Effects of Oral Administration of Berberine on Distribution and Metabolism of 2-Aminofluorene in Sprague-Dawley Rats

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Abstract. *The effects of berberine on the in vivo N-acetylation and metabolism of 2-aminofluorene (2-AF) in bladder, blood, colon, kidney, liver, feces and urine samples and brain tissues (cerebrum, cerebellum and pineal gland) of male Sprague-Dawley rats were investigated. Major metabolites, such as 1-OH-2-AAF, 3-OH-2-AAF, 8-OH-2-AAF and 9-OH-2-AAF were found in bladder tissues, 1-OH-2-AAF, 5-OH-2-AAF and 8-OH-2-AAF were found in blood samples, 1-OH-2-AAF, 3-OH-2-AAF, 5-OH-2-AAF, 8-OH-2-AAF and 9-OH-2-AAF were found in colon tissues, 1-OH-2-AAF, 3-OH-2-AAF and 9-OH-2-AAF were found in kidney tissues, 1-OH-2-AAF, 3-OH-2-AAF and 8-OH-2-AAF were found in liver tissues, 1-OH-2-AAF, 3-OH-2-AAF, 5-OH-2-AAF, 7-OH-2-AAF, 8-OH-2-AA and 9-OH-2-AAF were found in feces samples and 1-OH-2-AAF, 3-OH-2-AAF, 5-OH-2-AAF, 7-OH-2-AAF, 8-OH-2-AA and 9-OH-2-AAF were also found in urine samples, 1-OH-2-AAF, 3-OH-2-AAF and 8-OH-2-AAF were found in cerebrum tissues, 1-OH-2-AAF, 3-OH-2-AAF and 7-OH-2-AAF were found in cerebellum tissues. In the control group, however, only 2-AF and 2-AAF were found in pineal gland after rats had been orally treated with 2-AF (50 mg/kg) for 24 h. Pre-treatment of male rats with berberine (40 mg/kg) 24 h prior to the administration of 2-AF (50 mg/kg), as well as the co-administration of berberine and 2-AF led to a decrease in the amounts of 3-OH-2-AAF and an increase in the amounts of 8-OH-2-AAF in bladder tissues. In blood samples, there were significant decreases of 2-AF, 2-AAF, 1-OH-2-AAF and 8-OH-2-AAF, after rats were pre-treated with berberine for 24 h before the addition of 2-AF. However, co-administration of berberine and 2-AF led to an increase in the amounts of 5-OH-2-AAF. In colon tissues, there were significant decreases of 2-AF, 2-AAF, 1-OH-2-AAF and 8-OH-2-AAF in colon samples after rats were treated with berberine for 24 h before the addition of 2-AF. 2-AF, 1-OH-2-AAF, 3-OH-2-AAF and 9-OH-2-AAF levels were significantly different between control and the group treated with berberine and 2-AF at the same time. In kidney tissues, significant decreases of 2-AF and 2-AAF and of 3-OH-2-AAF were observed after rats were treated with both compounds separately and simultaneously. However, 24 h berberine pre-treatment followed by addition of 2-AF led to significant increase of 9-IH-2-AAF. In liver tissues, there were significant decreases of 2-AAF and 1-OH-2-AAF, after co-administration of berberine and 2-AF. The amounts of 2-AAF, 1-OH-2-AAF and 3-OH-2-AAF were significantly different between the control and the group pre-treated with berberine 24 h before the addition of 2-AF. In the feces samples, there were significant decreases of 2-AAF, 3-OH-2-AAF, 7-OH-2-AAF, 8-OH-2-AAF and 9-OH-2-AAF after co-administration of berberine and 2-AF. However, the berberine pre-treatment followed by addition of 2-AF led to a significant increase of 2-AF, 2-AAF and 1-OH-2-AAF levels. In urine samples, there were significant differences of 2-AF, 2-AAF, 1-OH-2-AAF, 3-OH-2-AAF, 5-OH-2-AAF, 8-OH-2-AAF and 9-OH-2-AAF after the co-treatment. However, berberine treatment followed by 2-AF led to significant differences in 1-OH-2-AAF and 5-OH-2-AAF levels. In the cerebrum samples, there were significant*

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differences in 1-OH-2-AAF and 8-OH-2-AAF after both berberine co-treatment and pre-treatment. In cerebellum samples, there were also significant differences in the 1-OH-2-AAF and 3-OH-2-AAF levels after both co- and pre-treatment. In pineal gland samples, there were significant differences in 2-AAF levels after co-treatment with berberine and 2-AF and 1-OH-2-AAF was also found in both groups. However, berberine pre-treatment followed by 2-AF led to different levels of 2-AF and 2-AAF, but not of 3-OH-2-AAF.

