in vivo *19*: 143-156 (2005)

Effects of Ellagic Acid by Oral Administration on Distribution and Metabolism of 2-Aminofluorene in Sprague-Dawley Rats

CHIN-CHIN HO[1], YI-SHYONG LAI[2], DER-YEAN WANG[3], YI-SHUAN CHEN[4], JAU-HONG LEE[5], NOU-ING TANG[6] and JING-GUNG CHUNG[7]

[1]Department of Nursing, Chungtai Institute of Health Sciences and Technology, 11, Pro-Tze Lane, 40605, Takun, Taichung, Taiwan;
[2]Department of Pathology, Hospital and Medical College of Chung Shan Medical University, 110, Section 1,Chien-Kuo North Road, Taichung 408, Taiwan;
Departments of [3]Orthopaedic and [5]Surgery, China Medical University Hospital, No 2,Yuh-Der Road, Taichung, Taiwan;
[6]Schools of Chinese Medicine, Departments of [4]Nutrition and [7]Microbiology, China Medical University, 91 Hsueh-Shih Road, Taichung, Taiwan, R.O.C.

Abstract. *The effects of ellagic acid on the in vivo N-acetylation and metabolism of 2-aminofluorene (2-AF) were investigated in bladder, blood, colon, kidney, liver, feces and urine samples from male Sprague-Dawley rats. Major metabolites such as 1-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 and 8-OH-2-AAF were found in feces samples and 1-OH-2-AAF, 3-OH-2-AAF, 5-OH-2-AAF and 8-OH-2-AAF were also found in urine samples after rats had been orally treated with 2-AF (50 mg/kg) for 24 h. Pretreatment of male rats with ellagic acid (10 mg/kg) 24 h prior to the administration of 2-AF (50 mg/kg) resulted in absence of 8-OH-2-AAF in bladder tissues, and there were significant decreases of 8-OH-2-AAF in blood and urine samples. In blood samples, amounts of 2-AAF and 8-OH-2-AAF were significantly decreased; in colon tissues, amounts of 2-AF, 1-OH-2-AAF and 3-OH-2-AAF, in liver tissues, amounts of 2-AAF, 1-OH-2-AAF and 3-OH-2-AAF, and in urine samples, amounts of 2-AF and 8-OH-2-AAF were significantly decreased in 24-h ellagic acid (EA)-treated rats before 2-AF was added to the diet. However, significantly increased 1-OH-2-AAF concentrations were found in urine samples in 24-h EA-treated rats before 2-AF was administered. In the EA and 2-AF rats, in the same time treated groups, bladder, colon and liver tissues, and feces and urine samples showed significant differences when compared to the ones without EA co-treatment. We saw significant decreases of the amounts of 2-AF and 1-OH-2-AAF in colon tissues. The feces samples showed increased amounts of 2-AAF in EA- and in 2-AF- treated rats in the same time groups, but urine samples showed a decreased amount of 8-OH-2-AAF in both EA-treated groups. The total amounts of 2-AF metabolites in bladder, blood, kidney and liver tissues showed significant difference between control and the group which was EA-treated 24 h before 2-AF was added. The total amounts of 2-AF metabolites in the liver, feces and urine showed significant decreases between control and EA-treated at the same time with 2-AF groups. This is the first report of EA affecting the N-acetylation and metabolism of 2-AF in rat tissues in vivo.*

Correspondence to: Dr. J-G Chung, Department of Microbiology, China Medical University, No 91, Hsueh-Shih Road, Taichung City 404, Taiwan, R.O.C. Tel: 886-4-2205-3366-8501, Fax: 886-4- 2205-3764, e-mail: jgchung@mail.cmu.edu.tw

Key Words: Ellagic acid (EA), 2-aminofluorene (2-AF), N-acetyl-2-aminofluorene (2-AAF), N-acetyltransferase (NAT), 2-AF metabolites.

Arylamine carcinogens require host-mediated metabolic activation to initiate carcinogenesis in target tissues (1-3). For example, an arylamine such as 2-aminofluorene (2-AF) is biotransformed to form corresponding arylamines such as N-acetyl-2-aminofluorene (2-AAF) *via* cytosolic N-acetyltransferase (NAT) (4,5). 2-AF has been demonstrated to be carcinogenic to liver, urinary bladder and other tissues of a variety of animal species (6-8). It has been shown that liver cells can metabolize AF into various metabolites *in vitro* (9). Previous studies have shown that Sprague-Dawley (SD) rats can acetylate 2-AF into 2-AAF *in vivo* (10). It was reported that 2-

Table I. *Distribution of 2-AF and 2-AF metabolites in bladder 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)	0.76 ±0.50	0.39 ±0.16	1.32 ±0.50	-- --	-- --	-- --	0.54 ±0.15	3.61 ±1.10
EA +2-AF	0.46 ±0.19	0.44 ±0.21	*3.35 ±1.13	-- --	-- --	-- --	**1.13 ±0.43	*1.83 ±1.11
EA 24 h +2-AF	0.74 ±0.23	0.36 ±0.12	1.61 ±0.71	-- --	-- --	-- --	-- --	*2.16 ±0.70

Values are mean ±SD n=6. -- = not detectable. Rats were administered with 2-AF similarly with or without EA or after being treated with EA for 24 hours. Then the bladder tissue was determined for 2-AF and metabolites. Data were analyzed by an unpaired Student's *t*-test. Difference between 2-AF-treated (control), 2-AF and EA co-treated at the same time, EA-treated for 24 h then 2-AF-treated groups. *$p<0.05$ **$p<0.01$.

Table II. *Distribution of 2-AF and 2-AF metabolites in blood 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)	0.07 ±0.03	0.12 ±0.03	1.38 ±0.36	-- --	0.05 ±0.01	-- --	0.23 ±0.04	-- --
EA +2-AF	0.07 ±0.04	0.12 ±0.09	0.89 ±0.29	-- --	0.05 ±0.00	-- --	0.25 ±0.09	-- --
EA 24 h +2-AF	0.05 ±0.01	***0.03 ±0.01	0.81 ±0.47	-- --	0.04 ±0.00	-- --	***0.12 ±0.02	-- --

Values are mean ±SD n=6. -- = not detectable. Rats were administered with 2-AF similarly with or without EA or after being treated with EA for 24 hours. Then the blood was determined for 2-AF and metabolites. Data were analyzed by an unpaired Student's *t*-test. *Difference between 2-AF-treated (control), 2-AF and EA co-treated at the same time, EA-treated for 24 h then 2-AF-treated groups. ***$p<0.001$.

