as nanomoles per milligram of creatinine. ‡ $P < 0.05$, week 3 vs. weeks 1, 6, and 9. § $P < 0.05$, FANFT vs. FANFT plus aspirin. ‖ $P < 0.01$, FANFT vs. FANFT plus aspirin.

apparatus without a kidney resulted in no detectable deformylation of FANFT to ANFT. Furthermore, whole organ homogenates yielded an activity of 1,783 nmol/kidney per h, which was abolished when the homogenate was heated for 5 min at 100°C.

The clearance of FANFT in the isolated perfused kidney appeared to follow first-order elimination for a single-compartment model in that a linear regression of the log FANFT concentrations over time had $r > 0.900$ (see Table I). The $t_{1/2}$ based on this linear regression was 41±3 min for FANFT and was not measurably different when aspirin was added to the perfusate (47±11 min).

The lack of an effect of aspirin on urinary excretion of FANFT or ANFT raised the question of whether these compounds were being excreted by organic acid transport, as has been reported for some nitrofurans (9, 10). To address this question, we perfused kidneys with 0.5 mM probenecid and 10 μM FANFT to examine the effect on FANFT and ANFT excretion. Results showed that probenecid had no measurable effect on FANFT excretion (0.14±0.04 nmol/min), ANFT excretion (0.77±0.04 nmol/min), or the $t_{1/2}$ for FANFT (42±9 min).

Rats were fed 0.2% FANFT with or without 0.5% aspirin added as part of the diet, and urinary levels of FANFT and ANFT were measured (Table II). There was no measurable FANFT in the urine. No FANFT deformylase activity was detected in rat urine (data not shown). Within the FANFT-fed group, no significant change was seen in urinary ANFT excretion during the 12-wk study. Within the group fed FANFT plus aspirin, week 3 showed a significant decline in urinary ANFT excretion when compared with weeks 1, 6, and 9. An ANOVA between the groups fed FANFT and FANFT plus aspirin revealed that although the latter consistently showed a higher mean excretion of ANFT, this reached statistical significance only at weeks 6 and 9 ($P < 0.05$ and $P < 0.01$, respectively). Control rats had no detectable ANFT or FANFT at any time.

Table III depicts clearance data in the isolated perfused kidney when ANFT with and without aspirin was perfused. There were no differences in GFR, Fe Na, ANFT excretion, or $t_{1/2}$ for ANFT between the two groups. The mean initial perfusate ANFT concentrations determined by HPLC were 8.3±1.0 μM for ANFT-perfused kidneys and 9.6±0.5 μM for kidneys perfused with ANFT and aspirin. Note that in contrast to FANFT perfusion, the ANFT excretion was fourfold lower for ANFT-perfused kidneys (0.25±0.05 vs. 1.06±0.22 nmol/min for FANFT-perfused kidneys, $P < 0.01$). In addition, the $t_{1/2}$ for ANFT in circulation was nearly four times longer than FANFT (41±3 vs. 147±21 min). This indicates that the total renal clearance (metabolic plus excretory) is markedly prolonged for ANFT as compared with FANFT.

Fig. 3 compares the clearance of ANFT in FANFT- and ANFT-perfused kidneys. Early in the FANFT perfusion, the clearance of ANFT exceeded GFR and progressively fell over the 60-min period, whereas the clearance of ANFT in ANFT-perfused kidneys remained constant over time (0.032±0.005 cm^3/min in the 0–20-min period vs. 0.029±0.003 cm^3/min in the 40–60 min period). The clearance of ANFT was always higher in FANFT perfusion than in ANFT perfusion ($P < 0.001$). The clearance of ANFT fell with time in FANFT-perfused kidneys because renal deformylation returned ANFT to the circulation and the clearance of perfusate ANFT was low (Table III). The

Table III. Isolated Perfused Kidney Clearance Data for ANFT and ANFT Plus Aspirin

Condition	GFR	Fe Na	ANFT excretion	$t_{1/2}$	r
	cm^3/min	*%*	*nmol/min*	*min*	
ANFT	0.59±0.06	9.5±2.3	0.25±0.05	147±21	0.901±0.03
ANFT plus aspirin	0.44±0.07	9.3±1.5	0.21±0.05	147±33	0.952±0.001

Isolated kidneys were perfused for 1 h under the conditions noted ($n = 4$). Data are expressed as the mean±SEM. $t_{1/2}$, ANFT excretion, and r were calculated as described in Table I. Original ANFT concentrations were ~10 μM and aspirin concentrations were 30 μM.

