in vivo *19*: 475-482 (2005)

Effects of Aspirin on the *In Vitro* and *In Vivo* Acetylation of 2-Aminofluorene in Sprague-Dawley Rats

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Abstract. *The present study was undertaken to determine the effect of acetylsalicylic acid acid on the in vitro N-acetyltransferase (NAT) enzyme activity and in vivo acetylation of 2-aminofluorene in laboratory rats. In the in vitro experiments, cytosols of blood, bladder, colon and liver cells, with or without acetylsalicylic acid co-treatment, showed different percentages of 2-aminofluorene acetylation. The data indicated that there was decreased NAT activity associated with increased acetylsalicylic acid in the cytosol reaction. In the in vitro experiments, values of apparent Km and Vmax decreased by 4% and 21%, respectively, for acetylation of AF in blood NAT, 28% and 31% for acetylation of AF in bladder NAT, 12% and 25% for acetylation of AF in colon NAT, and 50% and 35% for acetylation of AF in liver NAT. In the in vivo experiments, pretreatment with acetylsalicylic acid (50 mg/kg) 48 h prior to the administration of 2-aminofluorene (50 mg/kg) resulted in 24% and 28% decreases in the fecal and urinary recovery of N-acetyl-2-aminofluorene and a 26% decrease in the metabolic clearance of 2-aminofluorene to N-acetyl-2-aminofluorene. This is the first demonstration of acetylsalicylic acid (Aspirin) inhibition of arylamine N-acetyltransferase activity showing decreases in the N-acetylation of carcinogens in vivo.*

Exposure to environmental and occupational chemical carcinogens seems to be responsible for a number of human cancers. Drug metabolizing enzymes are of paramount importance in drug detoxification, as well as in chemical carcinogenesis, mutagenesis and toxicity *via* metabolic activation. Many of these carcinogens, once in the body, require metabolic activation by host enzymes in order to initiate carcinogenesis in specific target organs or tissues (1,2). Thus, genetically determined differences in the activity of these enzymes can influence individual susceptibility to adverse drug reactions, drug-induced diseases, and certain types of chemically-induced cancers. Arylamine carcinogens, such as 2-aminofluorene (AF), are N-acetylated to 2-acetylaminofluorene (AAF, which can undergo further activation or detoxification reactions. This metabolic pathway is catalyzed by cytosolic arylamine N-acetyltransferase (NAT) using acetyl coenzyme A as an acetyl donor (3).

NAT, an enzyme involved in several steps of both arylamine activation and detoxification (4), is found in many tissues of humans and laboratory animals (5-8). NAT is also active in nematodes (9,10), fresh water shrimp (11) and the bacterium *Helicobacter pylori* (12). There are distinct groups of rapid acetylators and slow acetylators (13,14). For humans, there are statistical and epidemiological studies that suggest an association between the rapid acetylator phenotype and colorectal cancer (15,16), as well as between the slow acetylator phenotype and bladder cancer (17). Thus, the acetylation of carcinogenic arylamine is involved in determining organ- or tissue- specific susceptibility to cancer. Other investigators indicated that elevated levels of NAT activity are associated with increased sensitivity to the mutagenic effects of many arylamines (18). Therefore, the genetically-mediated variation in NAT activities within target organs or tissues may indicate differential risks for arylamine-induced neoplasm among human populations.

Many immunomodulating agents have been shown to inhibit cytochrome P-450-dependent drug metabolism in humans and laboratory animals (19,20). It was reported that the ratio of acetylsulfamethazine to the total sulfamethazine

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Key Words: Acetylsalicylic acid, 2-aminofluorene, N-acetyl-2-aminofluorene, N-acetyltransferase, *p*-aminobenzoic acid, N-acetyl-*p*-aminobenzoic acid.