Table III. *Distribution of 2-AF and 2-AF metabolites in colon 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)	2.02 ±1.18	0.69 ±0.35	6.68 ±2.08	0.21 ±0.05	1.22 ±0.40	-- --	1.48 ±0.21	1.44 ±0.56
EA +2-AF	***0.18 ±0.12	*1.55 ±0.58	**3.28 ±1.30	0.21 ±0.01	0.84 ±0.20	-- --	3.42 ±2.02	1.46 ±0.23
EA 24 h +2-AF	***0.08 ±0.03	0.69 ±0.15	***1.30 ±0.35	***0.06 ±0.02	0.97 ±0.37	-- --	1.68 ±0.27	0.81 ±0.20

Values are mean ±SD n=6. -- = not detectable. Rats were administered with 2-AF similarly with or without EA or after being treated with EA for 24 hours. Then the colon tissue was determined for 2-AF and metabolites. Data were analyzed by an unpaired Student's *t*-test. Difference between 2-AF-treated (control), 2-AF and EA co-treated at the same time, EA-treated for 24h then 2-AF-treated groups. . $p<0.05$ **$p<0.01$ ***$p<0.001$.

AAF is hepatocarcinogenic in adult SD rats after repeated exposure (1,11). Human, and several other mammalian species, have been demonstrated to have N-acetylation polymorphism. Therefore, individuals can be divided into rapid, intermediate and slow acetylator phenotypes (5,12,13). It has been demonstrated that the higher incidence of bladder cancer in slow acetylators of human populations is associated with exposure to arylamine carcinogens (12).

Table IV. *Distribution of 2-AF and 2-AF metabolites in kidney 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)	0.51 ±0.32	0.65 ±0.26	4.7 ±1.34	0.15 ±0.10	-- --	-- --	-- --	1.33 ±0.40
EA +2-AF	0.37 ±0.12	0.44 ±0.13	3.52 ±0.93	0.08 ±0.04	-- --	-- --	-- --	2.38 ±0.66
EA 24 h +2-AF	0.43 ±0.28	0.52 ±0.38	**2.37 ±0.96	0.06 ±0.04	-- --	-- --	-- --	1.23 ±0.43

Values are mean ±SD n=6. -- = not detectable. Rats were administered with 2-AF similarly with or without EA or after being treated with EA for 24 hours. Then the kidney tissue was determined for 2-AF and metabolites. Data were analyzed by an unpaired Student's *t*-test. Difference between 2-AF-treated (control), 2-AF and EA co-treated at the same time, EA-treated for 24h then 2-AF-treated groups. $^{**}p<0.01$.

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

2-AF Metabolites / Treatment	2-AF	2-AAF	1-OH-2-AAF	3-OH-2-AAF	7-OH-2-AAF	8-OH-2-AAF	9-OH-2-AAF
Control (2-AF only)	0.33 ±0.09	1.01 ±0.33	4.83 ±1.69	0.10 ±0.03	-- --	0.74 ±0.13	-- --
EA +2-AF	0.45 ±0.06	*0.61 ±0.10	3.18 ±1.22	0.12 ±0.03	-- --	0.85 ±0.18	-- --
EA 24 h +2-AF	0.43 ±0.11	**0.52 ±0.12	***1.27 ±0.55	***0.01 ±0.00	-- --	0.65 ±0.33	-- --

Values are mean ±SD n=6. -- = not detectable. Rats were administered with 2-AF similarly with or without EA or after being treated with EA for 24 hours. Then the liver tissue was determined for 2-AF and metabolites. Data were analyzed by an unpaired Student's *t*-test. *Difference between 2-AF-treated (control), 2-AF and EA co-treated in the same time, EA-treated for 24 h then 2-AF-treated groups. $^{*}p<0.05$ $^{**}p<0.01$ $^{***}p<0.001$.

Ellagic acid (EA) (2,3,7,8-tetrahydroxy[I]benzopyrano-[5,4,3-cde][I]benzopyran-5,10-dione), a naturally occurring polyphenol compound present in plants, has been reported to possess anticarcinogenic activity in animal studies (14,15). Dietary treatment with EA in male AVI/N rats decreased the incidence of *N*-2-fluorenylacetamide-induced hepatocellular neoplasms (16). It was also reported that EA decreased the number of esophageal preneoplastic and neoplastic lesions in mice and rats induced by *N*-nitrosomethylbenzylamine (17,18). Other investigators reported that orally administered EA, during or after carcinogen treatment of rats, could significantly reduce the incidence and number of small intestinal tumors (19). It was reported that dietary EA led to decreased hepatic and esophageal mucosal cytochrome P450 and phase II enzyme activity in rats (20). It was also reported that EA inhibited chemically-induced tumors through multiple mechanisms against different classes of chemical carcinogens and in different organs (21-24) Our recent work demonstrated that orally administered EA decreased NAT activity and 2-AF acetylation and metabolism and excretion in SD rats (25).

Materials and Methods

Chemicals and reagents. Ellagic acid (EA), leupeptin, 2-aminofluorene (2-AF), N-acetyl-2-aminofluorene (2-AAF), acetyl carnitine, Tris, carnitine acetyltransferase, dithiothreitol (DTT), phenylmethylsulfonylfluoride (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 Sprague-Dawley (SD) rats, weighing 160-180g, were housed in individual cages and were maintained at 25˚C on a 12-h light/dark cycle, as previously described (10). EA

Table VI. *Distribution of 2-AF and 2-AF metabolites in feces 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)	2.66 ±0.72	22.07 ±4.89	66.52 ±8.26	1.16 ±0.50	15.16 ±3.65	-- --	17.72 ±2.00	-- --
EA +2-AF	3.82 ±1.83	*36.63 ±6.02	69.40 ±10.46	2.85 ±1.00	19.18 ±6.32	-- --	24.73 ±4.75	-- --
EA 24 h +2-AF	4.17 ±1.22	16.67 ±1.88	84.65 ±26.40	1.24 ±0.16	18.47 ±4.90	-- --	15.77 ±0.98	-- --

Values are mean ±SD n=6. -- = not detectable. Rats were administered with 2-AF similarly with or without EA or after being treated with EA for 24 hours. Then the feces was determined for 2-AF and metabolites. Data were analyzed by an unpaired Student's *t*-test. *Difference between 2-AF-treated (control), 2-AF and EA co-treated at the same time, EA-treated for 24h then 2-AF-treated groups. *$p<0.05$.

Table VII. *Distribution of 2-AF and 2-AF metabolites in urine 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)	14.41 ±6.43	1.08 ±0.25	13.51 ±6.96	0.38 ±0.11	12.54 ±3.30	-- --	39.07 ±8.62	-- --
EA +2-AF	13.14 ±3.96	0.79 ±0.35	**2.68 ±0.15	0.25 ±0.50	***3.31 ±0.56	-- --	***5.21 ±0.20	-- --
EA 24 h +2-AF	**3.03 ±0.67	0.62 ±0.27	***33.47 ±6.15	0.26 ±0.11	14.62 ±4.57	-- --	***15.00 ±3.70	-- --

Values are mean ±SD n=6. -- = not detectable. Rats were administered with 2-AF similarly with or without EA or after being treated with EA for 24 hours. Then the urine was determined for 2-AF and metabolites. Data were analyzed by an unpaired Student's *t*-test. *Difference between 2-AF treated (control), 2-AF and EA co-treated at the same time, EA-treated for 24h then 2-AF-treated groups. **$p<0.01$ ***$p<0.01$.

