in vivo *19*: 399-406 (2005)

N-Acetyltransferase is Involved in Baicalein-induced N-Acetylation of 2-Aminofluorene and DNA-2-Aminofluorene Adduct Formation in Human Leukemia HL-60 Cells

YU-CHING LI[1], YU-SHENG TYAN[2], YIH-MIN LEE[1], TANG-YI TSAO[3], JING-YUAN CHUANG[4], HSIU-MAAN KUO[5], TE-CHUN HSIA[6], JEN-HUNG YANG[7] and JING-GUNG CHUNG[8]

[1]Department of Medical Technology, Chungtai Institute of Health Sciences and Technology, Taichung 406; Departments of [2]Radiology and [7]Dermatology, Chung Shan Medical University Hospital, Taichung 402; [3]Department of Pathology, Tungs' Taichung Metroharbor Hospital, [6]Department of Internal Medicine, China Medical University Hospital, Taichung; [4]School of Medical Laboratory Science and Biotechnology, Departments of [5]Parasitology and [8]Microbiology, China Medical College, Taichung 400, Taiwan, R.O.C.

Abstract. *Many arylamine and hydrazine drugs are acetylated by cytosolic N-acetyltransferase (NAT). The human promyelocytic leukemia cell line (HL-60) has been shown to acetylate arylamine and contain NAT activity. The purpose of this study was to determine whether or not baicalein could affect N-acetylation of 2-aminofluorene (AF) in HL-60 cells. Acetylated and nonacetylated AF were determined by using high performance liquid chromatography. Baicalein displayed a dose-dependent inhibition of cytosolic and intact cells' NAT activity and reduced the number of viable cells. Time-course experiments showed that N-acetylation of AF, measured from intact HL-60 cells, was inhibited by baicalein for up to 48 h. Baicalein also decreased AF-DNA adduct formation in the examined cells. The effects of baicalein on NAT were examined by flow cytometry and NAT gene expression was examined by polymerase chain reaction. The results demonstrated that baicalein inhibited NAT1 mRNA gene expression and reduced the level of NAT in HL-60 cells. These results show that baicalein can affect the NAT activity of human leukemia cells in vitro.*

The bioactivation and detoxification of many arylamine drugs and carcinogens have been reported among various species (1, 2). Arylamine N-acetyltransferase (NAT), a ubiquitous enzyme found in the cytosol fraction of the liver and other tissues or organs, participates in the biochemical metabolism of arylamines (1, 3, 4). Two distinct NAT genes (*NAT1* and *NAT2*) in humans have been identified and sequenced, encoding for two different human liver arylamine NATs (5, 6). Individuals are classified as rapid or slow acetylators and susceptibility to arylamine carcinogens has been associated with acetylator phenotypes. Rapid acetylation has been linked to an increased risk of colorectal cancer (7, 8). Slow acetylation has been linked to increased drug toxicity from sulphonamide (9) and isoniazid (1), and increased susceptibility to occupational bladder cancer (10). Therefore, the genetically mediated variation in NAT activities within target organs or tissues for arylamine-induced neoplasm may indicate different risks among human populations (11).

Scutellariae radix is the dried root of *Scutellaria baicalenesis* Georgi, and has been widely used for centuries in traditional herbal medicine in Japan and China. This herb and its ingredients, such as baicalein, have been used for treating upper respiratory infections, diphtheria, nephritis, dysentery, scarlet fever and hepatitis (12). Baicalein (5,6,7-trihydroxy-2-phenyl-4H-1-benzopyran-4-one) has been demonstrated to have anti-bacterial (12), anti-viral (13, 14), anti-oxidant (15), anti-inflammatory (16), anti-thrombotic (17, 18) and anti-tumor (19-23) properties. Baicalein also showed a suppressive effect on the proliferation of hepatic stellate cells (24). However, there is no available information which addresses the effects of baicalein on NAT activity in human leukemia cells (HL-60). Thus, the present study was performed to determine whether baicalein could affect N-acetylation of AF in human leukemia cells.

Correspondence to: Dr. Jing-Gung Chung, Department of Microbiology, China Medical University, 91 Hsueh-Shih Road, Taichung 400, Taiwan, Republic of China. Tel: (04) 2322-7569, Fax: (04) 2205-3764, e-mail: jgchung@mail.cmu.edu.tw

Key Words: N-acetyltransferase, baicalein, leukemia cells, arylamine, 2-aminofluorene.

