The effect of α-solanine on the activity, gene expression, and kinetics of arylamine N-acetyltransferase in HepG2 cells

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Abstract. α-solanine is one of the major components of *Solanum nigrum* Linn., the fruits of which are used in China for food. In the present study, α-solanine was selected to assess the inhibition of arylamine N-acetyltransferase (NAT) activity and mRNA expression of NAT and kinetics in HepG2 cells. NAT activity was examined by HPLC. The double-reciprocal plot was contrived to yield a regression equation to calculate K_m and V_{max}. NAT mRNA expression was determined by PCR. The results revealed that α-solanine could significantly decrease NAT activity in intact HepG2 cells or the cytoplasm. K_m did not differ either for intact HepG2 cells or for the cytoplasm, however V_{max} was significantly different. α-solanine could decrease the expression of NAT1 mRNA and NAT2 mRNA. In summary, α-solanine was a non-competitive inhibitor of NAT in HepG2 cells. It decreased NAT activity through non-competitive inhibition of NAT activity and by decreasing the expression of NAT1 mRNA and NAT2 mRNA.

Introduction

Epidemiological studies have revealed that 70% of tumors in humans result from environmental hazards and that among the many environmental hazards, 70-90% are chemical carcinogens, of which arylamines are an important type. Most arylamines are not active carcinogens themselves, but are transformed into carcinogens in the human body by relevant enzymes such as arylamine N-acetyltransferases (NATs). Furthermore, NATs represent the first and the rate-limiting step in the reactions leading to the activation of arylamines. Arylamine N-acetyltransferasesv (NATs) are phase II metabolizing enzymes that can transfer the acetyl group from AcCoA onto arylamines, activating arylamines into carcinogens. Thus, the activity of NATs in the human body is closely associated with the susceptibility to tumors. Tiang *et al* revealed that NAT1 is a novel target for breast cancer treatment (1), and concomitantly we reported that the NAT enzyme is a new inhibitor of apoptosis (2). Sugamori *et al* revealed that NAT deficiency reduces the risk for ABP-induced liver tumors (3). To date, studies of active ingredients on NAT activity have been focused on lung (4), colon (5-7), prostate cancer (8), and mouse leukemia (9), while relatively little research has been performed on the relationship between the occurrence of hepatocarcinoma and NATs. Furthermore, researchers reported that aqueous extract of *Solanum nigrum* L. affected the NAT enzyme activity and gene expression in human gastric and colon cancer (10,11). α-solanine, as displayed in Fig. 1 which reveals its molecular structure, is one of the chemical components in *Solanum nigrum* L. (12,13). The question arised as to whether α-solanine could affect NAT enzyme activity. We previously revealed that α-solanine had a cytotoxic effect on HepG2 cells (14), therefore it was speculated that α-solanine could affect the NAT activity in HepG2 cells and may be a target of proliferation inhibition. Subsequently, in the present study, we observed the effect of α-solanine on the activity, gene expression, and kinetics of arylamine N-acetyltransferase in HepG2 cells.

Materials and methods

Cell line and cell culture. The human liver cancer HepG2 cell line was obtained from the Institute of Cancer Prevention and Control of Harbin Medical University (Harbin, Heilongjiang, China). The cells were grown in RPMI-1640 medium containing 10% fetal bovine serum at 37˚C in a humidified atmosphere containing 5% CO_2. Cells were harvested by

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Key words: α-solanine, arylamine N-acetyltransferase, human liver cancer HepG2 cells, gene expression, enzyme kinetics

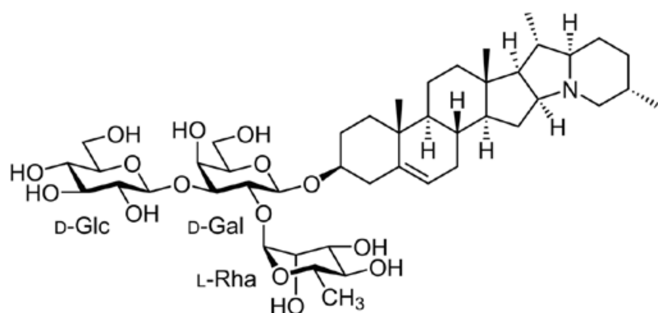

Figure 1. Molecular structure of α-solanine.

trypsinization with 0.25% trypsin solution and suspended in culture medium before use.

Chemicals and reagents. α-solanine, 2-aminofluorene (2-AF), acetylcarnitine, carnitine acetyltransferase, 2-acetylaminofluorene (2-AAF), Tris, DTT, leupeptin, AcCoA, BSA, DEPC, DTNB and agarose were obtained from Sigma-Aldrich (St. Louis, MO, USA). PMSF and EB were obtained from Amresco, LLC (Solon, OH, USA). RPMI-1640, trypsin and TRIzol were obtained from Thermo Fisher Scientific, Inc. (Waltham, MA, USA). Fetal bovine serum was purchased from GE Healthcare Life Sciences (Pittsburgh, PA, USA). The NAT primer was obtained from Sangon Biotech Co., Ltd. (Shanghai, China) and the RT-PCR kit was obtained from Takara Bio, Inc. (Kusatsu, Shiga, Japan).