(50 mg/kg) was administered by gauge (10, 25) in isotonic saline (1 ml of saline administered). Control animals received 1% DMSO containing no EA. 2-AF was administered at 50 mg/kg in 1% DMSO to rats. In addition, other rats were treated with 2-AF (control) or were treated with EA and 2-AF at the same time. 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 and tissues were immediately extracted twice with ethyl acetate/methanol (95:5), the solvent evaporated and the residue redissolved in methanol and assayed. 2-AF, 2-AAF and 2-AF metabolites were quantified by HPLC as described previously (10). Briefly, an aliquot of the extracted 2-AF and 2-AAF or 2-AF metabolites 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. For 2-AF and 2-AAF, the solvent system was 20 mM KH_2PO_4, pH 4.5/ CH_3CN (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.

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

Results

The effects of EA on the total 2-AF and 2-AF metabolites in the bladder, blood, colon, kidney, liver, feces and urine from SD rats are presented in Tables I-VII. The profiles of 2-AF metabolites in examined tissues are given in Figures 1-7. The comparison of total amounts of 2-AF and 2-AF metabolites from all examined tissues is presented in Figure 8. These figures indicate that EA affected 2-AF metabolites in the examined tissues. In the bladder tissues (Table I and Figure 1), the control (2-AF only), the EA-treated for 24 h before addition of 2-AF and the EA- and 2-AF-treated at the same

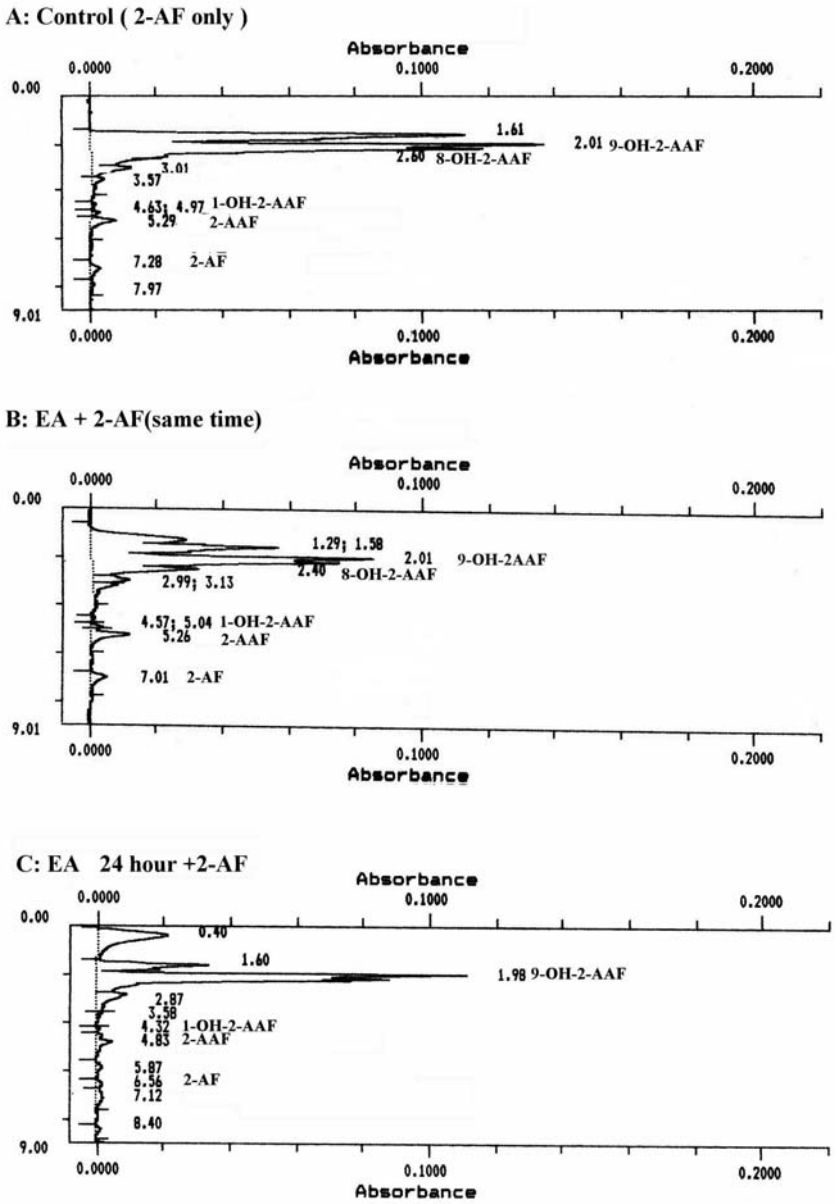

Figure 1. *Effect of ellagic acid on the 2-AF acetylation and metabolites in rat bladder tissues. Animals were treated with 2-AF only or EA for 24 h then treated with 2-AF or 2-AF- and EA- treated at the same time. Then animals were sacrificed and bladder tissue was collected for 2-AF and 2-AF metabolite analysis as described in Materials and Methods. Data are expressed as mean±SD, N=6.*

time did not display 3-OH-2-AAF, 5-OH-2-AAF and 7-OH-2-AAF. However, 2-AF, 2-AAF, 1-OH-2-AAF, 8-OH-2-AAF and 9-OH-2-AAF were shown in the three examined groups. The amounts of 1-OH-2-AAF, 8-OH-2-AAF and 9-OH-2-AAF showed a significant difference between the control and the EA and 2-AF group treated at the same time. 9-OH-2-AAF showed a significant decrease between the control and the EA-treated for 24 h before addition of 2-AF (Table I). In the blood samples (Table II and Figure 2), the control (2-AF only), the EA-treated for 24 h before addition of 2-AF and the EA- and 2-AF- treated at the same time did not display 3-OH-2-AAF, 7-OH-2-AAF and 9-OH-2-AAF. However, 2-AF, 2-AAF, 1-OH-2-AAF, 5-OH-2-AAF and 8-OH-2-AAF occurred in the three examined groups. The amounts of 2-AAF and 8-OH-2-AAF showed a significant decrease between the control and the EA- treated for 24 h before addition of 2-AF (Table II). In the colon tissues (Table III and Figure 3), the control (2-AF only), the EA-treated for 24