It is well known that arylamine carcinogens require host-mediated metabolic activation to initiate carcinogenesis in target tissues (1-3). 2-Aminofluorene (2-AF) is bio-transformed to form corresponding arylamines (N-acetyl-2-aminofluorene, 2-AAF) *via* cytosolic N-acetyltransferase (NAT) (4, 5) to be further metabolized by cP450 enzymes to form 2-AF metabolite-DNA adducts in tissues. Especially in the liver cells, 2-AF can be metabolized into various metabolites *in vitro* (6). Much evidence shows 2-AF to be carcinogenic to liver, urinary bladder and other tissues of a variety of animal species (7-9). Previous *in vivo* studies have indicated that Sprague-Dawley (SD) rats can acetylate 2-AF into 2-AAF followed by further metabolism (6, 10, 11). Furthermore, 2-AAF is hepatocarcinogenic in adult SD rats after repeated exposure (1, 12). Many mammalian species, including humans, have been demonstrated to have N-acetylation polymorphism. Individuals can be divided into rapid, intermediate and slow acetylator phenotypes based on liver NAT activity (4, 13, 14). The higher incidence of bladder cancer in slow acetylators of human populations is associated with exposure to arylamine carcinogens (13).

Berberine, a yellow benzylisoquinoline alkaloid from *Berberis* species (15), has been reported to exert anti-inflammation (16), anti-diarrhetic and antimalaria functions (15). It also has a long history as a tonic remedy for liver (17). Berberine was also reported to have antimicrobial activity for Gram-positive and Gram-negative bacteria, as well as for other micro-organisms (18, 19). In addition, berberine was reported to display anticancer activity (20-23) that can induce apoptosis in promyelocytic leukemia HL-60 cells (24) and to down-regulate nucleophosmin/B23 and telomerase activity (25). Our earlier studies demonstrated that berberine inhibited NAT activities and gene expression in human bladder cancer T24 cells (26), colon cancer colo 205 cells (27), human leukemia HL-60 cells (28), malignant astrocytoma G9T/VGH and brain glioblastoma multiforme GBM 8401 cells (29) and mouse leukemia L 1210 cells (30). There are no statistics available to address the effects of berberine on the distribution and metabolism of 2-AF *in vivo*. Thus, the present study is focused on the effect of oral administration of berberine on the distribution and metabolism of 2-AF in an animal model.

Materials and Methods

Chemicals and reagents. Berberine, 2-aminofluorene (2-AF), N-acetyl-2-aminofluorene (2-AAF), acetyl carnitine, leupeptin, Tris, carnitine acetyltransferase, dithiothreitol (DTT), phenylmethylsulfonyl fluoride (PMSF), bovine serum albumin (BSA) and acetyl-Coenzyme A (AcCoA) were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Acetonitrile, dimethyl sulfoxide (DMSO), ethyleacetate and potassium phosphates were from Merck Co. (Darmstadt, F.R. Germany). All chemicals used were reagent grade.

Animals and treatment. Male SD rats, weighing 160-180 g, were obtained from stock maintained in the animal center of the China Medical University, Taiwan. All animals were housed in individual cages and were maintained at 25˚C on a 12-h light/dark cycle, as previously described (10, 11). Berberine (50 mg/kg) was administered by gauge (10, 11) in isotonic saline (1 ml of saline administered). The control animals received 1% DMSO only. 2-AF was administered at 50 mg/kg in 1% DMSO to rats (18 animals). Then the animals were divided into 3 groups of 6 rats each. Group 1 was treated with 2-AF (control); group 2 was treated with berberine and 2-AF at the same time; group 3 was treated with 2-AF for 24 hours and then with berberine (4 mg/kg, dissolved in DMSO) (31).