Materials and Methods

Chemicals and reagents. Baicalein, ethylenediaminetetraacetic acid (EDTA), 2-aminofluorene (AF), N-acetyl-2-aminofluorene (AAF), acetylcarnitine, leupeptin, bovine serum albumin (BSA), phenylmethylsulfonylfluoride (PMSF), Tris, dimethyl sulfoxide (DMSO), dithiothreitol (DTT), acetyl-coenzyme A and carnitine acetyltransferase were obtained from Sigma Chemical Co. (St. Louis, MO, USA). RPMI 1640, fetal bovine serum, penicillin-streptomycin and glutamine were obtained from Gibco BRL (Grand Island, NY, USA). All the chemicals used were of reagent grade.

Human leukemia cell line (HL-60). The human promyelocytic leukemia cell line (HL-60) was obtained from the the Food Industry Research and Development Institute (Hsinchu, Taiwan). The cells were placed into 75-cm^3 tissue culture flasks and grown at 37˚C under a humidified 5% CO_2 atmosphere in RPMI 1640 medium supplemented with 10% fetal bovine serum, 1% penicillin-streptomycin (10,000 U/ml penicillin and 10 mg/ml streptomycin) and 1% glutamine.

Preparation of human leukemia cell cytosols. About 5 x 10^7 cells were placed in 1 ml of the 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), as previously described (25). The suspensions were centrifuged at 9000xg for 1 min in a model 3200 Eppendorf/Brinkman centrifuge, and the supernatant fraction was subsequently centrifuged at 10,000xg for 60 min. The supernatant was collected and kept on ice for NAT activity and protein determinations.

NAT activity determination. The determination of acetyl-CoA-dependent N-acetylation of AF was performed as previously described by Chung *et al*. (26).

Protein determination. Protein concentrations in the human leukemia cell cytosols were determined by the method of Bradford (27) with bovine serum albumin as the standard. All of the samples were assayed in triplicate.

Effects of various concentrations of baicalein on N-acetylation of AF in human leukemia cell cytosol. The reaction mixture consisted of 50 μl cytosol, 20 μl of recycling mixture (11.25 ml of 1M Tris, 4.5ml of 0.1M EDTA-Na_2, 69.4 mg of DTT, 267.5 mg of acetylcarnitine, and distilled water to 50 ml (pH 7.5)) containing AF at selected concentrations as substrate, and 10 μl of a selected concentration of baicalein. The reaction was started by the addition of acetyl coenzyme A (Ac-CoA). The control reaction had 20 μl distilled water in place of Ac-CoA. Following these steps, the NAT activity was determined as previously described (26, 28).

Effects of various concentrations of baicalein on N-acetylation of AF in human leukemia cells. 5x10^5 human leukemia cells per ml were incubated with 22.5 μM AF in individual wells of 24-well cell culture plate with or without baicalein. After incubation, the cells and media were removed and centrifuged. The supernatant was immediately extracted with ethyl acetate/ methanol (95:5), the solvent evaporated and the residue redissolved in methanol and assayed for AAF by using HPLC, as described previously (26, 28).

Table I. *Effects of baicalein on N-acetylation of AF in human leukemia HL-60 cell cytosol.*

Baicalein treatment (μM)	AAF (nmol/min/mg protein)
Control	0.98±0.30
0.05	0.86±0.22
0.5	0.72±0.24
5	0.62±0.20
50	0.40±0.12a
500	0.22±0.08ab
F Value	70.42*

N-acetylation of AF was determined by incubation of HL-60 cytosol with 22.5 μM AF (as substrate) and 50 μM acetyl CoA for 10 min at 37˚C. The amount of acetylated product was determined by HPLC as described in Materials and Methods. Values are mean±SD of three individual experiments. ANOVA analysis was used for comparison.
*$p<0.001$
[a]Significantly different, at p level of 0.05, when compared to control.
[b]Significantly different, at p level of 0.05, compared to 50 μM.

Time-course effects of baicalein on N-acetylation of AF in human leukemia cells. 5x10^5 human leukemia cells per ml in selected media were incubated with 22.5 μM AF in individual wells of 24-well cell culture plate with or without baicalein fo 6, 12, 18, 24 and 48 h of incubation. After incubation, the acetylated AF (AAF) and unacetylated AF were determined by HPLC, as described previously (26, 28).