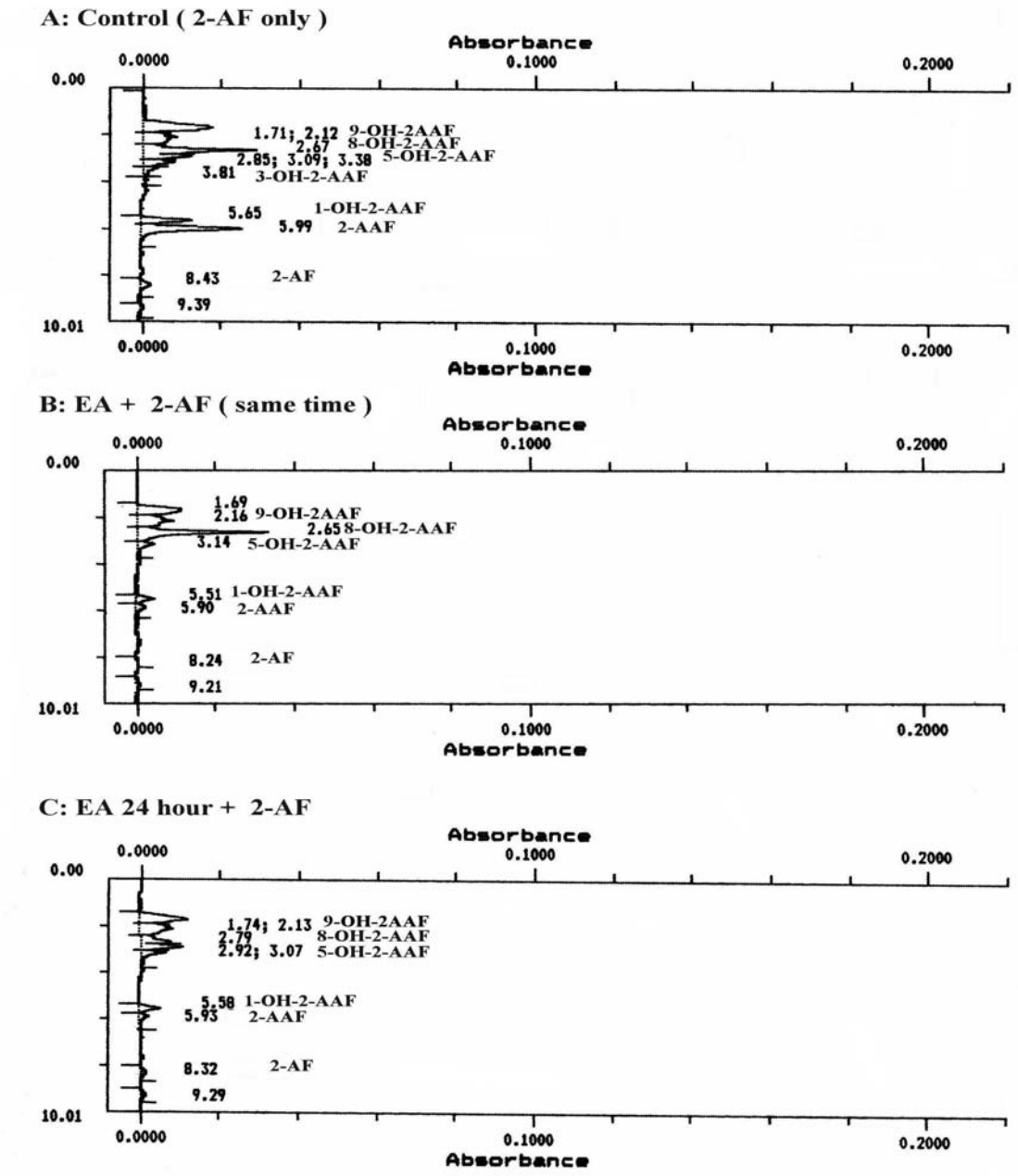

Figure 2. *Effect of ellagic acid on the 2-AF acetylation and metabolites in rat blood. Animals were treated with 2-AF only or EA for 24 h then treated with 2-AF or 2-AF- and EA- treated at the same time. Then animals were anesthetized and blood was collected for 2-AF and 2-AF metabolite analysis as described in Materials and Methods. Data are expressed as mean±SD, N=6.*

h before addition of 2-AF and the EA- and 2-AF- treated at the same time did not show 7-OH-2-AAF. 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 occurred in the three examined groups. The amounts of 2-AF, 1-OH-2-AAF, 3-OH-2-AAF showed a significant decrease between the control and the EA-treated for 24 h before addition of 2-AF. The control and EA- and 2-AF- treated at the same time groups showed significant variation of the 2-AF, 2-AAF and 1-OH-2-AAF (Table III). In the kidney tissues (Table IV and Figure 4), the control (2-AF only), the EA-treated for 24 h before addition of 2-AF and the EA- and 2-AF- treated at the same time did not show 5-OH-2-AAF, 7-OH-2-AAF and 8-OH-2-AAF. However, 2-AF, 2-AAF, 1-OH-2-AAF, 3-OH-2-AAF and 9-