All animals were maintained under the same conditions. Urine and feces samples were collected for 24 h. Blood samples and bladder, colon, kidney and liver tissues were collected 24 h after treatment. The samples of blood, urine, feces, bladder, colon, kidney and liver, cerebrum, cerebellum and pineal gland were immediately extracted twice with ethyl acetate/methanol (95:5), the solvent evaporated and the residue was redissolved in methanol and assayed (10, 11). 2-AF, 2-AAF and 2-AF metabolites were quantified by HPLC, as described previously (10, 11). Briefly, an aliquot of the extracted 2-AF and 2-AAF or 2-AF metabolites from each sample was injected into a C18 reversed-phase column (Spherisorb 4.6x250 nm) of a Beckman HPLC (pump 168 and detector 126) and eluted at a flow rate of 1.2 ml/min. For 2-AF and 2-AAF, the solvent system was 20 mM KH_2PO_4, pH 4.5/ CH_3 CN (53:47) with detection at 280 nm. The retention time was about 6.5 min for 2-AAF and 9 min for 2-AF. All compounds were quantified by comparison of the integrated area of the elution peak with that of known amounts of standards (2-AF, 2-AAF, 1-OH-2-AAF, 3-OH-2-AAF, .5-OH-2-AAF, 7-OH-2-AAF, 8-OH-2-AAF, and 9-OH-2-AAF). The total amounts of 2-AF metabolites were the sum of 2-AAF, 1-OH-2-AAF, 3-OH-2-AAF, .5-OH-2-AAF, 7-OH-2-AAF, 8-OH-2-AAF and 9-OH-2-AAF (10, 11, 32, 33).

Data analysis. The statistical analysis of the data was performed with an unpaired Student's *t*-test.

Results

The effects of berberine on the amounts of total 2-AF and 2-AF metabolites in the bladder, blood, colon, kidney, liver, feces, urine and brain tissues (cerebrum, cerebellum and pineal gland) from 3 groups of SD rats are presented in Tables I-X. The comparison of total amounts of 2-AF and 2-AF metabolites from all examined tissues of the 3 groups is presented in Figure 1. The data in Figure 1 indicate that
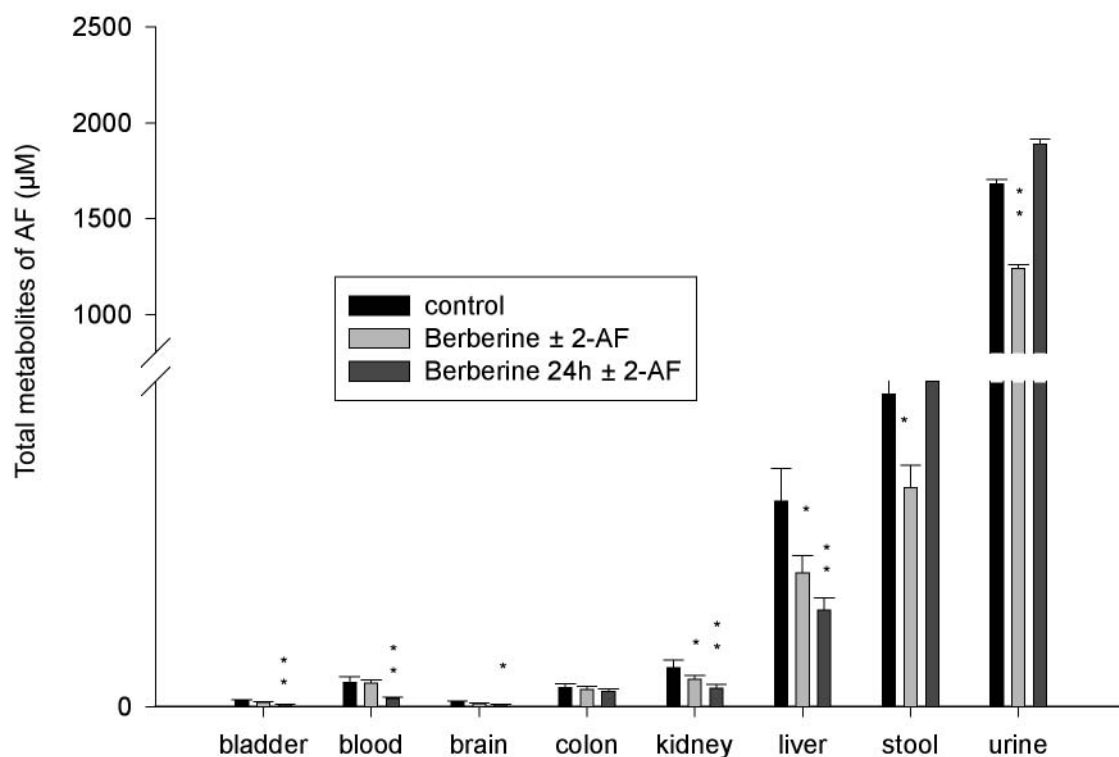
Figure 1. *The comparison of total amounts of 2-AF and 2-AF metabolites in organs from SD rats after co-treatment with berberine. Animals were treated with 2-AF only, or 2-AF and berberine at the same time or were pre-treated with berberine before administration of 2-AF. Total amounts of 2-AF were calculated and analyzed by unpaired Student's t-test. Data are expressed as mean±SD, N=6.*