Table I. *Effect of acetylsalicylic acid on blood, bladder, colon and liver N-acetyltranserase activity in vitro.*

	Blood		Bladder		Colon		Liver	
Treatment	AAF	NAPABA	AAF	NAPABA	AAF	NAPABA	AAF	NAPABA
Control (distilled water)	0.26±0.09	0.22±0.09	0.58±0.18	0.42±0.10	0.74±0.18	0.60±0.12	1.59±0.30	1.38±0.22
Concentration of acetylsalicylic acid								
0.004 μM	0.21±0.08	0.19±0.07	1.21±0.18	1.19±0.16	1.64±0.30	1.40±0.28	3.03±0.48	2.26±0.39
0.04 μM	[a]0.15±0.06	[a]0.19±0.04	[a]1.02±0.12	[a]0.94±0.14	[a]1.39±0.26	[a]1.21±0.24	[a]2.38±0.40	[a]1.94±0.28
0.4 μM	[b]0.13±0.05	[b]0.13±0.05	[b]0.84±0.12	[b]0.73±0.11	[b]1.08±0.17	[b]1.04±0.15	[b]2.08±0.43	[b]1.67±0.23
4 μM	[c]0.11±0.04	[c]0.10±0.03	[c]0.63±0.09	[c]0.57±0.06	[c]0.84±0.14	[c]0.72±0.13	[c]1.72±0.27	[c]1.41±0.14
40 μM	[d]0.09±0.04	[d]0.09±0.04	[d]0.39±0.06	[d]0.19±0.04	[d]0.70±0.12	[d]0.49±0.08	[d]1.42±0.19	[d]1.03±0.14

Values are mean ±SD of activity (nmol/min/mg protein), n=3.
[a]differs between 0.004 μM acetylsalicylic acid and control. $p<0.05$
[b]differs between 0.04 μM acetylsalicylic acid and control. $p<0.02$
[c]differs between 0.4 μM acetylsalicylic acid and control. $p<0.005$
[d]differs between 4.0 μM acetylsalicylic acid and control. $p<0.001$

secreted in the urine of rats was significantly increased after the administration of Freund's adjuvant. Other investigators also demonstrated that pretreatment with Freund's adjuvant increased the acetylation of sulfamethazine in rapid- and slow- acetylating rabbits by 60% and 135%, respectively (21). Other reports demonstrated that pretreatment with tilorone 48 h prior to the administration of procainamide resulted in a 32% increase in the urinary content of N-acetylprocainamide and a 35% increase in the metabolic clearance of procainamide to N-acetylprocainamide (22). In other words, tilorone increased the N-acetylation of the drugs *in vivo*.

Acetylsalicylic acid, in common with most non-steroidal anti-inflammatory drugs, has a marked inhibitory effect on cyclooxygenase *in vitro* (23). It has been reported that acetylsalicylic acid inhibits platelet function *via* cyclooxygenase inhibition (24). The pharmacological action of acetylsalicylic acid is exerted by its conversion to salicylic acid within 15-20 min after oral application, and involves anti-inflammatory, antipyretic and analgesic activities. However, the platelet inhibitory, *i.e.*, the antithrombotic, action of acetylsalicyclic acid is only due to this compound itself (25). Therefore, acetylsalicylic acid has been proposed for primary and secondary prevention of myocardial infarction and possible strokes (26).

The effect of acetylsalicylic acid *per se* on acetylation of carcinogens has not been previously reported. In the present study, the influence of acetylsalicylic acid on the acetylation of 2-AF was examined *in vitro* and *in vivo* in laboratory rats.

Materials and Methods

Chemicals and reagents. Acetylsalicylic acid (Aspirin), ethylenediaminetetraacetic acid (EDTA), leupeptin, *p*-aminobenzoic acid (PABA), N-acetyl-*p*-aminobenzoic acid (N-Ac-PABA), 2-acetylaminofluorene (AAF), phenylmethylsulfonylfluoride (PMSF), 2-aminofluorene (AF), Tris, dithiothreitol (DTT), carnitine acetyltransferase, acetyl carnitine, bovine serum albumin (BSA) and acetyl-Coenzyme A (AcCoA) were obtained from Sigma Chemical Co. (St. Louis, MO, USA). Acetic acid, acetonitrile, dimethyl sulfoxide (DMSO) and potassium phosphates were obtained from Merck Co. (Darmstadt, Germany). All chemicals used were of reagent grade.