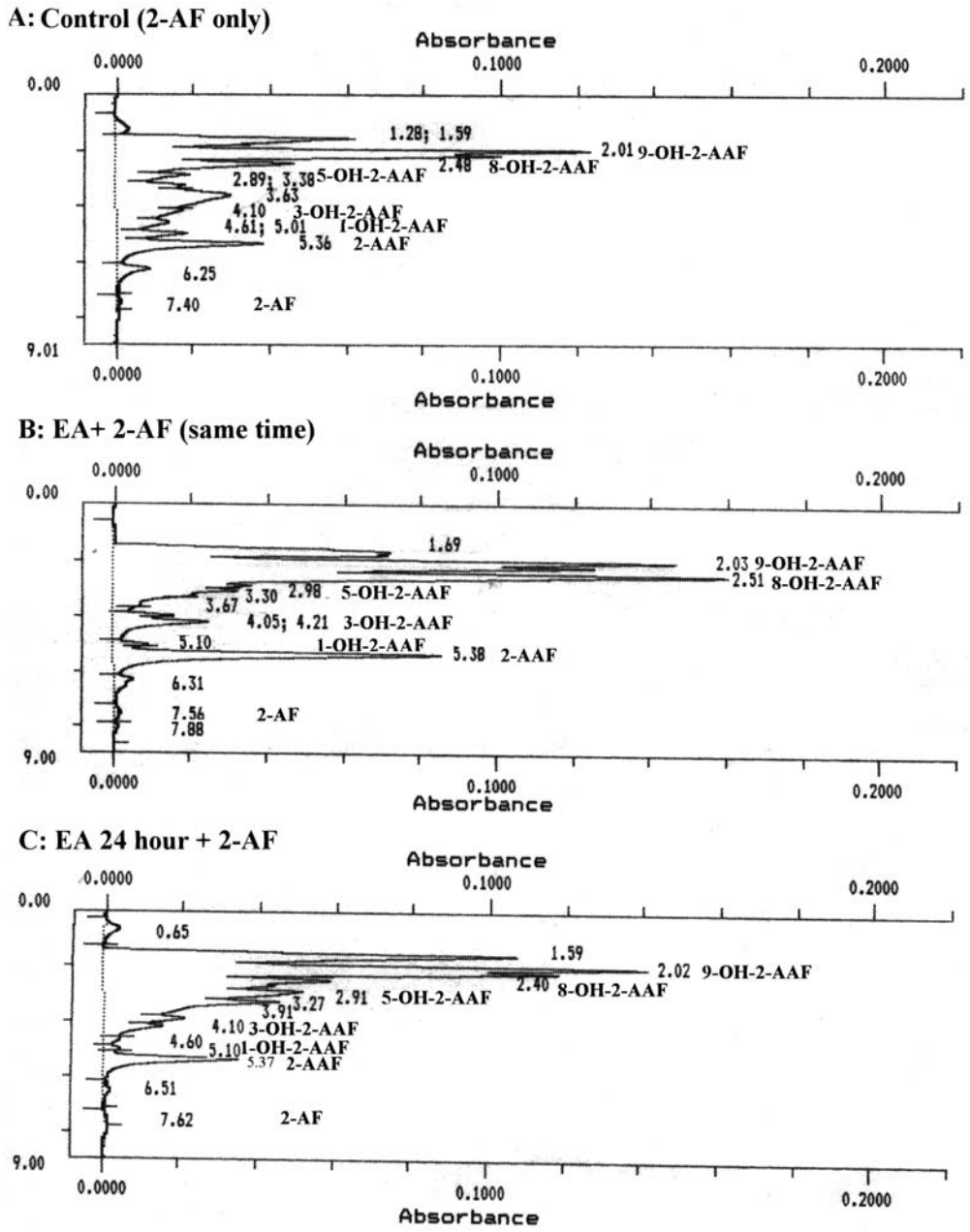

Figure 3. *Effect of ellagic acid on the 2-AF acetylation and metabolites in rat colon tissues. Animals were treated with 2-AF only or EA for 24 h then treated with 2-AF or 2-AF and EA at the same time. Then animals were sacrificed and colon tissue was collected for 2-AF and 2-AF metabolite analysis as described in Materials and Methods. Data are expressed as mean±SD, N=6.*

OH-2-AAF occurred in the three examined groups. The amounts of 1-OH-2-AAF showed significant decrease between the control and the EA-treated for 24 h before addition of 2-AF goup. However, the amounts of 2-AF metabolites did not show significant differences between the control and the EA- and 2-AF- treated at the same time group (Table IV). In the liver tissues (Table V and Figure 5), the control (2-AF only), the EA-treated for 24 h before addition of 2-AF and the EA- and 2-AF- treated at the same time groups did not show 7-OH-2-AAF and 9-OH-2-AAF. However, 2-AF, 2-AAF, 1-OH-2-AAF, 3-OH-2-AAF and 8-OH-2-AAF occurred in the three examined groups. The amounts of 2-AAF showed a significant decrease between the control and the EA- and 2-AF- treated at the same time

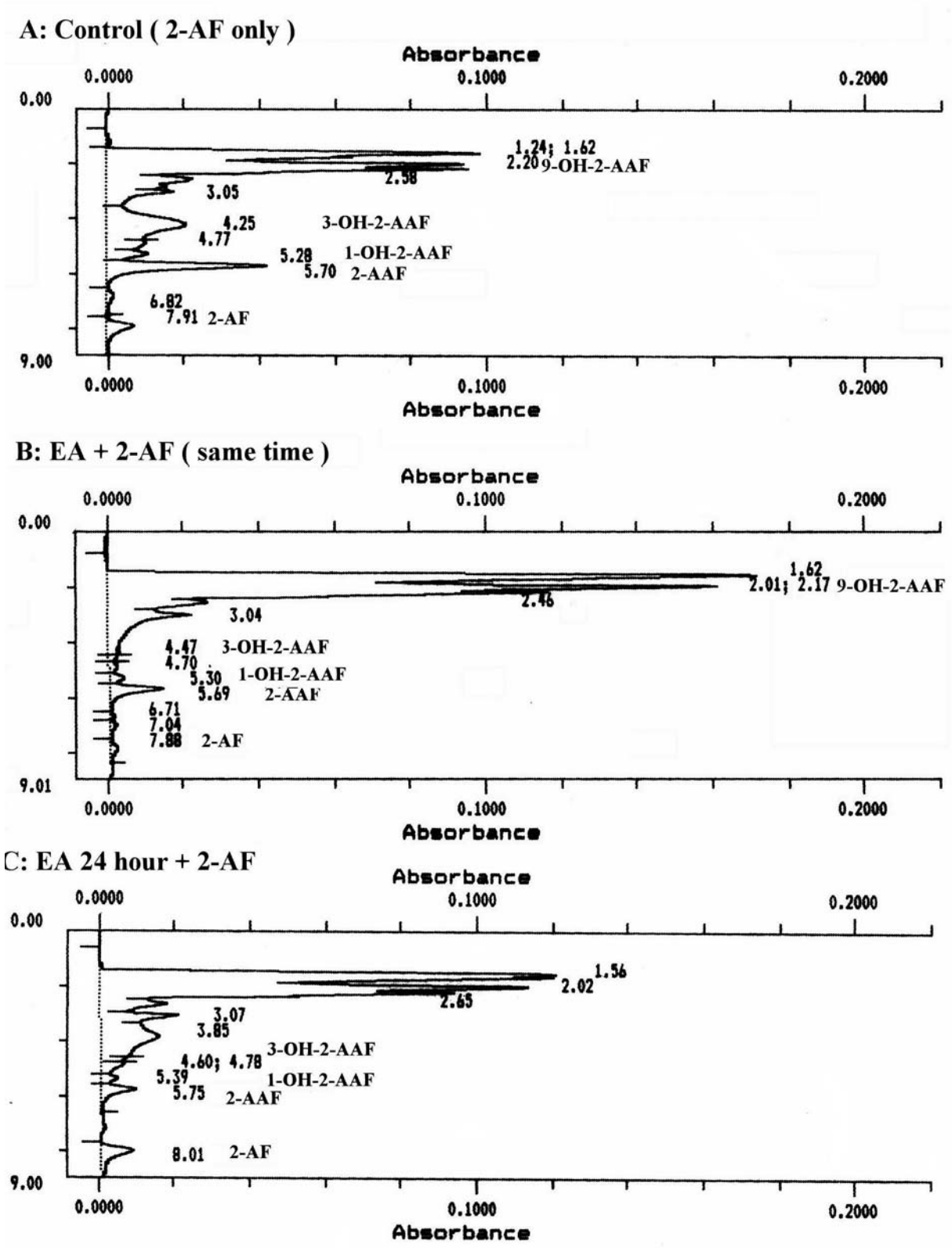

Figure 4. *Effect of ellagic acid on the 2-AF acetylation and metabolites in rat kidney tissues. Animals were treated with 2-AF only or EA for 24 h then treated with 2-AF or 2-AF and EA at the same time. Then animals were sacrificed and kidney tissue was collected for 2-AF and 2-AF metabolite analysis as described in Materials and Methods. Data are expressed as mean±SD, N=6.*