berberine affected the levels of the 2-AF metabolites in the examined tissues and urine and feces samples

Neither the control bladder tissues (2-AF only) (Table I), nor the berberine pre-treated nor the co-treated tissues displayed 5-OH-2-AAF or 7-OH-2-AAF. However, 2-AF, 2-AAF, 1-OH-2-AAF, 3-OH-2-AAF, 8-OH-2-AAF and 9-OH-2-AAF were detected in all three examined groups. The amounts of 3-OH-2-AAF, 8-OH-2-AAF and 9-OH-2-AAF were significantly different between the control and the berberine and 2-AF co-treated groups. 3-OH-2-AAF and 9-OH-2-AAF levels were significantly decreased in the berberine pre-treated group compared to the control. 3-OH-2-AAF levels were also significantly decreased in the berberine pte-treated group (Table I).

In the blood samples (Table II), neither 3-OH-2-AAF, 7-OH-2-AAF nor 9-OH-2-AAF were detected in any of the three groups. However, 2-AF, 2-AAF, 1-OH-2-AAF, 5-OH-2-AAF and 8-OH-2-AAF were found in all rats examined. The amounts of 2-AF, 2-AAF, 1-OH-2-AAF and 8-OH-2-AAF were significantly decreased in the berberine pre-treated groups compared to the control (Table II).

In the colon tissues (Table III), 7-OH-2-AAF was not detected in any of the three groups. However, 2-AF, 2-AAF, 1-OH-2-AAF, 3-OH-2-AAF, 5-OH-2-AAF, 8-OH-2-AAF and 9-OH-2-AAF were detected in all groups examined. The amounts of 2-AF and 1-OH-2-AAF were significantly lower in co-treated and berberine pre-treated groups compared to the control. The co-treated groups showed a higher 8-OH-2-AAF production than that of the control. The berberine pre-treated rats displayed a higher 9-OH-2-AA production than that of the control (Table III).

In the kidney tissues (Table IV), none of the three groups were positive for 5-OH-2-AAF, 7-OH-2-AAF or 8-OH-2-AAF. 2-AF, 2-AAF, 1-OH-2-AAF, 3-OH-2-AAF and 9-OH-2-AAF, however, were detected in all three groups. The amounts of 2-AF, 2-AAF and 3-OH-2-AAF were significantly lower in the co-treated groups compared to the control and the levels 1-OH-2-AAF and 3-OH-2-AAF were also significantly lower in the berberine pre-treated groups compared to the control. The amount of 9-OH-2-AAF was lower in the co-treated group as were the levels of 1-OH-2-AAF in the berberine pre-treated groups.

The presence of the 7-OH-2-AAF and 9-OH-2-AAF metabolites was not detected in any liver smples from any group (Table V). However, 2-AF, 2-AAF, 1-OH-2-AAF, 3-OH-2-AAF and 8-OH-2-AAF were detected in all three examined groups. The levels of 2-AAF were significantly

Table I. *Distribution of 2-AF and 2-AF metabolites in bladder samples from male SD rats (μM).*