Detection and measurement of DNA adducts in HL-60 cells. HL-60 cells were incubated with various concentrations (15, 30, 60 and 90 μM) of AF and/or 50 μM baicalein for 24 h and the cells were recovered by centrifugation (28). Then DNA-AF adducts were determined as previously described (28).

Preparation of polyclonal antibody. We immunized 6-week-old female BALB/c mice with the prepared recombinant protein (NAT). Each mouse was initially injected with 0.5 ml of pristane. About 100 μg of antigen, mixed with an equal volume of complete Freund's adjuvant, were applied *s.c.*, for 10-15 days. The antigen was emulsified with incomplete Freund's adjuvant, and injected *i.p.* for 10-20 days and boosted again. The serum-free myeloma cells (1x10^6/ml) in PBS were then injected *i.p.* into the mouse. The ascites fluids, which normally accumulated after 1 week, were collected daily for 5-8 days before the polyclonal antibody (anti-NAT) was used for further experiments (29).

Detection of NAT protein by flow cytometry. The level of intracellular NAT of the HL-60 cells was determined by flow cytometry (Becton Dickinson, FACS Calibur), using the prepared polyclonal antibody mentioned above (27). Cells were co-treated with various concentrations (0, 0.05, 0.5, 5, 50 and 500 μM) of baicalein for 24 h to detect the intracellular NAT. The cells were washed twice, re-suspended in 100 μl of ice-cold 1% formaldehyde for 5 min, and mixed with 100 μl of ice-cold 99% methanol for 30 min. Then the cells were washed three times with 0.1% BSA in PBS and mixed