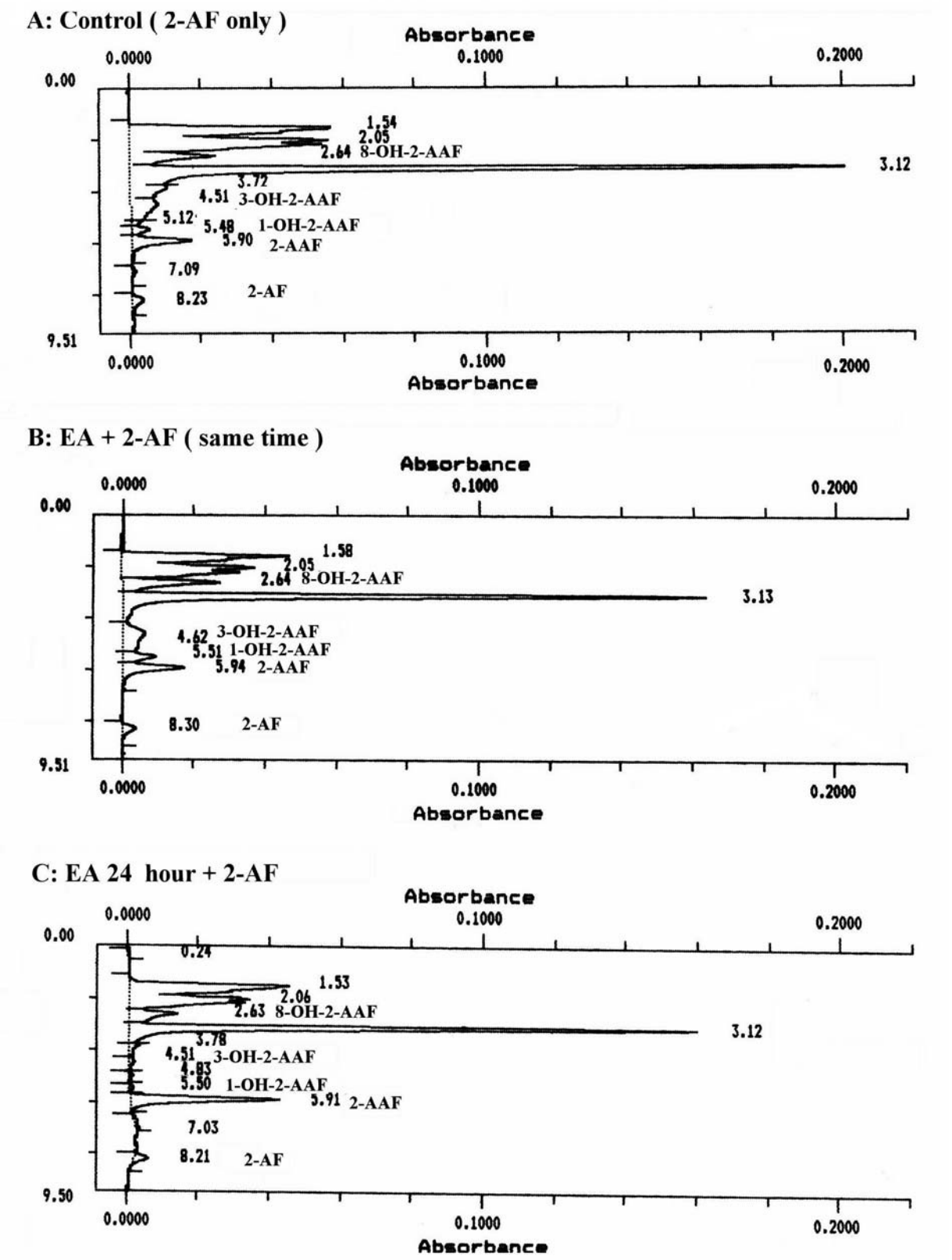

Figure 5. *Effect of ellagic acid on the 2-AF acetylation and metabolites in rat liver tissues. Animals were treated with 2-AF only or EA for 24 h then treated with 2-AF or 2-AF and EA at the same time. Then animals were sacrificed and liver tissue was collected for 2-AF and 2-AF metabolite analysis as described in Materials and Methods. Data are expressed as mean±SD, N=6.*

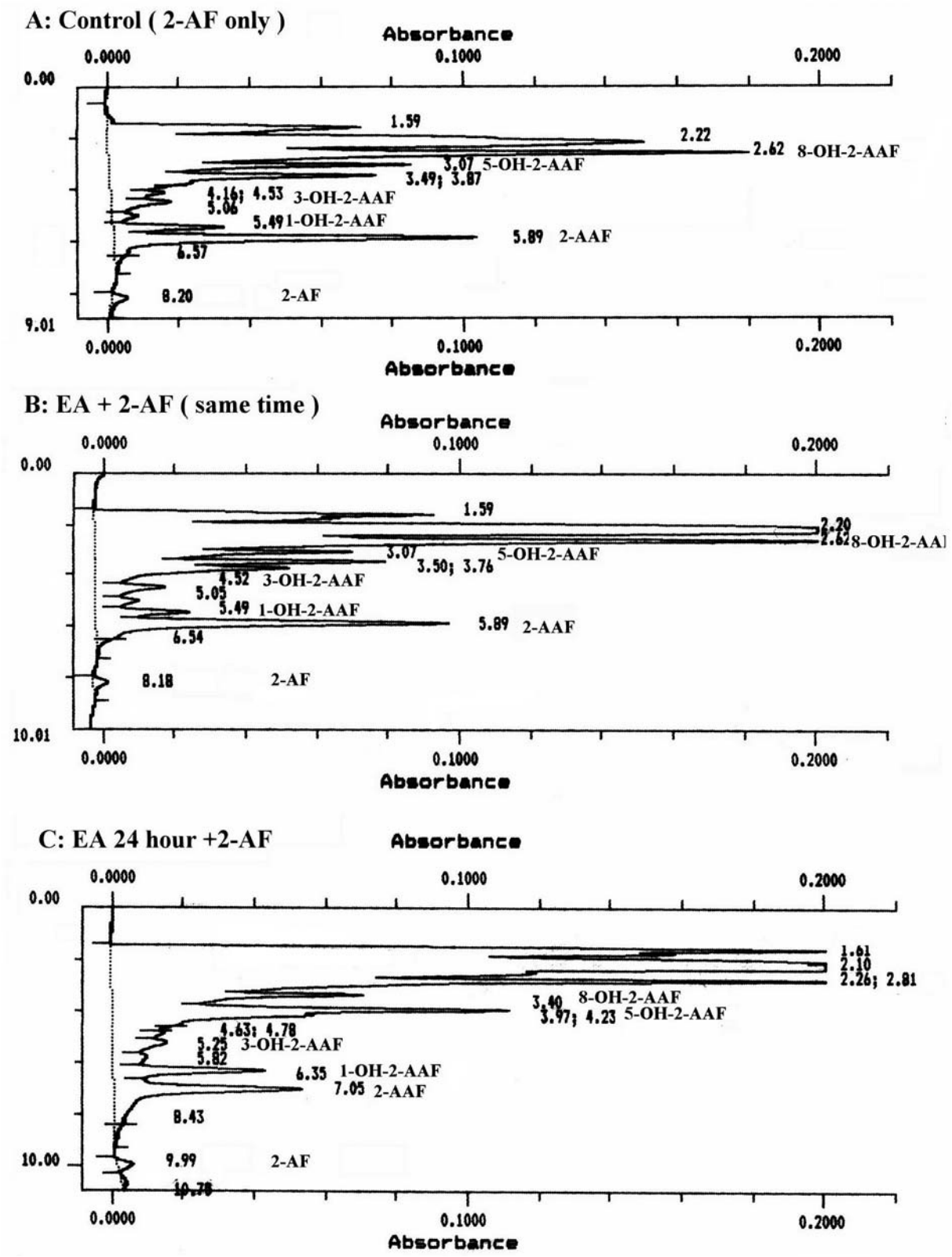

Figure 6. *Effect of ellagic acid on the 2-AF acetylation and metabolites in rat feces. Animals were treated with 2-AF only or EA for 24 h then treated with 2-AF or 2-AF and EA at the same time. Feces were collected for 2-AF and 2-AF metabolite analysis as described in Materials and Methods. Data are expressed as mean±SD, N=6.*