				2-AF Metabolites				
Treatment	2-AF	2-AAF	1-OH-2-AAF	3-OH-2-AAF	5-OH-2-AAF	7-OH-2-AAF	8-OH-2-AAF	9-OH-2-AAF
Control (2-AF only)	0.88±0.56	0.41±0.19	1.26±0.27	0.26±0.08	N.D.	N.D.	0.56±0.12	3.79±0.49
Berberine+2-AF	0.51±0.26	0.46±0.22	3.89±0.54	*0.04±0.04	N.D.	N.D.	**2.07±0.57	*2.14±0.37
Berberine 24 h+2-AF	0.91±0.34	0.39±0.18	1.82±0.46	*0.08±0.04	N.D.	N.D.	*1.46±0.27	2.96±0.54

Values are mean±SD n=6. N.D.=not detectable. Difference between 2-AF-treated (control), 2-AF and berberine co-treated and pre-treated groups; *$p<0.05$, **$p<0.01$.

Table II. *Distribution of 2-AF and 2-AF metabolites in blood samples from male SD rats (μM).*

				2-AF Metabolites				
Treatment	2-AF	2-AAF	1-OH-2-AAF	3-OH-2-AAF	5-OH-2-AAF	7-OH-2-AAF	8-OH-2-AAF	9-OH-2-AAF
Control (2-AF only)	0.09±0.06	0.13±0.08	1.44±0.423	N.D.	0.06±0.03	N.D.	0.25±0.065	N.D.
Berberine+2-AF	0.06±0.06	0.14±0.06	0.64±0.08	N.D.	*0.09±0.02	N.D.	0.26±0.08	N.D.
Berberine24 h+2-AF	*0.02±0.04	*0.04±0.04	**0.50±0.04	N.D.	0.07±0.03	N.D.	**0.09±0.04	N.D.

Values are mean±SD n=6. N.D.=not detectable. Difference between 2-AF-treated (control), 2-AF and berberine co-treated and pre-treated groups; *$p<0.05$, **$p<0.01$.

Table III. *Distribution of 2-AF and 2-AF metabolites in colon samples from male SD rats (μM).*

				2-AF Metabolites				
Treatment	2-AF	2-AAF	1-OH-2-AAF	3-OH-2-AAF	5-OH-2-AAF	7-OH-2-AAF	8-OH-2-AAF	9-OH-2-AAF
Control (2-AF only)	2.10±0.07	0.66±0.30	6.74±2.18	0.27±0.07	1.16±0.44	N.D.	1.49±0.27	1.48±0.70
Berberine+2-AF	***0.20±0.09	*1.76±0.50	**2.57±0.66	0.24±0.06	0.70±0.38	N.D.	**4.96±0.54	1.26±0.50
Berberine24+2-AF	***0.12±0.04	0.74±0.40	***1.80±0.42	**0.02±0.06	0.91±0.27	N.D.	1.86±0.27	*0.27±0.04

Values are mean±SD n=6. N.D.=not detectable. Difference between 2-AF-treated (control), 2-AF and berberine co-treated and pre-treated groups; *$p<0.05$, **$p<0.01$.

Table IV. *Distribution of 2-AF and 2-AF metabolites in kidney samples from male SD rats (μM).*

				2-AF Metabolites				
Treatment	2-AF	2-AAF	1-OH-2-AAF	3-OH-2-AAF	5-OH-2-AAF	7-OH-2-AAF	8-OH-2-AAF	9-OH-2-AAF
Control (2-AF only)	0.57±0.36	0.68±0.27	4.86±1.06	0.18±0.06	N.D.	N.D.	N.D.	1.36±0.27
Berberine+2-AF	*0.20±0.08	*0.34±0.16	3.27±0.64	*0.04±0.06	N.D.	N.D.	N.D.	*2.56±0.30
Berberine24 hrs+2-AF	0.46±0.20	0.46±0.20	*0.46±0.16	*0.08±0.04	N.D.	N.D.	N.D.	1.27±0.40

Values are mean±SD n=6. N.D.=not detectable. Difference between 2-AF-treated (control), 2-AF and berberine co-treated and pre-treated groups; *$p<0.05$, **$p<0.01$.