Preparation of HepG2 cell lysates. HepG2 cells ($1x10^7$) were placed in 2 ml of lysis buffer [20 mM Tris-HCl (pH 7.5), 1 mM DTT, 1 mM EDTA, 50 μM PMSF and 10 μm leupeptin] and ultrasonicated for 20 min at 4˚C. The suspension was centrifuged for 1 min at 9,000 x g and then the supernatant was centrifuged for 60 min at 10,000 x g. Subsequently, the supernatant was kept on ice for NAT activity. The Bradford assay was used for protein determination.

NAT activity determination by HPLC. NAT activity was calculated by total amounts of acetylated 2-AF (2-AAF), which were determined by HPLC (Waters Corp., Milford, MA, USA). The HPLC conditions were: a Symmetry Shield RP C_{18} column (4.6 mm x 250 mm, 5 μm, 100 Å), a mobile phase consisting of 20 mM KH_2PO_4 (pH 4.5):CH_3CN = 53:47, a 2487 UV detector (wavelength 288 nm) and a column temperature of 25±5˚C. The peak area was automatically integrated using the Empower workstation software. The standard curve was plotted using GraphPad Prism 5 software, with the concentration of the standard sample 2-AAF and the peak area as the horizontal and the vertical axis, respectively. A regression equation for the standard curve was calculated using GraphPad Prism 5 software.

α-solanine affects NAT activity in HepG2 intact cells. HepG2 cells at the logarithmic growth phase were incubated with 45 μM of 2-AF as the substrate and different concentrations of α-solanine (0, 0.016, 0.08, 0.4, 2 and 10 μg/ml) for 24 h, or 2 μg/ml α-solanine at different treatment time-points (12, 24, 36 and 48 h). Then, the culture medium was centrifuged for 10 min at 3,500 x g, and the supernatants were immediately extracted with equal volumes of ethyl acetate:methanol (95:5). The supernatants were then evaporated until the samples were dry, after which the residue was dissolved in 2 ml of methanol. The amounts of the 2-AAF in the samples were assessed using HPLC with an automatic sample injection of 20 μl. The retention time was about 13.5 min for 2-AAF and about 17 min for 2-AF. NAT activity was examined through HPLC analysis of the yield of 2-AAF and expressed as nmol acetylated substrate/10^6 cells.

α-solanine affects NAT acivity in HepG2 lysates. The amount of 2-AAF (2-AF acetylated by AcCoA-dependent NAT) was used as a measurement for NAT activity in HepG2 lysates. The total volume of the reaction mixture was 550 μl, including 250 μl of the cytoplasm solution and 100 μl of the cycling mixture [50 mM Tris-HCl (pH 7.5), 0.2 mM EDTA, 2 mM DTT, 15 mM acetylcarnitine, and 2 U/ml carnitine acetyltransferase]. A suitable amount of 2-AF and α-solanine were added to reach a final concentration of 0.04 mM for 2-AF, and 0.016, 0.08, 0.4, 2 and 10 μg/ml, respectively, for α-solanine. The reaction was started by adding AcCoA (0.45 mM, final concentration). Distilled water (200 μl) was added instead of AcCoA in the blank control reactions. The mixtures were incubated for 6 h at 37˚C and 900 μl of acetonitrile was added to terminate the reaction. Then the mixtures were filtered and the NAT activity was determined as aforementioned.

α-solanine affects the kinetic constants of NAT in intact HepG2 cells or lysates. For the intact cells, HepG2 cells co-treated with or without 2 μM α-solanine and different concentrations of AF (5.625, 11.25, 22.5, 45 and 90 μM) were used to assess NAT activity as aforementioned. For the cytoplasm groups, cell lysates co-treated with or without 2 μM α-solanine and different concentrations of AF (20.45, 40.90, 81.80, 163.60 and 327.20 μM) were used to assess NAT activity as aforementioned. The data was used to draw the Lineweaver-Burk's double-reciprocal plot and calculate the Michaelis-Menten constant (K_m) and maximum reaction rate (V_{max}) according to

$$V_0 = \frac{V_{max}[S]}{K_m + [S]} \qquad \frac{1}{V_0} = \frac{K_m + [S]}{V_{max}[S]} = \frac{K_m}{V_{max}}\frac{1}{[S]} + \frac{1}{V_{max}}$$

the following formula:

The S is the concentration of the substrate (2-AF), the V_0 is the reaction rate, which is associated with the amount of 2-AAF produced.

