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Berberine inhibits arylamine *N*-acetyltransferase activity and gene expression in mouse leukemia L 1210 cells

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Abstract

N-acetyltransferases (NATs) are recognized to play a key role in the primary step of arylamine compounds metabolism. Polymorphic NAT is coded for rapid or slow acetylators, which are being thought to involve cancer risk related to environmental exposure. Berberine has been shown to induce apoptosis and affect NAT activity in human leukemia cells. The purpose of this study is to examine whether or not berberine could affect arylamine NAT activity and gene expression (NAT mRNA) and the levels of NAT protein in mouse leukemia cells (L 1210). *N*-acetylated and non-*N*-acetylated AF were determined and quantited by using high performance liquid chromatography. NAT mRNA was determined and quantited by using RT-PCR. The levels of NAT protein were examined by western blotting and determined by using flow cytometry. Berberine displayed a dose-dependent inhibition to cytosolic NAT activity and intact mice leukemia cells. Time-course experiments indicated that *N*-acetylation of AF measured from intact mice leukemia cells were inhibited by berberine for up to 24 h. The NAT1 mRNA and NAT proteins in mouse leukemia cells were also inhibited by berberine. This report is the first demonstration, which showed berberine affect mice leukemia cells NAT activity, gene expression (NAT1 mRNA) and levels of NAT protein.
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Keywords: *N*-acetyltransferase; Berberine; Mouse leukemia cells; Arylamines (*N*-acetyl-2-aminofluorene and 2-aminofluorene)

Introduction

The *N*-acetyltransferases (NATs) play an important role in the metabolism of arylamine chemicals and carcinogens that catalyze both detoxifying *N*-acetylation and *O*-acetylation that generate intermediate metabolites in electrophiles which then bind to DNA and form DNA adduct formation that finally generates cancer in specific target organs or tissues (Hein, 1988; Fretland et al., 2002). *N*-acetyltransferase-1 (NAT1) and *N*-acetyltransferase-2 (NAT2) are encoded by NAT1 and NAT2 in human chromosome 8 (Blum et al., 1990). The genetic determined the variants of both enzymes of individuals which then lead to the specific NAT activity for rapid or slow acetylation

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(Weber and Hein, 1985; Evans, 1989; Weber, 1987). Based on the NAT activity (*N*-acetylation of substrate) the individuals are classified into rapid and slow acetylators. From the epidemiological statistic analysis, it was demonstrated that slow acetylators have an increased bladder cancer risk after exposed to smoking-derived or occupational carcinogens (Risch et al., 1995; Brockmoller et al., 1996; Cartwright, 1982) but rapid acetylator with high intake of cooked meat containing carcinogenic heterocyclic amines could be a potential risk factor for colorectal cancer (Lang, 1986; Roberts-Thomson et al., 1996; Chen et al., 1998). Lenkemia usually occurs in animals and children. Usually, mutagen by oral administration was absorbed from the intestinal system, and then passed through the circulation system to the whole body. The *in vivo* studies of our research colleagues had been evaluated, and these mutagen and intermediates were deposited in the blood and had been accumulated in other tissues for a long time. Recently, comprehensive reviews on the molecular genetic analysis and epidemiology of the both NATs acetylation polymorphisms have been reported (Brockton et al., 2000; Cascorbi and Roots, 1999; Hein et al., 2000).

Berberine (5,6-Dihydro-9,10-dimethoxybenzo[g]-1,3-benzodioxolo[5,6-a]quinolizinium), a benzodioxoloquinolozine alkaloid present in the plant genera *Berberis* and *Coptis*, and also in many other plants, has been widely used to treat gastroenteritis and diarrhea patients in the Chinese population for a long time (Tang and Eisenbrand, 1992). Berberine acts as an antimicrobial (Amin et al., 1969), antidiarrhea (Tai et al., 1981; Yamamoto et al., 1993), and antineoplastic agent (Hoshi et al., 1976; Zhang et al., 1990) and it also has a high binding affinity for mast cells (Berlin and Enerback, 1983) and influenced mast cell-mediated chloride secretion in rat colons (Taylor and Baird, 1993). Recently, it was also demonstrated that berberine could be used as an anti-inflammatory agent which may arise in part from the inhibition of DNA-synthesis in human activated peripheral lymphocytes (Weber and Hein, 1985; Ckless et al. 1995). In our laboratory, we also found out that berberine affected NAT activity of human colon (Chung et al., 1999) and bladder (Lin et al., 1999a, b) tumor cells. However, there is no available information to address berberine effects on NAT activity and gene expression in the mouse leukemia cells. Thus, the initial studies were focused on the effects of berberine on the NAT activity and gene expression of a mouse leukemia L 1210 cell line.

Materials and methods

Chemicals and reagents

Berberine, ethylenediaminetetraacetic acid (EDTA), 2-aminofluorene (AF), *N*-acetyl-2-aminofluorene (AAF), dithiothreitol (DTT), Tris, acetylcarnitine, leupeptin, bovine serum albumin (BSA), phenylmethylsulfonylfluoride (PMSF), dimethyl sulfoxide (DMSO), acetyl-coenzyme A (Acetyl-CoA) and carnitine acetyltransferase were obtained from Sigma Chemical Co. (St. Louis, MO). All of the chemicals used were reagent grade.

Mouse leukemia cell line

Mouse lymphocytic leukemia cell line (L 1210) was obtained from the Food Industry Research and Development Institute (Hsinchu, Taiwan). The cells (1×10^6) were placed into 75 cm^3 tissue culture flasks and grown in Dulbecco's modified Eagle's Medium (DMEM) (Gibco BRL, Grand Island, NY) supplemented with 10% horse serum (Gibco BRL, Grand Island, NY), 2% penicillin-streptomycin (10 U/ml penicillin and 10 mg/ml streptomycin) in the incubator for 37 °C, 10% CO_2.

Preparation of mouse leukemia cell cytosols

About 5×10^7 cells were placed in 2 ml of the lysis buffer (20 mM Tris/HCl, pH 7.5, 1 mM DTT, 1 mM EDTA, 50 μM PMSF, and 10 μM leupeptin) previously described (Chung et al., 2000). The suspensions were centrifuged at 9000*g* for 1 min at 4 °C in a model 3200 Eppendorf/Brinkman centrifuge, and the supernatant fraction was subsequently centrifuged at 10,000*g* for 60 min 4 °C. The supernatant was kept on ice for NAT activity and protein determinations.

NAT activity determinations

The determination of Acetyl-CoA-dependent *N*-acetylation of AF was performed as previously described by Chung et al. (2000).

Determination of the levels of protein

Protein concentrations in the mouse leukemia cell cytosols were determined by the method of Bradford with BSA as the standard (Bradford, 1976). All of the samples were assayed in triplicate.

Intact cell NAT activity determination

Mouse leukemia cells (in 1 ml DMEM media with glutamine and 10% fetal calf serum) were incubated with arylamine substrate (AF) at 1×10^6 cells/ml in individual wells of a 24-well cell culture plate with or without various concentrations of berberine co-treatment for 24 h at 37 °C in 95% air 5% CO_2. At the conclusion of incubation, the cells and media were removed and centrifuged. The supernatant was immediately extracted with ethyl acetate/methanol (95:5), the

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