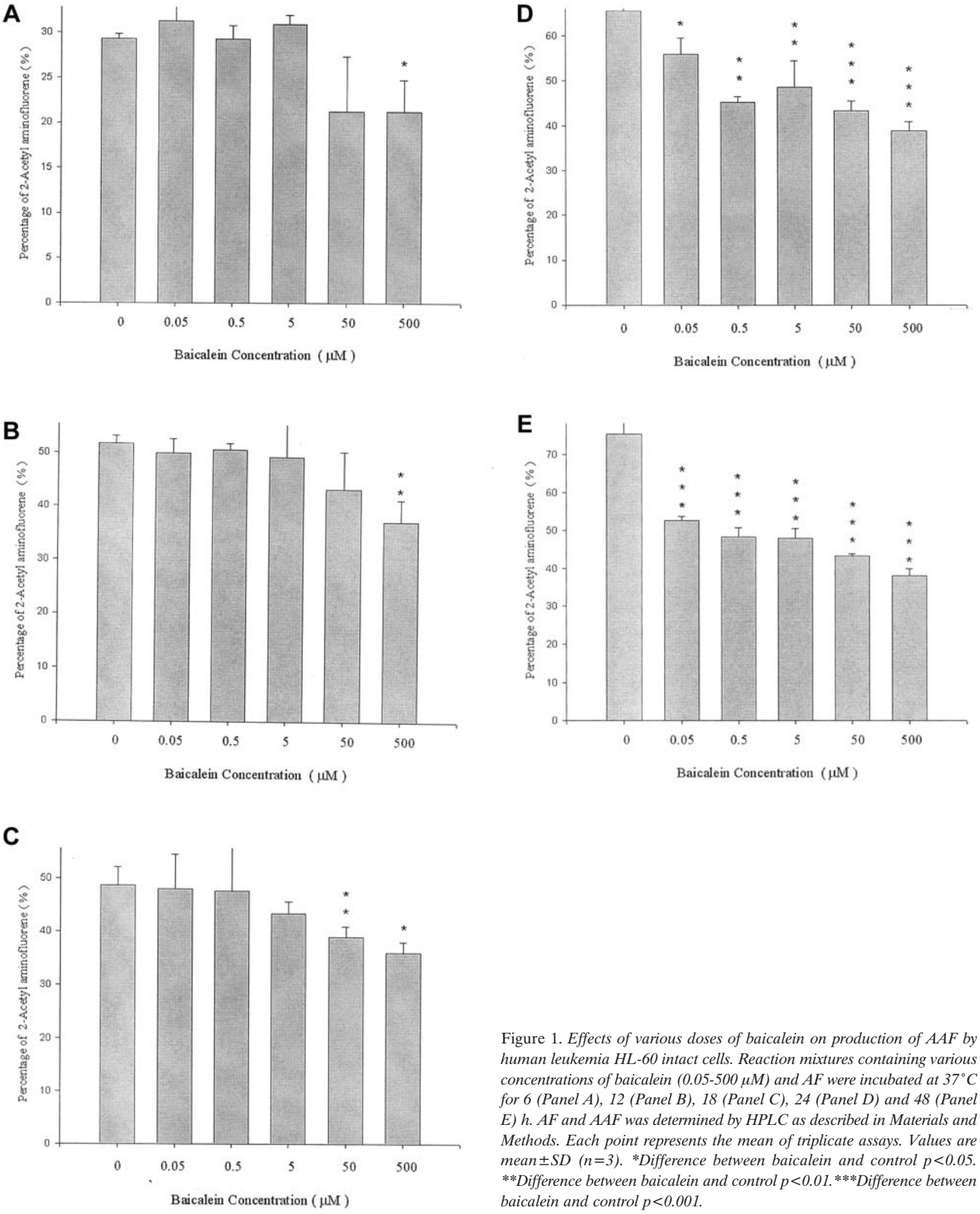


Figure 1. *Effects of various doses of baicalein on production of AAF by human leukemia HL-60 intact cells. Reaction mixtures containing various concentrations of baicalein (0.05-500 μM) and AF were incubated at 37°C for 6 (Panel A), 12 (Panel B), 18 (Panel C), 24 (Panel D) and 48 (Panel E) h. AF and AAF was determined by HPLC as described in Materials and Methods. Each point represents the mean of triplicate assays. Values are mean±SD (n=3). *Difference between baicalein and control p<0.05. **Difference between baicalein and control p<0.01.***Difference between baicalein and control p<0.001.*

Table II. *AF-DNA adduct formation (pmol adduct/mg DNA) following 24-h incubation of human leukemia HL-60 cells with or without 50 μM baicalein.*

	Adducts (pmol/mg DNA)			
Cancer cell	15 μM 2-AF	30 μM 2-AF	60 μM 2-AF	90 μM 2-AF
HL-60 cells	0.21±0.06	0.46±0.10	0.72±0.16	0.94±0.20
HL-60 cells + baicaelin	[a]0.10±0.04	[a]0.22±0.12	[a]0.41±0.11	[a]0.52±0.18

[a]Difference between control and baicaelin cotreatment groups using SAS program for analysis. F value is 12.06. Pr >F is 0.0071.
Values are means±SE of six separate preparations (HL-60 cells, incubation with AF, DNA preparation, postlabelling and HPLC).

Table III. *Percentage of HL-60 cells stained by the NAT antibody after treatment with balcalein.*

Baicalein (μM)	Percentage of cells stained by anti-NAT
0 (control)	50.30±7.89
50	*9.29±2.10

Values are mean±S.D. n=3. The HL-60 cells ($1x10^6$ cells/ml) were co-treated with or without 50 μM baicalein. The zero concentration was defined as control. The percentage of cells stained by NAT antibody, and the stained cells were determined by flow cytometry as described in the Materials and Methods section.
*difference between baicalein and control $p<0.05$.

with 100 μl of 0.1% Triton X-100 in PBS with 0.1% sodium citrate on ice for 45 min. After being washed three times with the same buffer, the cells were incubated with polyclonal antibody at 4˚C for 2.5 h, and then washed three times with 0.1% BSA in PBS. The cells were then stained with FITC-labelled secondary antibody (goat anti-mouse IgG; Jackson ImmunoResearch Laboratories, West Grove, PA, USA) at 4˚C for 35 min. Again, the cells were washed three times, re-suspended in PBS and analyzed by flow cytometry (29).

Reverse transcriptase polymerase chain reaction (RT-PCR). The total RNA was extracted from HL-60 cells by using the Qiagen RNeasy Mini Kit at 24 h following baicalein treatment. The entire protocol had been descibed prevously (30).