α-solanine affects the gene expression of NAT1 and NAT2. Total RNA was extracted from HepG2 cells 24 h after treatment with different concentrations (0.016, 0.08, 0.4, 2 and 10 μg/ml) of α-solanine. TRIzol (1 ml) was added after the cells were rinsed with DEPC (diethyl pyrocarbonate)-treated water and after 30 min, the suspension was placed in 1.5-ml Eppendorf tubes. Chloroform (0.2 ml) was added, and the tubes were vigorously shaken for 15 sec to allow thorough mixing. The aqueous phase was then placed in other Eppendorf tubes and the same volume of isopropanol was added before the samples were centrifuged for 15 min at 12,000 x g. The supernatant was discarded, and the precipitation was the total RNA. The subsequent procedures

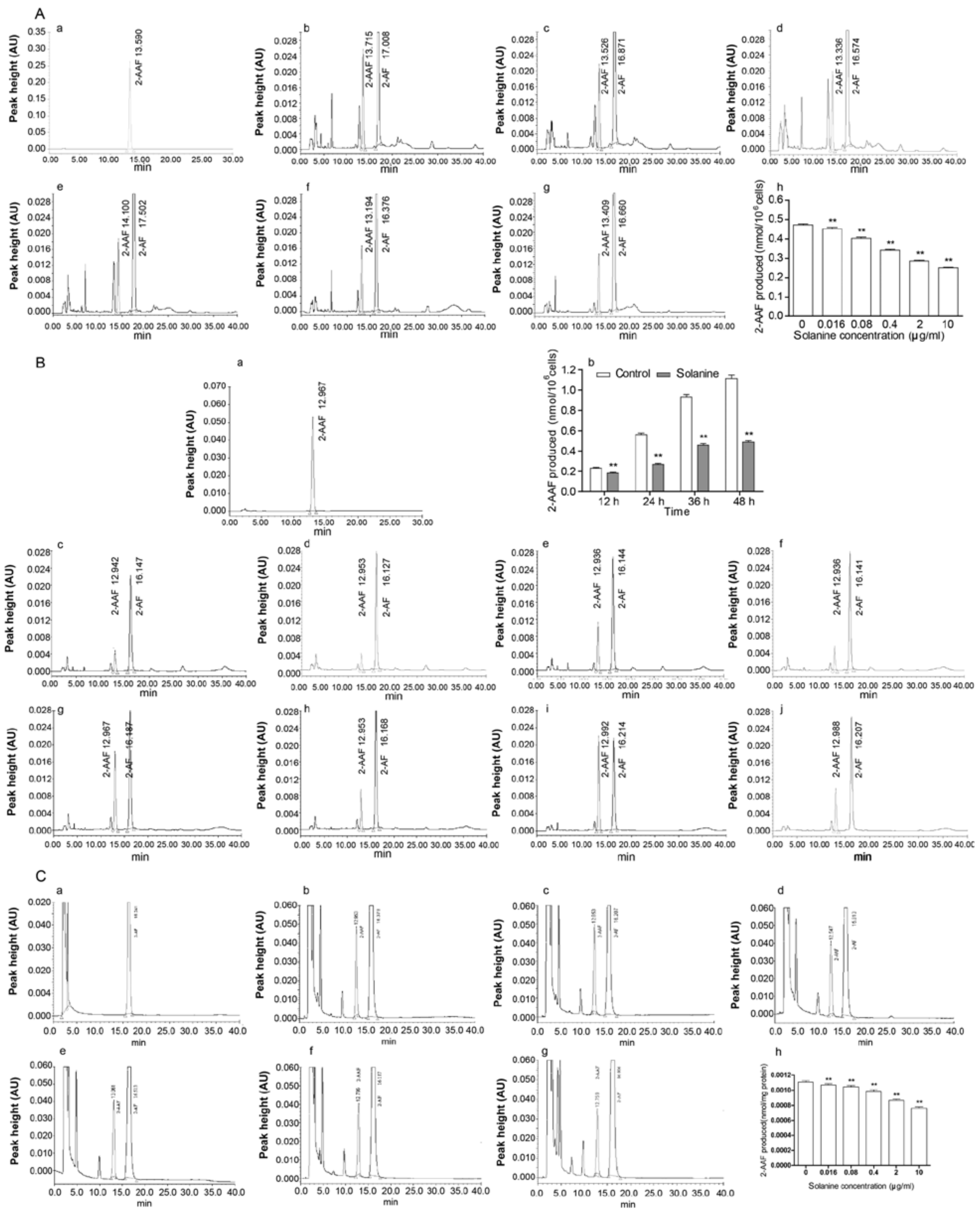

Figure 2. Effect of α-solanine on NAT activity in HepG2 cells. The amounts of 2-AAF (the acetylated AF) were used to represent NAT activity. (A) The effect of α-solanine at various concentrations on NAT activity in intact HepG2 cells. (a) 2-AAF standard substance, (b) control, (c) 0.016 μg/ml α-solanine, (d) 0.08 μg/ml α-solanine, (e) 0.4 μg/ml α-solanine, (f) 2 μg/ml α-solanine, (g) 10 μg/ml α-solanine and (h) histogram of 2-AAF produced. (B) The effect of α-solanine at various treatment times on NAT activity in intact HepG2 cells. (a) 2-AAF standard substance, (b) histogram of 2-AAF produced. (c) 12-h control, (d) 12 h of α-solanine, (e) 24-h control, (f) 24 h of α-solanine, (g) 36-h control, (h) 36 h of α-solanine, (i) 48-h control, (j) 48 h of α-solanine. (C) The effect of α-solanine on NAT activity in the cytoplasm of HepG2 cells. (a) blank control, (b) negative control, (c) 0.0032 μg/ml α-solanine, (d) 0.016 μg/ml α-solanine, (e) 0.08 μg/ml α-solanine, (f) 0.4 μg/ml α-solanine, (g) 2.0 μg/ml α-solanine and (h) histogram of 2-AAF produced.

for conducting reverse transcription and PCR were exactly the same as those in the instruction manual [Takara RNA PCR kit (AMV) Ver.3.0; Takara Bio, Otsu, Japan]. Each amplification for NAT1 was performed for 30 cycles, one cycle profile consisted of denaturation at 94˚C for 30 sec, annealing at 58˚C for 30 sec and extension at 72˚C for