Table V. *Distribution of 2-AF and 2-AF metabolites in liver samples from male SD rat (µM).*

Treatment	2-AF	2-AF Metabolites						
	2-AF	2-AAF	1-OH-2-AAF	3-OH-2-AAF	5-OH-2-AAF	7-OH-2-AAF	8-OH-2-AAF	9-OH-2-AAF
Control (2-AF only)	0.36±0.08	1.18±0.36	4.96±1.76	0.14±0.04	N.D.	N.D.	0.78±0.20	N.D.
Berberine+2-AF	0.46±0.09	**0.39±0.10	*2.06±0.46	0.11±0.06	N.D.	N.D.	*1.04±0.10	N.D.
Berberine24 h+2-AF	0.48±0.11	**0.16±0.08	**1.08±0.67	***0.02±0.02	N.D.	N.D.	0.64±0.18	N.D.

Values are mean±SD n=6. N.D.=not detectable. Difference between 2-AF-treated (control), 2-AF and berberine co-treated and pre-treated groups; *$p<0.05$, **$p<0.01$.

Table VI. *Distribution of 2-AF and 2-AF metabolites in feces from male SD rats (µM).*

Treatment	2-AF	2-AF Metabolites						
	2-AF	2-AAF	1-OH-2-AAF	3-OH-2-AAF	5-OH-2-AAF	7-OH-2-AAF	8-OH-2-AAF	9-OH-2-AAF
Control (2-AF only)	2.69±0.54	24.56±5.17	71.4±5.16	1.28±0.34	16.42±4.4	32.26±4.4	17.90±5.6	48.76±7.8
Berberine+2-AF	3.46±0.67	*38.46±412	70.8±6.17	*3.54±0.46	18.71±2.9	*54.49±6.8	*36.40±3.7	*72.32±9.2
Berberine24h+2-AF	*4.94±0.29	*12.74±1.54	*89.6±7.4	1.24±0.38	17.40±2.2	33.68±5.1	18.0±4.4	44.49±5.8

Values are mean±SD n=6. N.D.=not detectable. Difference between 2-AF-treated (control), 2-AF and berberine co-treated and pre-treated groups; *$p<0.05$, **$p<0.01$.

Table VII. *Distribution of 2-AF and 2-AF metabolites in urine from male SD rats (µM).*

Treatment	2-AF	2-AF Metabolites						
	2-AF	2-AAF	1-OH-2-AAF	3-OH-2-AAF	5-OH-2-AAF	7-OH-2-AAF	8-OH-2-AAF	9-OH-2-AAF
Control (2-AF only)	0.24±0.06	1.54±0.26	0.06±0.08	1.09±0.18	3.09±0.40	12.89±2.57	14.64±2.02	36.28±6.18
Berberine+2-AF	*0.86±0.14	**4.28±0.62	*0.88±0.22	*3.18±0.54	*7.26±0.69	14.86±3.24	**6.54±1.87	*46.44±8.16
Berberine 24 h+2-AF	0.28±0.08	2.67±0.12	*0.56±0.20	0.48±0.04	*1.06±0.40	13.68±2.14	12.48±2.28	34.92±3.46

Values are mean±SD n=6. N.D.=not detectable. Difference between 2-AF-treated (control), 2-AF and berberine co-treated and pre-treated groups; *$p<0.05$, **$p<0.01$.

Table VIII. *Distribution of 2-AF and 2-AF metabolites in cerebrum samples from male SD rats (µM).*

Treatment	2-AF	2-AF Metabolites						
	2-AF	2-AAF	1-OH-2-AAF	3-OH-2-AAF	5-OH-2-AAF	7-OH-2-AAF	8-OH-2-AAF	9-OH-2-AAF
Control (2-AF only)	0.41±0.08	0.08±0.04	0.30±0.14	0.20±0.10	N.D.	N.D.	11.04±4.12	N.D.
Berberine+2-AF	0.48±0.10	0.07±0.08	*0.09±0.08	0.12±0.08	N.D.	N.D.	**0.69±0.10	N.D.
Berberine24 h+2-AF	*0.10±0.04	0.07±0.08	*1.27±0.24	0.16±0.04	N.D.	N.D.	*0.29±0.14	N.D.

Values are mean±SD n=6. N.D.=not detectable. Difference between 2-AF-treated (control), 2-AF and berberine co-treated and pre-treated groups; *$p<0.05$, **$p<0.01$.