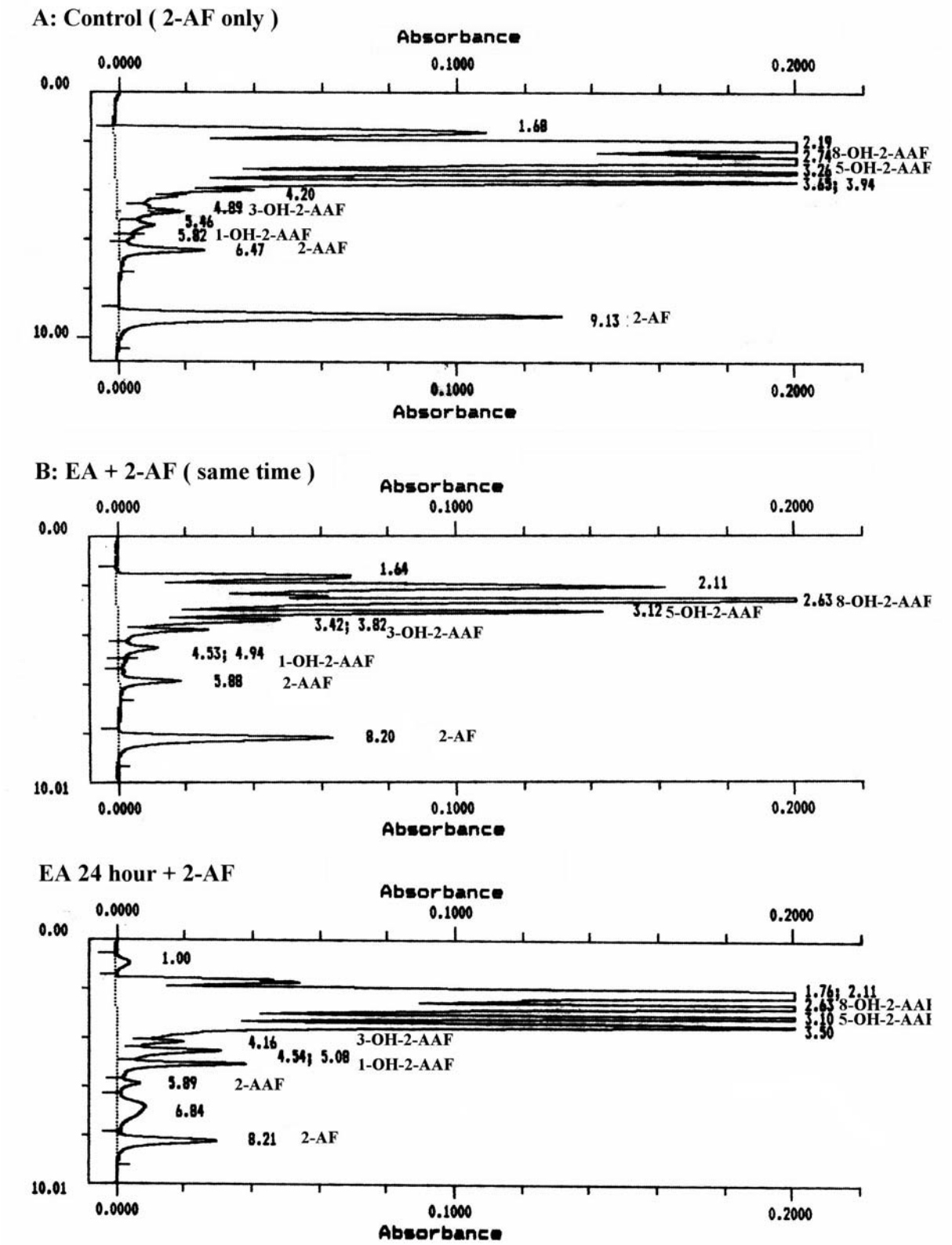

Figure 7. *Effect of ellagic acid on the 2-AF acetylation and metabolites in rat urine. Animals were treated with 2-AF only or EA for 24 h then treated with 2-AF or 2-AF and EA at the same time. Urine was collected for 2-AF and 2-AF metabolite analysis as described in Materials and Methods. Data are expressed as mean±SD, N=6.*