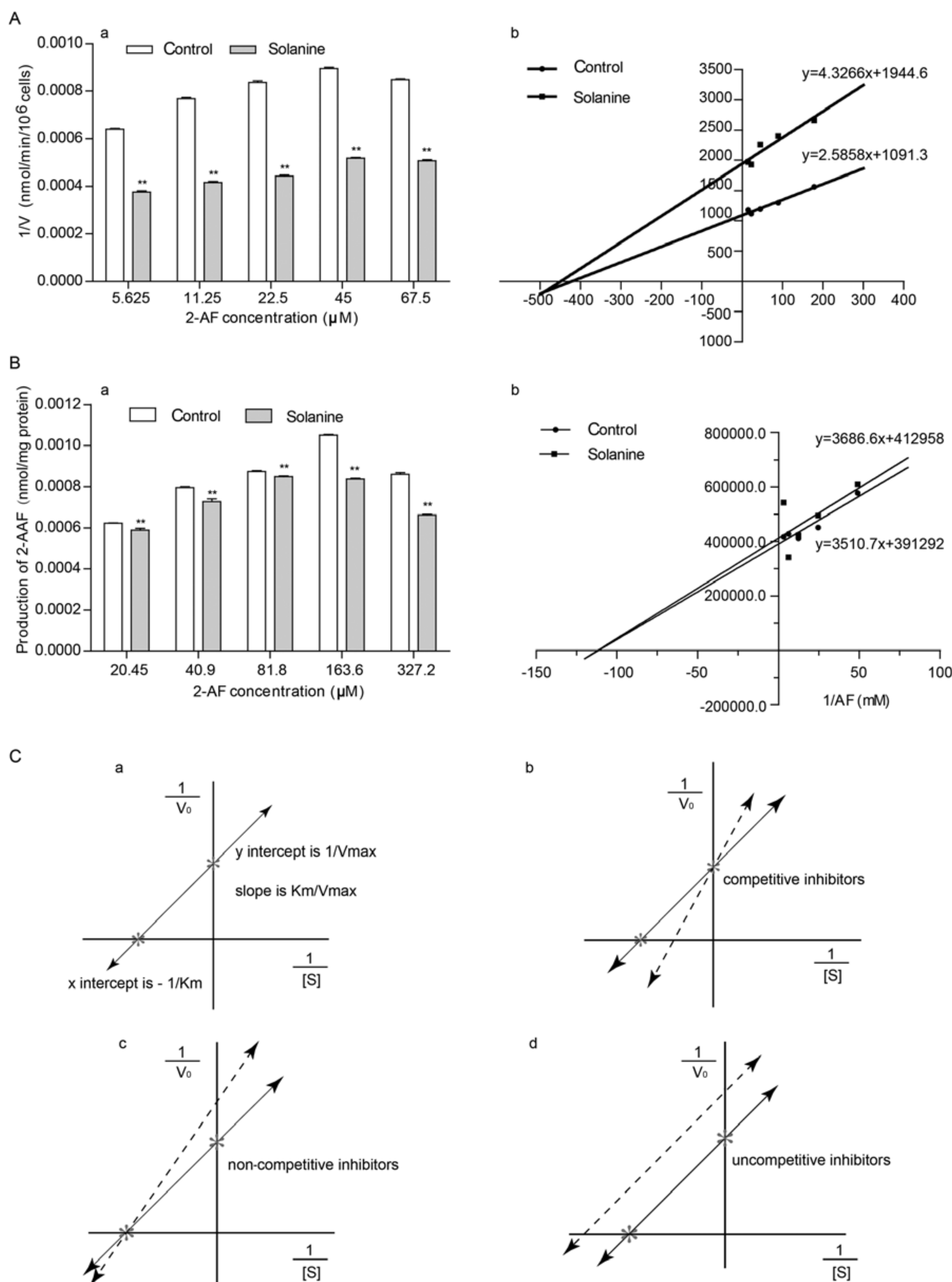

Figure 3. Effect of α-solanine on the kinetic constants of NAT in HepG2 cells. (A) Intact HepG2 cells. (a) Effect of α-solanine on the production of 2-AAF at various substrate (2-AF) concentrations in intact HepG2 cells. (b) Lineweaver-Burk's double-reciprocal plot of NAT activity in HepG2 intact cells. (B) The cytoplasm of HepG2 cells. (a) Effect of α-solanine on the production of 2-AAF at various substrate (2-AF) concentrations in the cytoplasm of HepG2 cells. (b) Lineweaver-Burk's double-reciprocal plot of NAT activity in the cytoplasm of HepG2 cells. (C) Lineweaver-Burk's double reciprocal plot of enzyme inhibitor. (a) Lineweaver-Burk's double-reciprocal plot. (b) Double-reciprocal plot of competitive inhibitors. (c) Double-reciprocal plot of non-competitive inhibitors. (d) Double-reciprocal plot of uncompetitive inhibitors.

80 sec. For NAT2 each amplification was performed for 26 cycles, one cycle profile consisted of denaturation at 94˚C for 30 sec, annealing at 58˚C for 30 sec and extension at 72˚C for 60 sec. PCR products were visualized by eletrophoresis using 1.5% agarose gels and quantified with Tianneng GIS gel analyzing software (Tianneng, Shanghai, China). Parallel reactions were run using human-actin as a control for RT-PCR.

Table I. The effect of α-solanine on the production of 2-AAF in intact HepG2 cells at different substrate (2-AF) concentrations.