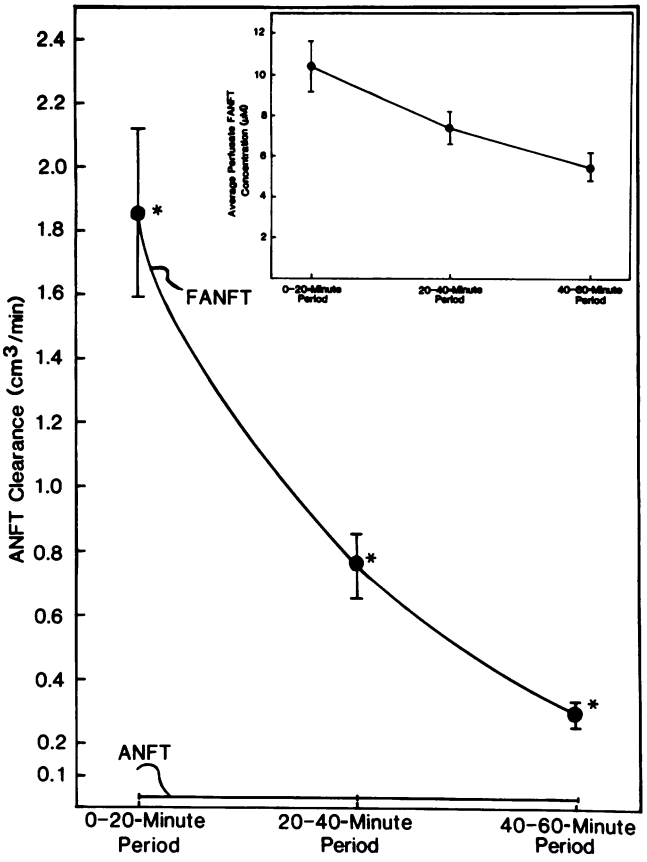

Figure 3. ANFT clearance in each time period for FANFT- and ANFT-perfused kidneys. Kidneys were perfused with either 10 μM FANFT or 10 μM ANFT for 60 min. Mean perfusate ANFT concentrations and urine ANFT concentrations were determined for each 20-min experimental period by HPLC assay (see Methods). Standard clearance calculations were performed for each time period. Data are expressed as mean±SEM for each time period (n = 5 for FANFT and n = 4 for ANFT). *, Significant difference at $P < 0.001$ level by ANOVA; FANFT vs. ANFT for each corresponding time period. *Inset,* average perfusate FANFT concentration for each time period in FANFT-perfused kidneys.

observed fall in the clearance of ANFT in FANFT-perfused kidneys closely parallels the fall in perfusate concentration of FANFT over the 60-min perfusion (see *inset,* Fig. 3). These data clearly indicate that most of the excreted ANFT in FANFT-perfused kidneys was derived from deformylated FANFT and that deformylation enhanced the excretion of ANFT. ANFT excretion was greater in FANFT-perfused kidneys than in ANFT-perfused kidneys.

NFTA is a weaker urinary tract carcinogen than either FANFT or ANFT (2). NFTA has been reported to be poorly deacetylated to ANFT in whole organ homogenates (20). The renal handling was examined by perfusing kidneys with NFTA. No ANFT was seen in perfusate or urine at any time point up to 120 min of perfusion. The NFTA urinary excretion rate (0.75±0.11 nmol/min) was higher than that seen for FANFT (0.15±0.06 nmol/min) and ANFT in ANFT-perfused kidneys (0.25±0.05 nmol/min). The original perfusate NFTA levels determined by HPLC were 15±1.8 μM. The $t_{1/2}$ was also prolonged compared with FANFT and comparable to the $t_{1/2}$ for ANFT (171±34 min for NFTA vs. 41±3 min for FANFT and 147±21 min for ANFT).