Animals and treatments

In vitro studies: Male Sprague-Dawley rats weighing 180-200 g were obtained from stock maintained at the animal center of China Medical University, Taiwan. The rats were housed in cages and maintained at 25˚C on a 12-h light/dark cycle. The animals had free access to water and chow. All animals were at least 12 weeks of age at the time of sacrifice. Blood, liver, colon and bladder were removed, and NAT activity determined. Tissues from 3 rats were assayed individually for each activity determination.

Preparation of cytosols

a) Blood samples: Whole blood from the tail vein (50 μl) was hemolyzed immediately in 950 μl of lysis buffer [20 mM Tris-HCl, pH 7.5 (at 4˚C), 1 mM DTT, 1 mM EDTA, 50 μM PMSF and 10 μM leupeptin]. The hemolysate was kept on ice until assayed for NAT activity.

b) Other tissues (liver, colon and bladder): Individual tissues were removed, trimmed and placed in 5 volumes of the lysis buffer as described above. Tissues were homogenized in ice with a Polytron homogenizer set at number 5 for 20 sec (twice). The homogenates were centrifuged for 8 min at 9000 x g and the supernatant kept on ice for NAT activity determination.

NAT activity determination. AcCoA-dependent N-acetylation of PABA and AF was assayed as described by Chung *et al.* (5). Incubation mixtures in the assay system consisted of a total volume of 90 μl: tissue cytosol diluted as required in 50 μl of lysis buffer [20 mM Tris-HCl, pH 7.5, 1 mM DTT, 1 mM EDTA, 1 mM acetylcarnitine], and AF or PABA at specific concentrations as substrate. The reaction was started

Table II. *Kinetics data of acetylation of aminofluorene in blood, bladder, colon and liver cytosol.*

	Blood		Bladder		Colon		Liver	
Treatment	Km(mM)	Vmax	Km(mM)	Vmax	Km(mM)	Vmax	Km(mM)	Vmax
Control (distilled water)	1.04±0.54	1.12±0.26	3.80±0.46	1.16±0.11	3.00±0.18	3.33±0.64	4.09±0.54	15.8±2.04
Acetylsalicylic acid	[a]0.87±0.04	[a]0.89±0.05	[d]1.00±0.15	[b]1.78±0.28	[a]1.92±0.23	[b]2.50±0.29	[d]1.69±0.32	[c]7.64±0.59

Values are mean±SD, n=6. The AcCoA and acetylsalicylic acid concentrations were 0.1 mM and 0.4 μM, respectively, and kinetic constants were calculated from the modified HYPER Program of Cleland (1967).
[a]differs between 0.4 mM acetylsalicylic acid and control. $p<0.12$
[b]differs between 0.4 mM acetylsalicylic acid and control. $p<0.05$
[c]differs between 0.4 mM acetylsalicylic acid and control. $p<0.01$
[d]differs between 0.4 mM acetylsalicylic acid and control. $p<0.001$

Table III. *Kinetic data for acetylation of 2-aminofluorene in blood cytosol.*

	In Vitro	
Cytosol treatment	Km(mM)	Vmax(nmol/min/mg protein)
Control (distilled water)	0.92±0.04	1.12±0.26
Acetylsalicylic acid	[a]0.87±0.04	[b]0.89±0.05

Values are mean±SD n=6. The AcCoA and acetylsalicylic acid concentrations were 0.1 mM and 0.4 μM, respectively, and the kinetic constants were calculated from the modified HYPER Program of Cleland (1967).
[a]differs between 0.4 mM acetylsalicylic acid and control. $p<0.12$
[b]differs between 0.4 mM acetylsalicylic acid and control. $p<0.05$

Table IV. *Kinetic data for acetylation of 2-aminofluorene in bladder cytosol.*

	In Vitro	
Cytosol treatment	Km(mM)	Vmax(nmol/min/mg protein)
Control (distilled water)	1.38±0.42	1.12±0.26
Acetylsalicylic acid	[a]1.00±0.15	[b]1.78±0.28