	2-AAF peak areas (AU)		Production of 2-AAF (nmol/10^6 cells)	
2-AF (μM)	Control	α-solanine	Control	α-solanine
5.625	818641.00±1098.50	479033.33±1635.47	$6.40x10^{-4}$±$3.97x10^{-6}$	$3.76x10^{-4}$±$4.39x10^{-6b}$
11.25	984393.33±2024.22	531009.33±2731.91	$7.68x10^{-4}$±$4.69x10^{-6}$	$4.16x10^{-4}$±$5.24x10^{-6b}$
22.5	1071720.00±4632.22	565358.67±1996.34	$8.36x10^{-4}$±$6.72x10^{-6}$	$4.43x10^{-4}$±$4.67x10^{-6b}$
45	1147756.67±3051.63	661883.33±701.21	$8.95x10^{-4}$±$5.49x10^{-6}$	$5.18x10^{-4}$±$3.66x10^{-6b}$
67.5	1086149.00±13513.75	648047.33±8991.60	$8.48x10^{-4}$±$1.32x10^{-6}$	$5.07x10^{-4}$±$1.01x10^{-6b}$

[b]P<0.01 vs. the control.

Table II. The effect of α-solanine on the yield of 2-AAF in the cytoplasm of HepG2 cells at different substrate concentrations.

	2-AAF peak areas (AU)		Production of 2-AAF (nmol/mg protein)	
2-AF (μM)	Control	α-solanine	Control	α-solanine
20.45	64401±116	60684±957	$6.23x10^{-4}$±$1.06x10^{-6}$	$5.89x10^{-4}$±$8.71x10^{-6b}$
40.90	83284±484	75797±1413	$7.95x10^{-4}$±$4.41x10^{-6}$	$7.27x10^{-4}$±$1.29x10^{-5b}$
81.80	91959±56	89241±306	$8.74x10^{-4}$±$5.10x10^{-7}$	$8.49x10^{-4}$±$2.78x10^{-6b}$
163.60	111603±231	87926±447	$1.05x10^{-3}$±$2.10x10^{-6}$	$8.37x10^{-4}$±$4.07x10^{-6b}$
327.20	90585±828	68741±1274	$8.61x10^{-4}$±$7.54x10^{-6}$	$6.62x10^{-4}$±$1.16x10^{-6b}$

[b]P<0.01 vs. the control.