Discussion

These results document that aspirin does not act to decrease the excretion of FANFT or its deformylated derivative, ANFT. This was true in both the whole animal and the perfused kidney preparation. Indeed, in animals fed 0.2% FANFT, aspirin tended to increase ANFT excretion. Thus, the effect of aspirin to decrease the incidence of bladder cancer in FANFT-fed rats does not appear to be secondary to any change in the urinary excretion of the carcinogen. When combined with the results of the probenecid plus FANFT–perfused kidneys studies, it would suggest that FANFT, in contrast to certain other 5-nitrofurans (9, 10) is not excreted by the organic acid pathway.

A close examination of the rat feeding study data shows that aspirin significantly increased the urinary excretion of ANFT in 2 wk of the study (weeks 6 and 9, Table II). Two possible explanations for these results are the following: aspirin could have acted to increase renal excretion of ANFT, and/or aspirin could have decreased the metabolism of ANFT. The data from the isolated perfused kidney provide no evidence that aspirin directly altered renal excretion of ANFT. Previous reports (8) showing a decreased incidence of uroepithelial cancer in rats fed FANFT plus aspirin suggested that this effect of aspirin was due to decreased chemical carcinogenicity of FANFT, perhaps when metabolic activation of the more proximal carcinogen ANFT was decreased. Prostaglandin H synthase has been shown to activate carcinogens such as benzidine (21, 22) and ANFT (23) to electrophilic intermediates capable of binding macromolecules such as protein and DNA. Arachidonic acid–initiated activation of carcinogens by prostaglandin H synthase is inhibited by aspirin (21–23). Therefore, the reduction in the incidence of FANFT-induced bladder cancer could be due to aspirin inhibition of activation by prostaglandin H synthase. Decreased metabolism of ANFT by prostaglandin H synthase would in part explain the increased content of ANFT in urine from FANFT plus aspirin–fed rats as reported in Table II.

Aspirin had no effect on renal excretion or deformylation of FANFT in the isolated perfused kidney. This is most convincingly shown by the absence of any change in $t_{1/2}$ for FANFT with aspirin. $t_{1/2}$ is related to clearance (for first-order elimination) by the following expression: $t_{1/2} = (0.693)(V_d/\text{clearance})$, where V_d is the volume of distribution for a particular drug. In the isolated perfused kidney, the V_d is held constant, so that $t_{1/2}$ is directly proportional to the clearance. Clearance is an expression not only of renal excretory clearance of a compound but of total metabolism, including renal and hepatic metabolism. In the isolated perfused kidney, the hepatic factor is absent; thus the absence of any change in $t_{1/2}$ implies that no change occurred in renal excretion or metabolism of the compound when aspirin was added.

A surprising finding was the degree to which the kidney participated in the deformylation of FANFT to ANFT. Previous studies in whole organ homogenates have demonstrated deformylase activity in rat liver and rat kidney; however, liver by far contained the greatest activity (20). In the present study, the kidney appeared to be capable of deformylating FANFT to a significant degree, such that the $t_{1/2}$ was only 41±3 min. If these results were extrapolated to the in vivo situation with two kidneys and with improved function over the isolated perfused kidney, this would suggest that the kidney contributes significantly to total FANFT metabolism. To our knowledge, no studies have examined the $t_{1/2}$ of FANFT in the whole animal. The charac-