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

Results

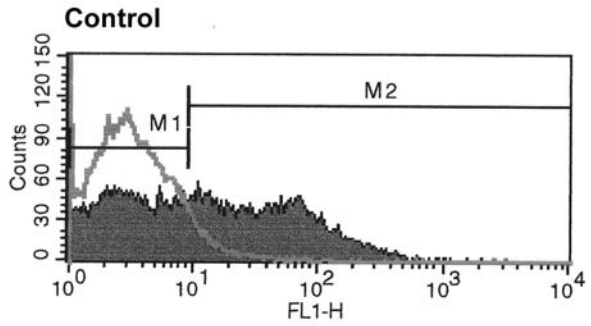
Effects of various concentrations of baicalein on human leukemia HL-60 cell cytosol. The effects of baicalein on N-acetylation of AF in HL-60 cells cytosols were examined by HPLC, assessing the percentage of acetylation of AF. The means±SD values of AF N-acetylation by HL-60 cell cytosol, with or without baicalein, are given in Table I. The data indicated that there was decreased N-acetylation of AF associated with increased baicalein in the examined HL-60 cell cytosol.

Time- and dose- dependent inhibition of AF N-acetylation by baicalein in intact human leukemia HL-60 cells. Human leukemia HL-60 cells were incubated with AF to produce AAF in the culture media, whereas cells without AF and AF without cells did not lead to any detectable AAF in the media in any examined time (Figure 1A, B, C, D and E). However, AAF production was found to increase with incubation time up to 48 h (see Figure 1A-E with no baicalein added). In the presence of 0.05-500 μM baicalein, AF N-acetylation was found to decrease with increasing incubation time and baicalein concentrations.

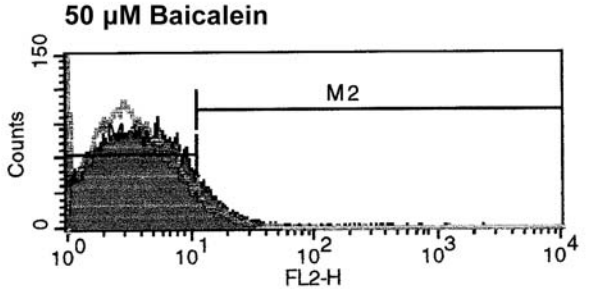
Effects of 50 μM baicalein on DNA-AF adduct formation on human leukemia HL-60 cells. Following 24-h incubation of human leukemia cells with AF, the cells were harvested and DNA was prepared, hydroylzed to nucleotides, and the adducted nucleotides were extracted into butanol and analyzed by HPLC. The results indicated that HL-60 cells activated AF to a metabolite able to bind covalently with DNA (Table II). In the presence of 50 μM baicalein, the AF-DNA adduct formation decreased at various concentrations of AF.

Effects of baicalein on the NAT- Ag-Ab reaction in HL-60 cells. The variations of NAT protein were measured by the NAT antibody. The amount of NAT-Ag-Ab complex was measured by flow cytometry (Figure 2 and Table III). The expression of the NAT-Ag-Ab complex in the cells treated with various concentrations of baicalein led to a dose-dependent decrease in the NAT-Ag-Ab complex in the HL-60 cells.

Dose-dependent effects of baicalein on NAT mRNA expression in intact HL-60 cells examined by polymerase chain reaction (PCR). The extracted RNA was subjected to RT-PCR analysis using specific primers for NAT1, NAT2 and β-actin, and then PCR-amplified cDNA derived from mRNA was applied to agarose gel-electrophoresis. The data presented in Figure 3A show that NAT1 mRNA levels decreased at 50 μM baicalein significantly differently from the control. The mRNA levels of NAT1 and β-actin on gel-electrophoresis were quantified by densitometric analysis of gel-photographs and expressed as NAT1/β-actin (Figure 3B).



Marker	Left, Right	Events	% Gated	% Total	Mean
All	1, 9910	20000	100.00	100.00	32.04
M1	1, 9	9970	49.85	49.85	3.33
M2	9, 9910	10059	50.30	50.30	60.43



Marker	Left, Right	Events	% Gated	% Total	Mean
All	1, 9910	20000	100.00	100.00	5.71
M1	1, 11	18180	90.90	90.90	3.84
M2	11, 9910	1858	9.29	9.29	24.08

Figure 2. *Effects of baicalein treatment on NAT expression in human HL-60 cell line. HL-60 cells (1x10^6/ml) were treated with 0 and 50 μM baicalein for 24 h followed by evaluation of NAT expression. Data were acquired and analyzed using flow cytometry.*