The sequence of the primers was as follows: B-MDIEA-NAT1, 5'-CACCCGGATCCGGGATCATGGACATTGAAGC-3', nt 435-454, GenBank accession no. X17059; VPKHGD-X-NAT1, 5'-GGTCCTCGAGTCAATCACCATGTTTGGGCAC-3', nt 1295-1278, GenBank accession no. X17059; FP1-NAT2, 5'-CTAGTTCCTGGTTGCTGGCC-3', nt 79-98, GenBank accession no. NM-000015; RP1-NAT2, 5'-TAACGTGAGGGTAGAGAGGA-3', nt 1073-1054, GenBank accession no. NM-000015; Act b1, 5'-GCTCGTCGTCGACAACGGCTC-3', nt 94-114, GenBank accession no. NM-001101; Act2 b2, 5'-CAAACATGATCTGGGTCATCTTCTC-3' and nt 446-422, GenBank accession no. NM-001101 (15).

Statistical analysis. The data are presented as the mean ± SD. Statistical analysis of group differences was performed using Student's t-test. A value of P<0.05 was considered to be statistically significant.

Results

α-solanine affects NAT activity in HepG2 intact cells. The effects of α-solanine on NAT activity in HepG2 intact cells were observed by HPLC. The amount of 2-AAF transformed from 2-AF was used to assess NAT activity. Suspensions of HepG2 cells treated with 45 μM of 2-AF and with various concentrations (0.016, 0.08, 0.4, 2 and 10 μg/ml) of α-solanine exhibited a decreased amount of 2-AAF produced when compared to the control (not treated with α-solanine) (Fig. 2A). The amount of 2-AAF produced gradually increased with the increase in treatment time (12, 24, 36 and 48 h). However, the amount of 2-AAF produced was significantly lower for α-solanine than for the control (Fig. 2B). The data indicated that α-solanine induced a dose- and time-dependent reduction of NAT activity in intact HepG2 cells.

α-solanine affects NAT acivity in HepG2 lysates. The effects of α-solanine on NAT activity in the lysates of HepG2 cells were observed by HPLC. The amount of 2-AAF transformed from 2-AF was used to assess the NAT activity. Lysates of HepG2 cells treated with a suitable amount of 2-AF and with various concentrations of α-solanine (0.016, 0.08, 0.4, 2 and 10 μg/ml) exhibited decreased amounts of 2-AAF produced than the control (not treated with α-solanine) (Fig. 2C). The results indicated that α-solanine induced dose-dependent reduction of NAT activity in the lysates of HepG2 cells.

α-solanine affects the kinetic constants of NAT in intact HepG2 cells or lysates. The effect of α-solanine on the production of 2-AAF in intact HepG2 cells or lysates with various substrate (2-AF) concentrations (5.625, 11.25, 22.5, 45 and 67.5 μM in intact HepG2 cells, and 20.45, 40.90, 81.80, 163.60 and 327.20 μM for the cytoplasm of HepG2 cells) was examined by HPLC. The results revealed the same pattern for both intact cells and the cytoplasm, namely, the amount of 2-AAF produced and the concentration of the substrate 2-AF gradually increased within a certain range and that with the same substrate concentration, α-solanine could inhibit the formation of 2-AAF (Fig. 3A-a and B-a;

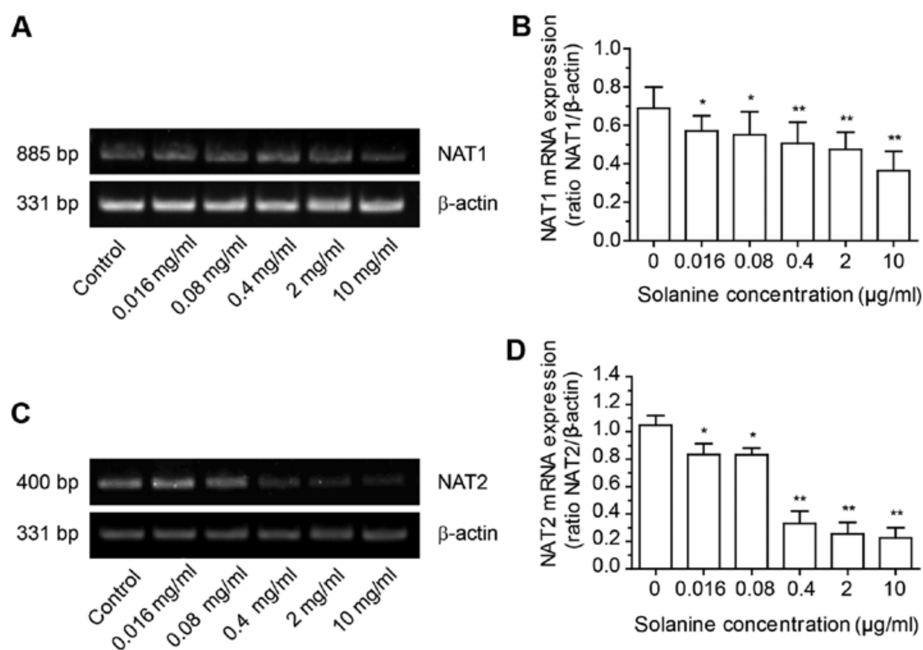

Figure 4. Effect of α-solanine on the expression of NAT mRNA. (A) The effect of α-solanine on the expression of NAT1 mRNA. (B) Histogram of the expression of NAT1 mRNA. (C) The effect of α-solanine on the expression of NAT2 mRNA. (D) Histogram of expression of NAT2 mRNA.