teristics of this renal deformylase enzyme have not been studied. This enzyme would belong to a group of enzymes generically known as formamidases or amidohydrolases. Similar enzymes have been described that hydrolyze *N*-formylkynurenine (a metabolite of tryptophan) to kynurenine. Certain of these enzymes are inhibited by such compounds as paraoxon, EDTA, and *p*-chloromercuribenzoate. Many of these inhibitors bind the enzyme at the active site, which is thought, in the case of enzymes inhibited by paraoxon, to contain an essential serine moiety (24). Aspirin is also known to irreversibly inhibit prostaglandin synthesis by acetylating a serine moiety in the fatty acid cyclooxygenase component of prostaglandin H synthase (25). Preliminary results with renal 100,000-*g* supernatants have demonstrated a lack of inhibition by 0.3 mM aspirin or 0.1 mM paraoxon on FANFT deformylase. The failure of aspirin or paraoxon to inhibit FANFT deformylation suggests that FANFT deformylase is not a serine esterase. An inhibitor of FANFT deformylase would be expected to decrease the incidence of FANFT-induced uroepithelial cancer by decreasing urinary excretion of the putative carcinogen ANFT. Further studies on this enzyme are needed. Previous studies (26) have shown that arylesterases such as paraoxonase are stimulated by high salt concentrations. Preliminary studies in our laboratory have shown deformylase activity is stimulated with 0.25 M NaCl. Such high salt concentrations would be expected in the inner medulla and papilla of the kidney. Because washout of this high solute content in the isolated perfused kidney is probable, as evidenced by previous reports (27) of a failure to concentrate urine in response to antidiuretic hormone in the isolated perfused kidney and by high perfusate flow rates, this enzyme activity may be much greater in the in vivo situation as compared with our data in the isolated perfused kidney.

The coupling of renal deformylation of FANFT to urinary excretion of ANFT is a significant observation of this study. This coupling resulted in a fourfold increase in ANFT excretion when equimolar FANFT or ANFT was perfused. There has been a recent report (28) of similar metabolic/excretory coupling for dopa and dopamine also seen in the isolated perfused kidney. Many studies of drug metabolism have centered on hepatic metabolism to more polar compounds enhancing renal excretion of drugs (29). The kidney has been shown to have the capacity to metabolize xenobiotics by such enzymes as NADPH-cytochrome *c* reductase (30), glutathione S transferase (31, 32), prostaglandin H synthase (5, 9, 21), glucuronidase (33, 34), sulfatase (34), deacetylase (31), and beta lyase (31, 35, 36). The renal deformylase described in this report would appear to be a pivotal enzyme in urinary excretion of the putative carcinogen ANFT. In preliminary studies we found deformylase activity in human renal tissue, which suggests that similar metabolic/excretory coupling could occur in humans. This metabolic/excretory coupling may explain the differential uroepithelial carcinogenicity of FANFT compared with ANFT. Previous hypotheses have suggested that the liver was responsible for deformylation of FANFT with subsequent renal excretion of ANFT (2). Our data indicate that urinary levels of ANFT are dependent upon renal deformylation of FANFT. Hepatic deformylation of FANFT would result in ANFT rather than FANFT being presented to the kidney for excretion. The low ANFT excretion and the long $t_{1/2}$ (Table III) would allow further hepatic metabolism of circulating ANFT by pathways such as nitroreduction (30), which would further contribute to decreased urinary excretion of ANFT. Renal deformylation followed by glomerular filtration of ANFT does not appear to be the route of ANFT excretion in FANFT-induced uroepithelial cancer (Table I, Figs. 2 and 3). The mechanism for enhanced urinary excretion of ANFT cannot be ascertained with the present data, but possibilities include deformylation distal to tubular reabsorption, competition for reabsorption between FANFT and ANFT that has been deformylated from FANFT, or tubular secretion of ANFT after FANFT uptake for deformylation. Future studies will address these issues.

The specificity of the renal deformylase is shown by the failure of the rat kidney to measurably deacetylate NFTA. This is in disagreement with previous reports showing low grade deacetylation of NFTA in whole rat kidney homogenates (20) and renal slices (37). Despite 2 h of total perfusion, no conversion of NFTA to ANFT could be measured in perfusate or urine. These differences may reflect different analytical techniques or may represent a failure of broken cell and slice preparations to reflect whole organ metabolism. The failure of deacetylation in the isolated kidney suggests that the renal deformylase is not a nonspecific deacylase but has specificity for the formamide group of FANFT.