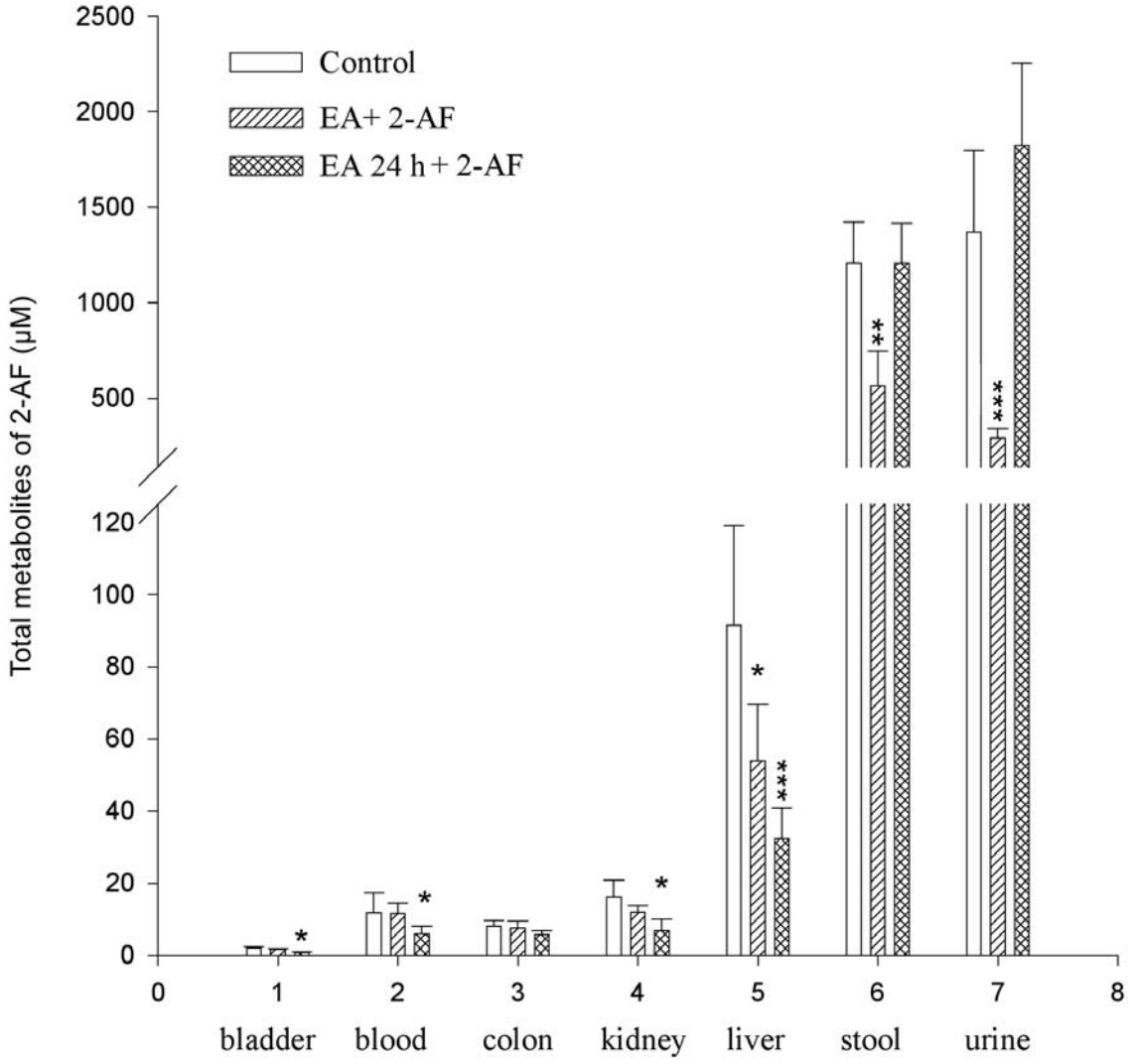

Figure 8. *The comparison of total amounts of 2-AF and 2-AF metabolites in SD rats' different organs during co-treatment with EA. Animals were treated with 2-AF only or EA for 24 h then treated with 2-AF or 2-AF and EA at the same time. Total amounts of 2-AF N- acetylation were calculated and analyzed by unpaired Student's t-test. Data are expressed as mean±SD, N=6.*

group. The amounts of 2-AAF, 1-OH-2-AAF and 3-OH-2-AAF showed a significant decrease between the control and the EA-treated for 24 h before addition of 2-AF group (Table V). In the feces samples (Table VI and Figure 6), the control (2-AF only), the EA-treated for 24 h before addition of 2-AF and the EA- and 2-AF- treated at the same time groups did not show 7-OH-2-AAF and 9-OH-2-AAF. However, 2-AF, 2-AAF, 1-OH-2-AAF, 3-OH-2-AAF, 5-OH-2-AAF and 8-OH-2-AAF occurred in the three examined groups. The amounts of 2-AAF showed a significant difference between the control and the EA- and 2-AF- treated at the same time group; the control and the EA-treated for 24 h before addition of 2-AF to groups did not show significant differences (Table VI). In the urine samples (Table VII and Figure 7), the control (2-AF only), the EA-treated for 24 h before addition of 2-AF was added and the EA- and 2-AF- treated at the same time groups did not show 7-OH-2-AAF and 9-OH-2-AAF. However, 2-AF, 2-AAF, 1-OH-2-AAF, 3-OH-2-AAF, 5-OH-2-AAF and 8-OH-2-AAF occurred in the three examined groups. The amounts of 1-OH-2-AAF, 5-OH-2-AAF and 8-OH-2-AAF showed a significant decrease between the control and the EA- and 2-AF- treated at the same time group. The amounts of 2-AF, 1-OH-2-AAF and 8-OH-2-AAF showed a significant difference between the control and the EA- treated for 24 h before addition of 2-AF group (Table VII).

Discussion

This study demonstrated that EA can affect N-acetylation and the metabolism of 2-AF *in vivo* in the examined rat tissues. Many studies have demonstrated that the AcCoA-dependent arylamine NAT enzyme, which acetylates 2-AF, is present in many tissues from experimental animals and humans, and that NAT has been shown to be involved in chemical carcinogenesis (26,27). The sensitivity of individuals to toxicity during exposure to arylamine chemicals is associated with genetically controlled rapid or slow NAT acetylation (5,28). Increased levels of NAT activity are associated with increased sensitivity to the mutagenic effects of arylamine carcinogens (29). Decreased NAT activity of the liver is associated with several disease processes such as breast and bladder cancer (5, 28). Much evidence for the formation of N-acetyl derivatives comes from arylamines in mammalian species (28). Thus, NAT plays an important role in 2-AF-induced malignancy in mammals. But, as to the *in vivo* metabolic conversion of arylamines to N-formyl derivatives, only a few reports have been published. Dietary EA inhibits the enzymic activity of CYP1A1 without altering hepatic concentrations of CYP1A1 mRNA (30). But there is no available information about the effect of dietary EA on N-acetylation and metabolism of 2-AF *in vivo* in rat tissues. Although it was reported that murine extrahepatic tissues showed N-acetylation of 2-AF and p-aminobenzoic acid (31), there is no work published on the 2-AF and 2-AF metabolites after oral treatment of 2-AF in laboratory animals. Therefore, the present studies were focused on the effects of dietary EA on 2-AF N-acetylation and metabolism in bladder, blood, colon, kidney, liver, feces and urine samples from SD rats.

The dose of 10 mg/kg EA for the presented *in vivo* experiments was decided in previous dietary EA studies in rats (25). The reason for selecting pretreatment with EA for 24 h before addition of 2-AF was based on the report of Barch and Rundhaugen (30), which demonstrated that a day of dietary EA significantly induces the transcription of two other genes (glutathione S-transferase YA and NAD(P)H: quinone reductase) containing the Ah responsive regulatory element. Thus, one day of administration may suffice to induce large changes in phase II enzyme activity. The data of the N-acetylation of 2-AF *in vitro* indicated that EA induced an inhibition of arylamine carcinogen (2-AF) acetylation in the examined cerebrum, cerebellum and pineal gland of rats (32). The data from the present study showed change in the total amounts of 2-AF and 2-AF metabolites in all the examined tissues (bladder, blood, colon, kidney, liver feces and urine) with or without EA co-treatment. Other reports have already shown that the elimination pathways of procainamide include renal excretion of unchanged drug, acetylation and oxidative metabolism (33). The present data indicate that decrease in acetylation of 2-AF and in the total amounts of 2-AF metabolites may suggest that EA may inhibit the oxidative metabolism of 2-AF.

Other reports showed that several other compounds increased acetylation of drugs: (a) stimulation of the reticuloendothelial system by Freund's adjuvant led to increased rate of N-acetylation *in vivo* in both the rat (34) and the rabbit (35); (b) pretreatment with tilorone resulted in an increased rate of N-acetylation of procainamide (36) and 2-AF (10) *in vivo* in the rat; (c) chronic administration of hydrocortisone in the rabbit has been shown to enhance the acetylation of sulfamethazine (37). The present data also showed that EA affected 2-AF metabolism based on the changes of 2-AF metabolite profiles. Other urinary metabolites of 2-AAF could not be isolated, purified 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 EA, to SD rats, the 24 h samples extracted by HPLC indicated that EA decreased the rate of arylamine carcinogen acetylation in bladder, blood, colon, kidney and liver tissues. The present results may also indicate that predisposition to carcinogen toxicity may be influenced by specific chemopreventive agents. Future studies will focus on the mechanism of these changes and their significance in the toxicity of agents that undergo biotransformation *via* acetylation in tissues.

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Received July 26, 2004
Revised December 27, 2004
Accepted December 29, 2004