Table III. Kinetic data for acetylation of 2-aminofluorene (2-AF) in HepG2 cells.

	In intact cells		In the cytosol	
Groups	K_m (mM)	V_{max} (nmol/10^6 cells)	K_m (mM)	V_{max} (nmol/min/mg protein)
Control	$2.37x10^{-3}±8.37x10^{-5}$	$9.16x10^{-4}±7.54x10^{-5}$	$8.95x10^{-3}±2.61x10^{-4}$	$2.55x10^{-6}±1.92x10^{-8}$
α-solanine	$2.22x10^{-3}±9.05x10^{-5}$	$5.14x10^{-4}±3.72x10^{-5b}$	$9.48x10^{-3}±3.63x10^{-4}$	$2.43x10^{-6}±1.32x10^{-8a}$

[a]P<0.05, [b]P<0.01 vs. the control.

Tables I and II). A double-reciprocal plot was produced in order to observe the effect of α-solanine on the kinetic constants for NAT (Fig. 3A-b and B-b, Table III) and it was discovered that α-solanine could decrease V_{max} (the maximum reaction rate), but had no effect on K_m (Michaelis-Menten constant), which revealed that α-solanine is a non-competitive inhibitor (Fig. 3A-b, B-b and C).

α-solanine affects the gene expression of NAT1 and NAT2. Reverse transcription-polymerase chain reaction (RT-PCR) was used to assess the gene expression of NAT in HepG2 cells treated with α-solanine. The mRNA levels of NAT and β-actin were quantified by densitometric analysis of gel images and expressed as NAT/β-actin (Fig. 4). The results revealed that α-solanine could decrease the expression of NAT1 mRNA and NAT2 mRNA in HepG2 cells.

Discussion

The arylamine N-acetyltransferases (NATs) are phase II-drug metabolizing enzymes (16). Their main function being to transfer the acetyl group from AcCoA onto the nitrogen or oxygen atom in such substances such as arylamines (17), hydrazines (18), arylhydroxylamines (19) or arylhydrazines (17). These enzymes play an important role in the metabolism of drugs (20) and toxins as well as in detoxification (21,22). Using 2-AF as an example, the process by which arylamines cause cancer is as follows: first, NATs in the cell transform 2-AF into N-acetyl-2-aminofluorene (2-AAF) (23), then 2-AAF is further activated by other enzymes such as glucuronyltransferase, deacylase, sulfotransferase and acyltransferase (24,25), and ultimately transformed in the end to carcinogens nitrenium ion and arylamidonium ion. These can react with guanines to produce bulky adducts of N-deoxyguanaminofluorene and N-deoxyguanacetaminofluorene, which are DNA-carcinogenic compounds. Closely related to cytotoxicity, mutations, and carcinogenesis, the formation of DNA-carcinogenic compounds is a crucial step in the induction of cancer by arylamines (26). Researchers have pointed out that an increase in NAT activity can increase the sensitivity of the organism to many carcinogenetic arylamines.

α-solanine, isolated from fruits of nightshades (27) or potato tubers (28), is a steroid alkaloid. We previously determined that α-solanine can inhibit HepG2 cell proliferation (29) and induce

HepG2 cell apoptosis (30), which is related to the increase of $[Ca^{2+}]_i$ in the cytoplasm (14). Rodrigues-Lima *et al* speculated that NAT1 may be a drug target (31). Butcher and Minchin reviewed literature and thought that NAT1 may be a novel drug target in cancer development (32). In addition, researchers reported that aqueous extract of *Solanum nigrum* L. affected NAT enzyme activity and gene expression in human gastric and colon cancer (10,11). α-solanine is one of the chemical components in *Solanum nigrum* L. (12,13). The question arised as to whether α-solanine could affect NAT enzyme activity. Ragunathan *et al* reported that NAT1 is a new target of cisplatin in breast cancer cells (33). We also found that α-solanine could inhibit HepG2 cell proliferation (30), and thus, we speculated, whether NAT is a target of α-solanine in HepG2 cells. We determined the effect of α-solanine on NATs activity and the dosage of α-solanine in our previous studies (30). The experimental results revealed that α-solanine could lower the amount of 2-AAF produced in both intact HepG2 cells and their cytoplasm (34). The activities of NATs were indirectly determined by measuring the amount of 2-AAF transformed from 2-AF by NATs and the amount of 2-AAF was determined by HPLC. The potential for arylamines to be transformed into carcinogens depends on the levels of NATs in relevant tissues and their capacity for activating compounds (35). Human NATs include NAT1 and NAT2. 2-AF is a rather typical arylamine and also the common substrate for NAT1 and NAT2 and therefore it was chosen to be a suitable substrate for the present experiment. The experimental results in the present study revealed that α-solanine could decrease the amount of 2-AAF produced in both intact HepG2 cells and their cytoplasm, with the effect being dosage-dependent. With the increase in reaction time, the amount of 2-AAF produced gradually increased, but for the same amount of reaction time, the amount of 2-AAF produced was significantly lower in the α-solanine-treated groups than in the control. The experimental results revealed that α-solanine had an inhibitory effect on the activities of NATs, which demonstrated that α-solanine is an inhibitor of NATs.