The differential renal handling of FANFT, ANFT, and NFTA would explain the observed differences in potency of these urinary tract carcinogens. FANFT is a potent urinary tract carcinogen, causing malignancy in 100% of rats fed 0.2% FANFT in the diet (2). ANFT is of intermediate potency, causing bladder or renal pelvic malignancy in one-third of rats fed 0.166% ANFT (15). NFTA is the least potent, causing uroepithelial malignancy in only 2 of 56 rats fed 0.199% NFTA in the diet (2). Small chemical structure changes in the 5-nitrofuran cause marked changes in the metabolic/excretory handling by the kidney. By failing to excrete NFTA and ANFT (as exemplified by the prolonged half-lives of these two compounds), these compounds are present in circulation longer and are thus more likely to cause neoplasia at sites other than the kidney and urinary bladder. ANFT is a more potent urinary tract carcinogen than NFTA because it is a more proximate carcinogen (12–14). In contrast, the kidney is the essential organ in FANFT-induced uroepithelial cancer because it causes high concentrations of the proximate carcinogen to reach the target organ, the urinary bladder. The kidney may also play a pivotal role in species specificity of the 5-nitrofurans. For example, FANFT causes urinary bladder cancer in most species tested except the guinea pig (2). In contrast, NFTA is a weak transitional cell carcinogen in most species except the hamster, where it caused uroepithelial cancer in 17 of 24 animals (2). Renal metabolic/excretory coupling could explain these species specificities.

In summary, we have shown that aspirin does not decrease either the excretion or deformylation of FANFT. We present data that indicate that in contrast to some other 5-nitrofurans, FANFT is not excreted by the organic acid pathway. Most FANFT is deformylated before excretion. The kidney has the capability to contribute significantly to this deformylation. We have shown a unique metabolic/excretory coupling between renal deformylation and urinary excretion of ANFT. The finding of deformylase activity in human renal tissue implies that a similar mode of excretion could occur in humans. This unique coupling may explain the differential uroepithelial carcinogenicity of FANFT with the kidney playing a pivotal role in the urinary levels of the putative proximate carcinogen ANFT. These results are consistent with those finding aspirin inhibition of FANFT bladder carcinogenesis (8) due to aspirin inhibition of bladder

prostaglandin H synthase activation of ANFT. We propose that the kidney plays a central role in the potency and site specificity of 5-nitrofuran–induced cancer.

Acknowledgments

Judith Rubinstein and Mark Palmier provided invaluable technical assistance.

This research was supported by the Veterans Administration and by grant CA-28015 from the National Cancer Institute.

References

1. Bryan, G. T. 1978. Occurrence, production, and uses of nitrofurans. *In* Carcinogenesis: A Comprehensive Survey. G. T. Bryan, editor. Raven Press, New York. 1–11.

2. Cohen, S. M. 1978. Toxicity and carcinogenicity of nitrofurans *In* Carcinogenesis: A Comprehensive Survey. G. T. Bryan, editor. Raven Press, New York. 171–231.

3. Swaminathan, S., and G. M. Lower, Jr. 1978. Biotransformations and excretion of nitrofurans. *In* Carcinogenesis: A Comprehensive Survey. G. T. Bryan, editor. Raven Press, New York. 59–97.

4. Wang, C. Y., B. C. Behrens, M. Ichikawa, and G. T. Bryan. 1974. Nitroreduction of 5-nitrofuran derivatives by rat liver xanthine oxidase and reduced nicotinamide adenine dinucleotide phosphate-cytochrome *c* reductase. *Biochem. Pharmacol.* 23:3395–3404.

5. Zenser, T. V., and B. B. Davis. 1984. Enzyme systems involved in the formation of reactive metabolites in the renal medulla: cooxidation via prostaglandin H synthase. *Fundam. Appl. Toxicol.* 4:922–929.