Values are mean±SD, n=6. The AcCoA and acetylsalicylic acid concentrations were 0.1 mM and 40 μM, respectively, and the kinetic constants were calculated from the modified HYPER Program of Cleland (1967).
[a]differs between 0.4 mM acetylsalicylic acid and control. $p<0.05$
[b]differs between 0.4 mM acetylsalicylic acid and control. $p<0.05$

by addition of 20 μl of AcCoA. Control reactions had 20 μl deionized water in place of AcCoA. The final concentration of PABA or AF was 0.1 mM, and that of AcCoA was 0.5 mM. The reaction mixtures were incubated at 37˚C for 10 min and the reaction was stopped with 50 μl of 20% trichloroacetic acid for PABA and 100 μl of acetontrile for AF. All of the experimental reactions and the controls were run in triplicate. The amounts of acetylated product and remaining nonacetylated substrate were determined by HPLC (5,9). An aliquot of the NAT incubation mixture was injected onto a C18 reversed-phase column (Spherisorb 4.6 x 250 nm) of a Beckman HPLC (pump 168 and detector 126) and eluted at a flow rate of 1.2 ml/min. The solvent system for PABA and N-Ac-PABA was 50 mM acetic acid/CH_3CN (86:14) with detection at 266 nm. The retention time for PABA was 4 min and that for N-Ac-PABA was 6.5 min. The solvent system for AF and AAF was 20 mM KH_2PO_4, pH 4.5/CH_3CN (53:47) with detection at 280 nm. The retention time for AAF was 6.5 min and 9 min for AF. All compounds were quantitated by comparison of the integrated area of the elution peak with that of known amounts of standards. NAT activity is expressed as nmol acetylated per min per mg of cytosolic protein.

Acetylation catalyzed by NAT follows ping-pong kinetics (27), so that increasing the concentration of AcCoA in an assay system containing a saturated concentration of arylamine will increase both the apparent Km and Vmax of the arylamine substrate. The kinetic constant estimate with 0.1 mM AcCoA should, therefore, approximate that achieved *in vivo*.

Protein determination. Protein concentration in the cytosols from the blood and liver samples were determined by the method of Bradford (28) with bovine serum albumin as the standard. All samples were assayed in triplicate.

Statistical analysis. Statistical analysis of the data was performed with an unpaired Student's *t*-test. The kinetic constants were calculated with the Cleland HYPER Program (29) that performs linear regression using a least-squares method.

Animals and treatments

In vivo studies: Male Sprague-Dawley rats weighing 180-200 g were housed in individual cages and maintained at 25˚C on a 12-h light/dark cycle as described in the section on *in vitro* studies. Forty-eight hours prior to receiving AF, the animals were administered a single dose of isotonic saline or acetylsalicylic acid (50 mg/kg) dissolved in isotonic saline (final concentration 20 mg/ml) by gastric intubation at 8 a.m., as described in Svensson and Knowlton (22). On the morning of the study, the animals were individually housed in metabolism cages and AF (60 mM dissolved in DMSO) redissolved in isotonic saline (final concentration 60 μM) was infused through the canal at a rate of 0.34 ml/min from 8 to 9 a.m.).

Table V. *Kinetic data for acetylation of 2-aminofluorene in colon cytosol.*

	In Vitro	
Cytosol treatment	Km(mM)	Vmax(nmol/min/ mg protein)
Control (distilled water)	1.52±0.32	3.33±0.64
Acetylsalicylic acid	[a]1.35±0.23	[b] 2.50±0.29

Values are mean±SD, n=6. The AcCoA and acetylsalicylic acid concentrations were 0.1 mM and 40 μM, respectively, and the kinetic constants were calculated from the modified HYPER Program of Cleland (1967).
[a]differs between 0.4 μM acetylsalicylic acid and control. $p<0.05$
[b]differs between 0.4 μM acetylsalicylic acid and control. $p<0.05$