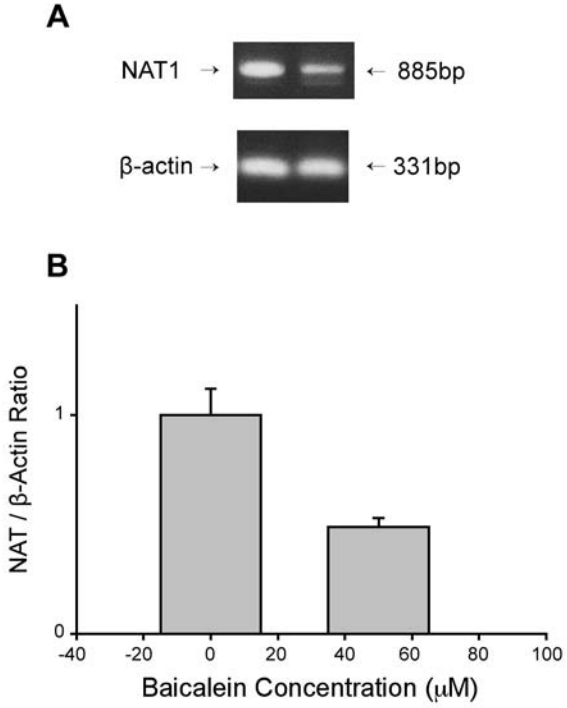


Figure 3. *Effect of baicalein on the expression of NAT mRNA in human HL-60 cells. The cells were incubated with or without 50 μM baicalein for 24 h. The cells were collected to extract RNA. The extracted RNA was subjected to RT-PCR analysis using specific primers for NAT and β-actin, and then PCR-amplified cDNA derived from mRNA was applied to agarose gel-electrophoresis (A). The mRNA levels of NAT and β-actin on the gel-electrophoresis were quantified by densitometric analysis of the gel-photograph and expressed as NAT/β-actin ratio (B).*

Discussion

In the present study, we examined the effects of baicalein on the N-acetylation of AF in human HL-60 leukemia cells including cytosols and intact cells. The results indicated that baicalein affected the N-acetylation of AF in cytosols (Table I) and intact cells (Figure 1A-E) based on two observations. First, the viable cells were decreased after cells were cotreated with baicalein and also this effect was dose-dependent. Second, the amounts of AAF production in cytosols and intact cell cultures were decreased and this effect also was dose-dependent. The decreasing NAT activity may have led to decreased tumor production which is based on two observations: (i) the decreasing of N-acetylation of AF is significant for decreasing tumor development because the attenuation of liver NAT activity is associated with breast and bladder cancer processes (1,31); and (ii) increased levels of NAT activity are associated with increased sensitivity to the mutagenic effects of many arylamines (32). On the other hand, it was shown that NAT enzyme has been involved in the role of some chemical carcinogenesis (33, 34).

The data from the human leukemia HL-60 cytosol and intact cell studies demonstrated that there were significant differences of N-acetylation of AF between the control and baicalein treatment groups based on the *p* values of the Student's *t*-test. Baicalein markedly inhibited the N-acetylation of AF in human leukemia in cytosol and intact cells, and the inhibition was dose-dependent. Our data also demonstrated that baicalein affected NAT1 mRNA gene expression and decreased the level of NAT, which may lead to a decrease of AF-DNA adduct formation. However, the nature of the interaction and the NAT protein domains from examined cells involved in this interaction remain unclear. In other words, the mechanism by which baicalein decreases the

amount of AAF production in HL-60 cells is still unclear. Obviously, further investigations are needed to determine whether this effect is competitive, noncompetitive or uncompetitive inhibition. It had been demonstrated that, after AF in animal cells is N-acetylated by NAT and then metabolized by CYP enzymes to form reactive metabolites, these bind to DNA before forming DNA-AF metabolite adducts, which finally lead to mutation and possibly to cancer development (35). Other important points which need to be considered for the future are based on the observations: (i) the cytochrome P450 enzyme was also reported to be involved in the metabolism of AAF; (ii) cytochrome P450-dependent formation of N-hydroxy-AAF is considered to be the initial rate-limiting step in the metabolism of AAF to mutagenic and potentially carcinogenic products (35); (iii) P4501A1 is particularly efficient for catalyzing the conversion of AAF to 7-OH-AAF (36).

The biological activity of baicalein is considered to be associated in part with its antioxidant potential. Other reports already demonstrated that maintenance of the flavonoid level in the blood might improve the antioxidant status *in vivo*.

The role of arylamine NAT activity in leukemia and tissue cells remains enigmatic. Studies in liver tissues suggest that NAT is involved in the detoxification of exogenous amines (30, 37). However, other investigations found that NAT is also involved in the acetylation of ocular drugs (38).