6. McCalla, D. R., A. Reuvers, and C. Kaiser. 1970. Mode of action of nitrofurazone. *J. Bacteriol.* 104:1126–1134.

7. Olive, P. L., and D. R. McCalla. 1977. Cytotoxicity and DNA damage to mammalian cells by nitrofurans. *Chem. Biol. Interact.* 16: 223–233.

8. Murasaki G., T. V. Zenser, B. B. Davis, and S. M. Cohen. 1984. Inhibition by aspirin of *N*-[4-(5-nitro-2-furyl)-2-thiazolyl]formamide–induced bladder carcinogenesis and enhancement of forestomach carcinogenesis. *Carcinogenesis (Lond.).* 5:53–55.

9. Zenser, T. V., T. M. Balasubramanian, M. B. Mattammal, and B. B. Davis. 1981. Transport of the renal carcinogen 3-hydroxymethyl-*l*-{[3-(5-nitro-2-furyl)allydidene]amino}hydantoin by renal cortex and cooxidative metabolism by renal prostaglandin endoperoxide synthetase. *Cancer Res.* 41:2032–2037.

10. Andersen, J., F. Kopko, E. G. Nohle, and A. J. Seidler. 1969. Intracellular accumulation of nitrofurantoin by rabbit renal cortical slices. *Am. J. Physiol.* 217:1435–1440.

11. Yü, T. F., and A. B. Gutman. 1955. Paradoxical retention of uric acid by uricosuric drugs in low dosage. *Proc. Soc. Exp. Biol. Med.* 90:542–547.

12. Klemencic, J. M., and C. Y. Wang. 1978. Mutagenicity of nitrofurans. *In* Carcinogenesis: A Comprehensive Survey. G. T. Bryan, editor. Raven Press, New York. 99–130.

13. Wang, C. Y., S. Hayashida, and G. T. Bryan. 1977. Mutagenicity of urine of various species fed *N*-[4-(5-nitro-2-furyl)-2-thiazolyl]-formamide (FANFT) or 2-amino-4-(5-nitro-2-furyl)thiazole (ANFT). *Fed. Proc.* 36:304. (Abstr.)

14. Wang, C. Y., and L. H. Lee. 1976. Mutagenic activity of carcinogenic and noncarcinogenic nitrofurans and of urine of rats fed these compounds. *Chem. Biol. Interact.* 15:69–75.

15. Wang, C. Y., Y. Kamiryo, and W. A. Croft. 1982. Carcinogenicity of 2-amino-4-(5-nitro-2-furyl)thiazole in rats by oral and subcutaneous administration. *Carcinogenesis (Lond.).* 3:275–277.

16. Bekersky, I. 1983. Use of the isolated perfused kidney as a tool in drug disposition studies. *Drug Metab. Rev.* 14:931–960.

17. Epstein, F. H., J. T. Brosnan, J. D. Tange, and B. D. Ross. 1982. Improved function with amino acids in the isolated perfused kidney. *Am. J. Physiol.* 243:F284–F292.

18. Führ, J., J. Kaczmarczyk, and C. D. Kruttgen. 1955. Eine einfache colorimetrishe methode zur inulinbestimmung für nieren-clearance-untersuchungen bei stoffwechselgesunden und diabetikern. *Klin. Wochenshr.* 33:1729–1730.

19. Owen, J. A., B. Iggo, F. J. Scandrett, and C. P. Stewart. 1954. The determination of creatinine in plasma and serum, and in urine; a critical examination. *Biochem. J.* 58:426–437.

20. Wang, C. Y., and G. T. Bryan. 1974. Deacylation of carcinogenic 5-nitrofuran derivatives by mammalian tissues. *Chem. Biol. Interact.* 9: 423–428.

21. Rapp, N. S., T. V. Zenser, W. W. Brown, and B. B. Davis. 1980. Metabolism of benzidine by a prostaglandin-mediated process in renal inner medullary slices. *J. Pharmacol. Exp. Ther.* 215:401–406.