Table IX. *Distribution of 2-AF and 2-AF metabolites in cerebellum samples from male SD rats (µM).*

Treatment	2-AF Metabolites							
	2-AF	2-AAF	1-OH-2-AAF	3-OH-2-AAF	5-OH-2-AAF	7-OH-2-AAF	8-OH-2-AAF	9-OH-2-AAF
Control (2-AF only)	0.08±0.08	1.44±0.45	4.41±0.58	2.06±0.48	N.D.	0.84±0.50	N.D.	N.D.
Berberine+2-AF	0.10±0.04	*4.76±0.85	**0.09±0.04	*0.44±0.08	N.D.	0.96±0.27	N.D.	N.D.
Berberine 24 h+2-AF	0.08±0.06	2.44±0.43	1.48±0.10	0.89±0.10	N.D.	*0.26±0.08	N.D.	N.D.

Values are mean±SD n=6. N.D.=not detectable. Difference between 2-AF-treated (control), 2-AF and berberine co-treated and pre-treated groups; *$p<0.05$, **$p<0.01$.

Table X. *Distribution of 2-AF and 2-AF metabolites in pineal gland samples from male SD rats (µM).*

Treatment	2-AF Metabolites							
	2-AF	2-AAF	1-OH-2-AAF	3-OH-2-AAF	5-OH-2-AAF	7-OH-2-AAF	8-OH-2-AAF	9-OH-2-AAF
Control (2-AF only)	0.27±0.14	0.08±0.08	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
Berberine+2-AF	0.16±0.11	*0.29±0.10	0.40±0.24	N.D.	N.D.	N.D.	N.D.	N.D.
Berberine24 h+2-AF	*0.04±0.08	*0.34±0.10	N.D.	0.27±0.08	N.D.	N.D.	N.D.	N.D.

Values are mean±SD n=6. N.D.=not detectable. Difference between 2-AF-treated (control), 2-AF and berberine co-treated and pre-treated groups; *$p<0.05$, **$p<0.01$.

lower in the co-treated group compared to the control. The amounts of 2-AAF, 1-OH-2-AAF and 3-OH-2-AAF were also significantly lower in the berberine pre-treated group.

In the feces samples (Table VI), 2-AF, 2-AAF, 1-OH-2-AAF, 3-OH-2-AAF, 5-OH-2-AAF, 7-OH-2-AAF and 9-OH-2-AAF were detected in all three groups. The amounts of 2-AF, 2-AAF, 3-OH-2-AAF, 7-OH-2-AAF, 8-OH-2-AAF and 9-OH-2-AAF were significantly higher in the co-treated group compared to the control. The amounts of 2-AF and 1-OH-2-AAF were significantly lower while that of 2-AAF was significantly higher in the berberine pre-treated group compared to the control.

In the urine samples (Table VII), 2-AF, 2-AAF, 1-OH-2-AAF, 3-OH-2-AAF, 5-OH-2-AAF, 7-OH-2-AAF, 8-OH-2-AA and 9-OH-2-AAF were all detected in all three groups with the exception of 2-OH-2-AAF. The levels of all examined compounds were significantly higher in the co-treated group compared to the control. However, only the amounts of 1-OH-2-AAF and 5-OH-2-AAF were significantly different (higher and lower, respectively) in the berberine pre-treated group.

In the cerebrum samples (Table VIII), 2-AF, 2-AAF, 1-OH-2-AAF, 3-OH-2-AAF, and 8-OH-2-AAF were detected in all the groups. The amounts of 1-OH-2-AAF and 8-OH-2-AAF were significantly lower in the berberine co-treated group compared to the control. The levels of the same metabolites were also significantly different in the berberine pre-treated group, but were increased in the case of 1-OH-2-AAF and decreased in the case of 2-OH-2-AAF.

The productions of 2-AF, 2-AAF, 1-OH-2-AAF, 3-OH-2-AAF and 7-OH-2-AAF were detected in the cerebellum samples from all the groups (Table IX). The amounts of 1-OH-2-AAF and 3-OH-2-AAF were significantly lower in the co-treated samples while that of 2-AAF was significantly higher compared to the control. Only the level of 7-OH-2-AAF was significantly different from the control (lower) in the berberine pre-treated samples.

The 2-AF and 2-AAF metabolites were detected in all three groups (Table X). The 2-AAF levels were significantly higher in both the co-treated and pre-treated groups compared to the control. The 2-AF levels were significantly lower in the pre-treated group. Finally, 1-OH-2-AAF was detected in both treated groups but not in the control.