References

1 Weber WW: The Acetylator Genes and Drug Response, Oxford University Press, NewYork, pp. 21-55, 1987.

2 Dybing E and Huitfeldt MS: Species differences in carcinogen metabolism and interspecies extrapolation. IARC Scientific Publications *116*: 501-522, 1992.

3 Hein DW, Doll MA, Gray KR *et al*: Metabolic activation of N-hydroxy-2-aminofluorene and N-hydroxy-2-acetylaminofluorene by monomorphic N-acetyltransferase (NAT1) and polymorphic N-acetyltransferase (NAT2) in colon cytosols of Syrian hamsters congenic at the NAT2 locus. Cancer Res *53*: 509-514, 1993a.

4 Hein DW, Doll MA, Rustan TD *et al*: Metabolic activation and deactivation of arylamine carcinogens by recombinant human NAT1 and polymorphic NAT2 acetyltransferase. Carcinogenesis *14*: 1633-1638, 1993b.

5 Blum M, Grant DM, McBride OW *et al*: Human arylamine N-acetyltransferase genes: isolation, chromosomal localization, and functional expression. DNA Cell Biol *9*: 193-203, 1990.

6 Ohsako S and Deguchi T: Cloning and expression of cDNAs for polymorphic and monomorphic N-acetyltransferase from human liver. J Biol Chem *265*: 4630-4634, 1990.

7 Lang NP, Chu DZJ, Hunter CF *et al*: Role of aromatic amine acetyltransferase in human colorectal cancer. Arch Surg *121*: 1259-1261, 1986.

8 Iletti KF, David BM, Detchon P *et al*: Acetylator phenotype in colorectal carcinoma. Cancer Res *47*: 1466-1469, 1987.

9 Rieder MJ, Shear NH, Kanee A, Spielberg SP *et al*: Prominence of slow acetylator phenotype among patients with sulfonamide hypersensitivity reactions. Clin Pharmacol Ther *49*: 13-17, 1991.

10 Cartwright RA, Glasham RW, Rogers HJ *et al*: Role of N-acetyltransferase phenotype in bladder carcinogenesis: a pharmacologenetic epidemiological approach to bladder cancer. Lancet *2*: 842-846, 1982.

11 Smith G, Stanley LA, Sim E *et al*: Metabolic polymorphisms and cancer susceptibility. Cancer Surv *25*: 27-65, 1995.

12 Huang KC: Antibacterial, antivirial, and antifungal herbs. *In*: Huang KC (Ed.), The Pharmacology of Chinese Herbs. CRC Press, Boca Raton. pp. 385-386, 1999.

13 Nagi T, Suzuki Y, Tomimori T *et al*: Antiviral activity of plant flavonoid, 5,7,4'-trihydroxy-8-methoxyflavone, from the roots of *Scutellaria baicalenesis* against influenza A (H3N2) and B viruses. Biol Pharm Bull *18*: 295-299, 1995.

14 Kitamura K, Honda M, Yoshizaki H *et al*: Baicalin, an inhibitor of HIV-1 production *in vitro*. Antiviral Res *37*: 131-140, 1998.

15 Gabrieska J, Oszmianski J, Zyka R *et al*: Antioxidant activity of flavones from *Scutellaria baicalensis* in lecitithin liposome. Z. Naturforsch *52*: 817-823, 1997.

16 Yano H, Mizoguchi A, Fukuda K *et al*: The herbal medicine Sho-saiko-to inhibits proliferation of cancer cell lines by inducing apoptosis and arrest at the GO/G1 phase. Cancer Res *54*: 448-454, 1994.

17 Kubo M, Matsuda H, Tani T *et al*: Studies on *Scutellariae radix.* XII. Antithrombic actions of various flavonoids from *Scutellariae radix.* Chem Pharm Bull *33*: 2411-2415. 1985

18 Kimura Y, Yokoi K, Matsushita, N *et al*: Effects of flavonoids isolation from *Scutellariae radix* on the production of tissue-type plasminogen activator and plasminogen activator inhibitor-1 induced by thrombin and thrombin receptor agonist peptide in cultured human umbilical vein endothelial cells. J Pharmacol *49*: 816-822, 1997.

19 Okita K, Li Q, Murakamio T *et al*: Anti-growth effects with components of Sho-sako-to (TJ-9) on cultured human hepatoma cells. Eur J Cancer Pre *2*: 169-175, 1993.