Table VI. *Kinetic data for acetylation of 2-aminofluorene in liver cytosol.*

	In Vitro	
Cytosol treatment	Km(mM)	Vmax(nmol/min/ mg protein)
Control (distilled water)	3.36±0.44	11.69±1.34
Acetylsalicylic acid	[a]1.69±0.32	[b]7.64±0.59

Values are mean±SD, n=6. The AcCoA and acetylsalicylic acid concentrations were 0.1 mM and 40 μM, respectively, and the kinetic constants were calculated from the modified HYPER Program of Cleland (1967).
[a]differs between 0.4 μM acetylsalicylic acid and control. $p<0.05$
[b]differs between 0.4 μM acetylsalicylic acid and control. $p<0.05$

An aliquot of the dosing solution was frozen and assayed for 2-AF content. Serial blood samples (200 μL) were obtained from a vein through the canal prior to and 5, 10, 20, 40, 80, 120, 160, 240 and 300 min after AF administration. The plasma was separated by centrifugation (10 min at 600g) and stored in polypropylene tubes at -20˚C until analyzed. Urine and feces were collected through 52 h and were immediately treated by extracting twice with ethyl acetate/methanol (95:5), evaporating the solvent and redissolving the residue in methanol and assayed (5). AF and AAF were quantitated by HPLC as described in the *in vitro* studies section.

Data analysis. The plasma concentrations *versus* time data were introduced to a biexponential equation using an unweighted nonlinear least-squares regression program to estimate the initial plasma concentration of AF (*i.e.*, concentration at time 0). The data were then analyzed using the LAGRAN program to obtain noncompartmental pharmacokinetics parameters (22).

Results

The possible effects of acetylsalicylic acid on the NAT activity in rat blood and liver *in vitro* were examined by high pressure liquid chromatography assessing the percentage of acetylation of AF and PABA. Blood was chosen because it is

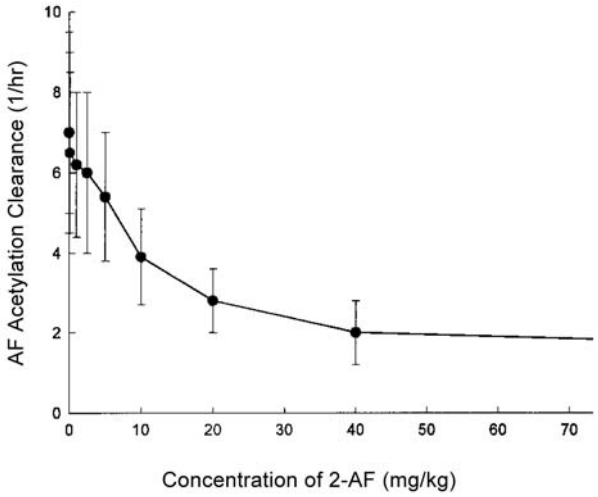

Figure 1. *Changes of clearances on different 2-AF concentration with a single dose of acetylsalicyclic acid 50 mg/kg in Sprague-Dawley rats. Values are mean±SD, N=3 as described in the text.*

the basis for a simple NAT phenotyping method in mice (6). The choice of liver was based on the importance of liver in total acetylation activity in mammals. The bladder and colon were examined because they are target tissues for arylamine in mammals and reported to differ in suspectibility to DNA damage, depending on the NAT phenotype (30,31). Cytosols of blood, colon, bladder and liver, with or without specific concentrations of acetylsalicylic acid co-treatment, showed different percentages of AF acetylation. Comparisons of the relative cytosolic NAT activity between with or without specific concentrations of acetylsalicylic acid are given in Table I. The data indicate a decreased NAT activity associated with increased acetylsalicylic acid in all tissue cytosols examined, *i.e.*, the higher the concentration of acetylsalicylic acid in the reaction mixtures, the higher the inhibition of NAT activity *in vitro*.