22. Zenser, T. V., M. B. Mattammal, R. W. Wise, J. R. Rice, and B. B. Davis. 1983. Prostaglandin H synthase-catalyzed activation of benzidine: a model to assess pharmacologic intervention of the initiation of chemical carcinogenesis. *J. Pharmacol. Exp. Ther.* 227:545–550.

23. Mattammal, M. B., T. V. Zenser, and B. B. Davis. 1981. Prostaglandin hydroperoxidase-mediated 2-amino-4-(5-nitro-2-furyl)[^{14}C]thiazole metabolism and nucleic acid binding. *Cancer Res.* 41: 4961–4966.

24. Augusteyn, R. C., J. de Jersey, E. C. Webb, and B. Zerner. 1969. On the homology of the active-site peptides of liver carboxylesterases. *Biochim. Biophys. Acta.* 171:128–137.

25. Roth, G. J., and C. J. Siok. 1978. Acetylation of the NH_2-terminal serine of prostaglandin synthetase by aspirin. *J. Biol. Chem.* 253:3782–3784.

26. La Du, B. N., and H. W. Eckerson. 1984. The polymorphic paraoxonase/arylesterase isozymes of human serum. *Fed. Proc.* 43:2338–2341.

27. Ross, B. D. 1978. The isolated perfused rat kidney. *Clin. Sci. Mol. Med.* 55:513–521.

28. Suzuki, H., H. Nakane, K. Minaka, M. Yoshizawa, E. Takeshita, and T. Sarota. 1984. Excretion and metabolism of dopa and dopamine by isolated perfused rat kidney. *Am. J. Physiol.* 247:E285–E290.

29. Reidenberg, M. M. 1977. The biotransformation of drugs in renal failure. *Am. J. Med.* 62:482–485.

30. Mattammal, M. B., T. V. Zenser, and B. B. Davis. 1982. Anaerobic metabolism and nuclear binding of the carcinogen 2-amino-4-(5-nitro-2-furyl)thiazole (ANFT). *Carcinogenesis (Lond.)* 3:1339–1344.

31. Elfarra, A. A., and M. W. Anders. 1984. Renal processing of glutathione conjugates; role in nephrotoxicity. *Biochem. Pharmacol.* 33: 3729–3732.

32. Mohandas, J., J. J. Marshall, G. G. Duggin, J. S. Horvath, and D. J. Tiller. 1984. Differential distribution of glutathione and glutathione-related enzymes in rabbit kidney. *Biochem. Pharmacol.* 33:1801–1807.

33. Rush, G. F., J. F. Newton, and J. B. Hook. 1983. Sex differences in excretion of glucuronide conjugates: the role of intrarenal glucuronidation. *J. Pharmacol. Exp. Ther.* 227:658–662.

34. Newton, J. F., W. E. Braselton, Jr., C.-H. Kuo, W. M. Kluwe, M. W. Gemborys, G. H. Mudge, and J. B. Hook. 1982. Metabolism of acetaminophen by the isolated perfused kidney. *J. Pharmacol. Exp. Ther.* 221:76–79.

35. Lock, E. A., J. Ishmael, and J. B. Hook. 1984. Nephrotoxicity of hexachloro-1,3-butadiene in the mouse: the effect of age, sex, strain, monooxygenase modifiers, and the role of glutathione. *Toxicol. Appl. Pharmacol.* 72:484–494.

36. Hassall, C. D., A. J. Gandolfi, and K. Brendel. 1983. Correlation of the *in vivo* and *in vitro* renal toxicity of *S*-(1,2-dichlorovinyl)-L-cysteine. *Drug. Chem. Toxicol.* 6:507–520.

37. Zenser, T. V., M. O. Palmier, M. B. Mattammal, and B. B. Davis. 1984. Metabolic activation of the carcinogen *N*-[4-(5-nitro-2-furyl)-2-thiazolyl]acetamide by prostaglandin H synthase. *Carcinogenesis (Lond.).* 5:1225–1230.