20 Motoo Y and Sawabu N: Antitumor effects of saikosaponins, baicalin and baicalein on human hepatoma cell lines. Cancer Lett *86*: 91-95, 1994.

21 Matsuzaki Y, Kurokawa N, Terai S *et al*: Cell death induced by baicalein in human hepatocellular carcinoma cell lines. Jpn J Cancer Res *87*: 170-177, 1996.

22 Fukutake M, Yokota S, Kawamura H *et al*: Inhibitory effect of *Coptidis rzhizoma* and *Scutellariae radix* on azoxymethane-induced aberrant crypt foci formation in rat colon. Biol Pharm Bull *21*: 814-817, 1998.

23 Kato M, Liu W, Asai HYN *et al*: The herbal medicine Sho-saki-to inhibits growth and metastasis of malignant meloanoma primarily developed in ret-transgenic mice. J Invest Dermatol *111*: 640-644, 1998.

24 Kayano K, Sakaida I, Uchida K *et al*: Inhibitory effects of the herbal medicine Sho-saiko-to (TJ-9) on cell proliferation and procollagen gene expressions in cultured rat hepatic stellate cells. J Hepatol *29*: 642-649, 1998.

25 Li YC, Hung CF, Yeh FT *et al*: Luteolin-inhibited arylamine N-acetyltransferase activity and DNA-2-aminofluorene adduct in human and mouse leukemia cells. Food Chem Toxicol *39*: 641-7, 2001.

26 Chung JG, Chang HL, Lin WC *et al*: Inhibitory of N-acetyltransferase activity and DNA-2-aminofluorene adducts by glycyrrhizic acid in human colon tumour cells. Food Chem Toxicol *38*: 163-172, 2000.
27 Bradford MM: A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding Anal Biochem *72*: 248-254, 1976.
28 Chung JG: Effects of butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) on the acetylation of 2-aminofluorene and DNA-2-aminofluorene adducts in the rat. Toxicol Sci *51*: 202-210, 1999.
29 Chung JG, Yeh KT, Wu SL *et al*: Novel transmembrane GTPase of non-small cell lung cancer identified by mRNA differential display. Cancer Res *61*: 8873-8879, 2001.
30 Yang CC, Chen GW, Lu HF *et al*: Paclitaxel (Taxol) inhibits the arylamine N-acetyltransferase activity and gene expression (mRNA NAT1) and 2-aminofluorene-DNA adduct formation in human bladder carcionoma cells (T24 and TSGH 8301). Pharmacol Toxicol *92*: 287-294, 2003.
31 Weber WW and Hein DW: N-acetylation pharmacogenetics. Pharmacol Rev *37*: 25- 99, 1985.
32 Einisto P, Watanabe M, Ishidate M *et al*: Mutagenicity of 30 chemicals in *S. typhimurium* possessing different nitroreductase or O-acetyltransferase activities. Mutation Res *259*: 95-102, 1991.
33 Grant DM, Blum M, Beer M *et al*: Monomorphic and polymorphic human arylamine N-acetyltransferase: a comparison of liver isozymes and expressed products of two cloned genes. Mol Pharmacol *39*: 184-191, 1991.
34 Minchin RF, Reeves PT, Teitel CH *et al*: N-and 0-acetylation of aromatic and heterocyclic amine carcinogens by human monomorphic and polymorphic acetyltransferase expressed in COS-1 cells. Biochem Biophys Res Commun *185*: 839-844, 1992.
35 Ferriola PC, Cody V, Middleton E Jr: Protein kinase C inhibition by plant flavonoids. Kinetic mechanisms and structure-activity relationships. Biochem Pharmacol *15*: 1617-1624, 1989.
36 Hayes MA, Roberts E, Safe SH *et al*: Influences of different polychlorinated biphenyls on cytocidal, mitoinhibitory, and nodule-selecting activities of N-2-fluorenylacetamide in rat liver. JNCI *76*: 683-690, 1986.
37 Evans DAP: N-acetyltransferase. Pharmacol Ther *42*: 157-234, 1989.
38 Campbell DA, Schoenwald RD, Duffel MW *et al*: Characterization of arylamine acetyltransferase in the rabbit eye. Invest Opththamol Vis Sci *32*: 2190-2200, 1991.

Received April 16, 2004
Revised December 27, 2004
Accepted January 12, 2005