The kinetic constants determined for blood, colon, bladder and liver NAT using AF as substrate, with or without 0.4 μM acetylsalicylic acid, are shown in Table II. In the *in vitro* experiments, the apparent values of Km and Vmax for acetylation of AF in the control blood cytosol were 0.92±0.04 mM and 1.12±0.26 nmol/min/mg protein, respectively, and those of co-treated with acetylsalicylic acid were 0.87±0.04 mM and 0.89±0.05 nmol/min/mg protein, respectively (Table III). In the control bladder cytosol, the apparent values of Km and Vmax for acetylation of AF were 1.38±0.42 mM and 2.56±0.72 nmol/min/mg protein, respectively, and after co-treatment with acetylsalicylic acid were 1.00±0.15 mM and 1.78±0.28 nmol/min/mg protein, respectively (Table IV). In the control colon cytosol, the apparent values of Km and Vmax for acetylation of AF of were 1.52±0.32 mM and 3.33±0.64 nmol/min/mg protein,

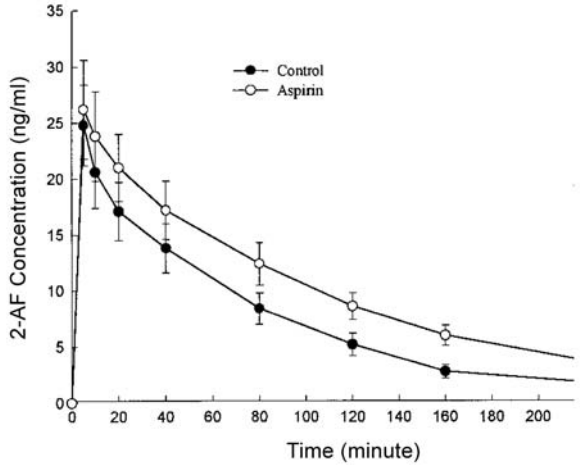

Figure 2. *Mean 2-aminofluorene plasma concentration versus time profile in acetylsalicylic acid pretreated (n=6) and control (n=6) animals. The data were obtained as described in Materials and Methods. Values are mean±SD.* ● *= control ,* ○ *= acetylsalicylic acid.*

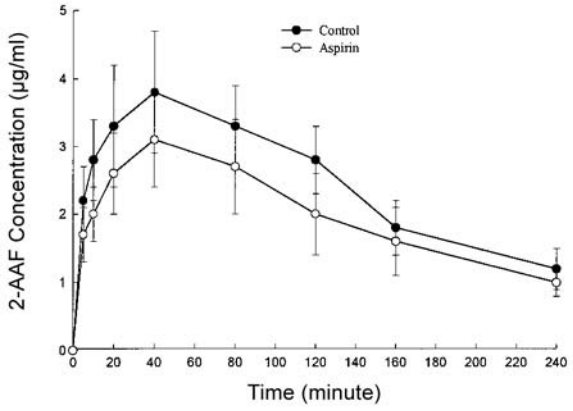

Figure 3. *Mean N-acetyl-2-aminofluorene plasma concentration versus time profile in acetylsalicylic acid pretreated (n=6) and control (n=6) animals. The data were obtained as described in Materials and Methods. Values are mean±SD.* ● *= control ,* ○ *= acetylsalicylic acid.*

respectively, and after co-treatment with acetylsalicylic acid were 1.35±0.23 mM and 2.50±0.29 nmol/min/mg protein, respectivelty (Table V). In the control liver cytosol, the apparent values of Km and Vmax for acetylation of AF were 3.36±0.44 mM and 11.69±1.34 nmol/min/mg protein, respectively, and after co-treatment with acetylsalicyclic acid were 1.69±0.32 mM and 7.64±0.59 nmol/min/mg protein, respectively (Table VI).