In enzyme kinetics, enzyme inhibitors are divided into competitive inhibitors, non-competitive inhibitors and uncompetitive inhibitors (Fig. 4). To determine the type of group α-solanine belonged to, as a NAT enzyme inhibitor, Lineweaver-Burk's double-reciprocal plot assay was adopted. Measurements in the kinetic experiments revealed that for both intact HepG2 cells and their cytoplasm, K_m did not differ significantly ($P>0.05$) between the α-solanine group and the negative control (Table III; Fig. 3A-b and B-b), while the maximum reaction rate V_{max} was decreased (Table III; Fig. 3A-b and B-b), revealing that α-solanine is a non-competitive inhibitor. This allowed us to deduce that the locus at which α-solanine acts on NATs differed from that for 2-AF and that the locus is located out of the enzyme active center. α-solanine does not compete with 2-AF for combination with NAT enzyme.

Active ingredients competing for the locus of the enzyme active center with enzyme substrates are similar to the enzyme substrates in structure. The structure of α-solanine and 2-AF is entirely different, thus revealing that it is impossible for α-solanine to be a competitive inhibitor of NAT and that it can only be a non-competitive inhibitor. The conclusion is consistent with our research results. It was speculated that NAT combines with 2-AF and α-solanine concurrently, which alters the enzyme conformation and decreases NAT enzyme activity. Therefore, α-solanine is a non-competitive inhibitor of NATs, the locus which α-solanine acts on NATs differs from that for 2-AF. The non-competitive inhibition of NAT activity by α-solanine is one of the mechanisms through which it inhibits the deterioration of HepG2 cells.

The capacity of NATs to activate arylamines is not only related to factors affecting NAT activity, but is closely related to the levels of expression of NAT proteins. For a unit of enzyme activity, the greater the amount of NAT proteins, the greater the NAT activity. The amount of NAT proteins is proportional to the expression of NAT mRNA, therefore in subsequent experiments, the effect of α-solanine on the expression of the mRNA of NATs was observed by RT-PCR.

NATs exist in many types of tissues in animal and human bodies. In the human body, the NAT family consists of 3 members, namely, NAT1, NAT2, and NATP. NATP is a pseudogene which does not code for any functional NAT, while NAT1 and NAT2 code for the NAT1 and NAT2 protein, respectively (36). Results from the RT-PCR experiment revealed that α-solanine could decrease the expression of NAT1 mRNA and NAT2 mRNA, which indicated that by decreasing the expression of NAT mRNA, α-solanine could decrease the amount of NAT expression, which in turn led to a decrease in the production of acetylated substrates. This resulted in an anti-HepG2 effect. RT-PCR analysis is used to detect gene expression and western blot analysis is used to detect protein expression. If the protein expression level of NAT was detected, this would be useful to draw a conclusion. However, due to the lack of commercialization of the antibodies of the NAT enzyme, the effects of α-solanine on the expression of the NAT protein are not currently available.

In conclusion, α-solanine achieves its inhibitory effect on HepG2 cells by inhibiting the expression of NAT1 mRNA and NAT2 mRNA in HepG2 cells as well as the activity of NAT. α-solanine is a non-competitive inhibitor of NAT. α-solanine does not compete for the locus of the NAT enzyme active center with 2-AF, NAT combines with 2-AF and α-solanine concurrently which alters the enzyme conformation and decreases NAT enzyme activity. This is one possibility. Another possibility is that the decreased NAT mRNA expression may be the reason for the decreased activity of NAT. Thus, the specific mechanism involved warrants further study.

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Availability of data and materials

The datasets used in the present study are available from the corresponding author upon reasonable request.

Author's contributions

SG, HT and YJ conceived and designed the study. SG, LL and JB performed the experiments. SG and YJ provided some financial support for the experiment. SG wrote the manuscript. HT reviewed and edited the manuscript. All authors read and approved the manuscript and agree to be accountable for all aspects of the research in ensuring that the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Ethics approval and consent to participate

The present study does not contain any studies with human participants or animals performed by any of the authors.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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