Discussion

The purpose of this study was to examine the distribution of 2-AF metabolites in berberine-treated Sprague-Dawley rats. The metabolism and mutagenesis of 2-AF and 2-AAF have previously been studied in animal microsomal, S9, and intact hepatocyte preparations (33).

AcCoA-dependent arylamine NAT enzyme, which acetylates 2-AF, is present in many tissues of animals and humans, and has been shown to be involved in chemical carcinogenesis (34, 35). It is well known that the sensitivity of individuals to toxicity during exposure to arylamine chemicals is associated with genetically controlled rapid or

slow NAT acetylation (4, 36). Increased levels of NAT activity are associated with increased sensitivity to the mutagenic effects of arylamine carcinogens (17). Decreased NAT activity of the liver is associated with several disease processes, such as breast and bladder cancer (4, 36). Thus, NAT plays an important role in 2-AF-induced malignancy in mammals. Nevertheless, on the *in vivo* metabolic conversion of arylamines to N-formyl derivatives, only a few reports have been published. Our previous studies showed that berberine affect NAT activity and gene expression in many human cancer cell lines. However, there is no available information about the effect of dietary berberine on the distribution and metabolism of 2-AF *in vivo* in rat tissues after oral 2-AF treatment. Murine extrahepatic tissues showed N-acetylation of 2-AF and p-aminobenzoic acid (37). The results from this study demonstrated that berberine could affect N-acetylation and the metabolism of 2-AF *in vivo* in the examined rat tissues including brain tissues.

The dose of 4 mg/kg berberine for the reported *in vivo* experiments was based on previous dietary berberine studies in rats (31, 38) and the 24-h pre-treatment with berberine before the addition of 2-AF was based on the report of Janbaza and Gilani (31), which demonstrated that a day of oral treatment with berberine had a significantly inhibitory effect on the microsomal drug metabolizing enzymes, cytochrome P450s CYPs of liver in albino Wistar rats (31). One day of administration may suffice to induce large changes in liver enzyme activity. Our previous studies also showed that the same family of compounds, such as ellagic acid, affected the N-acetylation of 2-AF *in vivo* in the examined cerebrum, cerebellum and pineal gland of rats (32, 39). The present study also showed that berberine affected the total amounts of 2-AF and 2-AF metabolites in all the examined tissues. The decrease in acetylation of 2-AF and of the total amounts of 2-AF metabolites may suggest that berberine inhibits the oxidative metabolism of 2-AF except N-acetylation, since the elimination pathways of procainamide include renal excretion of the unchanged drug, acetylation and oxidative metabolism (40). It was reported that co-incubation of cells with berberine or *C. rhizoma* inhibited *IL-6* mRNA expression induced by IL-1 in a dose-dependent manner. Therefore, anticachectic activity may be attributable to transcriptional inhibition of IL-6 in tumor cells after administration of this herb (41).

Previous reports have denoted that several other compounds increased drug acetylation. Stimulation of the reticuloendothelial system by Freund's adjuvant led to increased rates of N-acetylation *in vivo* in both the rat (32) and the rabbit (33); pre-treatment with tilorone resulted in an increased rate of N-acetylation of procainamide (34) and 2-AF (10) *in vivo* in the rat; and chronic administration of hydrocortisone in the rabbit has been shown to enhance the acetylation of sulfamethazine (35). The present data also

showed that berberine affected 2-AF metabolism based on the changes of 2-AF metabolite profiles. Other urinary metabolites of 2-AAF could not be isolated and identified because of their small quantities. Therefore, the nature of the metabolites corresponding to unknown peaks in all the examined samples remains unknown.

In conclusion, when 2-AF was given orally, with or without berberine pre-treatment to SD rats, berberine decreased the rate of 2-AF acetylation in bladder, blood, colon, kidney and liver tissues. Interestingly, berberine also affected the distribution of the metabolites in brain tissues. Therefore, we suggest that predisposition to carcinogen toxicity may be influenced by specific chemopreventive agents. Future studies focusing on the mechanism of these changes, involved enzymes and their significance in the toxicity of agents that undergo bio-transformation *via* acetylation in tissues are necessary.

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