Changes of clearances on different 2-AF concentration with a single dose 50 mg/kg of acetylsalicyclic acid in Sprague-Dawley rats are shown in Figure 1. The mean plasma 2-AF and 2-AAF concentrations *versus* time profile after oral administration of 60 µM of 2-AF in the control animals and those pretreated with a single dose of acetylsalicylic acid 50 mg/kg are shown in Figures 2 and 3. Mean data after the 240-min sample are not plotted because the AF plasma concentrations were below the limit of detection of the HPLC system. The pharmacokinetic parameters for AF in controls and in the acetylsalicylic acid-pretreated rats are given in Table VII. There was no significant difference in the pharmacokinetic parameters for AF acetylation between the two groups. The mean AAF concentration in the acetytlsalicylic acid-pretreated group was significantly lower than that in the control group from 10 to 240 min (Figure 3).

The urinary recovery of AF and AAF in the control and acetylsalicylic acid pretreated animals are given in Table VII. The percentage urinary recovery of AAF was decreased in the acetylsalicylic acid-pretreated rats by 37% (from 14.8±2.2 to 10.6±1.4; $p<0.05$). In contrast, the urinary recovery of AF was not significantly changed by acetylsalicylic acid pretreatment.

Table VII. *Effect of acetylsalicylic acid pretreatment on the disposition of 2-aminofluorene in rats*[a].

Pharmacokinetic parameter	Control (N=6)	Acetylsalicylic acid (N=6)
CL_s (ml/min/kg)	114±26	1118±18
$T_{1/2}$ (min)	48.9±5.0	44.8 ±5.8
V_{ss} (liters/kg)	4.53±2.43	4.84±1.05
Urinary recovery (% of dose)		
2-aminofluorene	32.9±6.6	38.6±6.9
N-acetyl -2-aminofluorene	14.8±2.2	[b]10.6±1.4
Fecal recovery (% of dose)		
2-aminofluorene	38.2±7.8	40.4±6.8
N-acetyl -2-aminofluorene	19.4±2.8	[c]14.6±2.0
CL_r(AF) (ml/min/kg)	76.5±20.4	68.2±16.9
CL_r(AAF)(ml/min/kg)	30.4±8.9	34.6±12.1
CL_m (ml/min/kg)	19.8±3.4	[d]14.5±1.8

[a]Acetylsalicylic acid (50mg/kg) was administered as a single dose 48 h prior to 2-aminofluorene (50 mg/kg) administration. Data are expressed as mean±SD. CL_s, systemic clearance; $T_{1/2}$, half-life; V_{ss}, steady-state volume of distribution; CL_r(AF),renal clearance of 2-aminofluorene; CL_r(AAF), renal clearance of N-acetyl -2-aminofluorene; CL_m, metabolic clearance of 2-aminofluorene to N-acetyl -2-aminofluorene.
[b,c]differs between acetylsalicylic acid and control. $p<0.05$
[d]differs between acetylsalicylic acid and control. $p<0.05$

The fecal recovery of AF and AAF in the control and acetylsalicylic acid-pretreated animals are also given in Table VII. The percentage fecal recovery of AAF was decreased in the acetylsalicylic acid-pretreated rats by 17% (from 19.4±2.8 to 14.6±2.0; $p<0.05$). In contrast, the fecal recovery of AF was not significantly changed by acetylsalicylic acid pretreatment.

The data in Table VII also show that the renal clearance of AF and AAF did not significantly differ between the

control and acetylsalicylic acid-pretreated rats. The partial clearance of AF and AAF was decreased by 26% (from 19.8±3.4 to 14.5±1.8; $p<0.01$) in the acetylsalicylic acid-pretreated rats compared to the control animals.

Discussion

There are many events which are likely to be prerequisites for the observed effects of acetylsalicylic acid (Aspirin) on NAT activity. First, the acetyl CoA-dependent arylamine NAT enzyme has been reported to exist in several experimental animals, as well as in humans, and NAT has been shown to be involved in chemical carcinogenesis (32,33). Second, rapid and slow acetylation has been demonstrated based on a predisposing factor for the sensitivity of individuals to the toxicity during exposure to arylamines (3,4). Therefore, the genetically-mediated variation in NAT activities within target tissues for arylamine-induced neoplasms may indicate differential risks among the human population. Third, some enzymes of enteric bacteria are known to contribute to the metabolic activation of chemical carcinogens in animals (34). Fourth, compounds containing amino, sulphydryl or hydroxyl groups may undergo biotransformation *via* acetylation. This pathway is a primary determinant in the elimination of several therapeutic compounds and arylamine. The capacity to acetylate amino-containing compounds may determine an individual's predisposition to toxicity from several agents (3). Fifth, NAT has been demonstrated to be present in Sprague-Dawley rats which belong to the rapid acetylator phenotypes (4). Sixth, N-acetyltransferase activity is particularly abundant in the reticuloendothelial system (3,4). Other investigators have reported that the stimulation of the reticuloendothelial system by immunomodulators reduces cytochrome P-450-dependent drug metabolism (35). It is reasonable to hypothesize that these agents may also alter the acetylation of arylamine carcinogens (AF). Therefore, the present studies focused on the effects of acetylsalicylic acid on NAT activity of the rat *in vitro* and *in vivo*.

The present data clearly indicated that acetylsalicylic acid, at concentrations from 0.004 μm to 4 μm in the *in vitro* tests, decreased the acetylated product of AF by the NAT in blood, bladder, colon and liver. Since acetylsalicylic acid can inhibit NAT activity of rat blood, bladder, colon and liver *in vitro*, the kinetic constants of NAT in these tissues were affected. In the *in vitro* experiments, different degrees of acetylsalicylic acid inhibition on the NAT enzyme were seen. This finding may provide information towards understanding the decreasing arylamine carcinogenicity in animals. Other reports have already demonstrated that elevated levels of NAT activity are associated with increased sensitivity to the mutagenic effects of many arylamines (18).

The data of the experiments on the urinary and fecal recovery of AAF and the partial clearance of AF to AAF indicated that pretreatment with acetylsalicylic acid resulted in inhibition of arylamine carcinogen acetylation *in vivo* during the examined time periods. However, changes were not shown in the total clearance of AF in the presence of a decrease in the partial metabolic clearance to AAF in the total examined time. This is most probably due to two opposing interactions of acetylsalicyclic acid such as the elimination pathways of procainamide including a) renal excretion of unchanged drug, (acetylation); and b) oxidative metabolism (22). Therefore, our results provided an opportunity to examine the effects of acetylsalicylic acid on another metabolic pathway, namely, acetylation.

The effects of acetylsalicylic acid on the urinary recovery of AF and its N-acetyl metabolite are most probably secondary to inhibition of the oxidative metabolism of AF. The significant changes in metabolite recovery with no significant changes in systemic clearance is most probably due to the wide variability in systemic clearance observed in the control rats. The quantitative contribution of oxidative metabolism to the elimination of AF in either humans or rats is not clear.

The results of the present study suggest that acetylsalicylic acid may inhibit the oxidative metabolism of AF. In other words, acetylsalicylic acid may also inhibit the c-P450 enzyme activity. Acetylation of AF and inhibition of AF oxidative metabolism, if of similar magnitude, may result in a net effect of no change in the total clearance. Indeed, AF acetylation activity has been demonstrated in many extrahepatic tissues (5). The quantitative contribution of these organs to the elimination of AF is unknown.

Other investigators have already demonstrated that: i) stimulation of the RES with Freund's adjuvant results in an increased rate of N-acetylation *in vivo* in both the rat (36) and the rabbit (21); ii) pretreatment with acetylsalicylic acid results in an decreased rate of N-acetylation of AF *in vivo* in the rat (22); iii) chronic administration of hydrocortisone in the rabbit has been shown to enhance the acetylation of sulfamethazine (37); and iv) tilorone pretreatment had no effect on the renal clearance of AF or AAF (22).

In conclusion, the present study demonstrated that acetylsalicylic acid decreases the rate of arylamine carcinogen (AF) acetylation *in vivo*. Future studies will focus on the mechanism of this effect and its significance in the toxicity of agents which undergo biotransformation *via* acetylation.

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Received June 30, 2004
Revised January 25, 2005
Accepted February 2, 2005