

## Effects of four chemicals on *N*-acetyltransferase activities in human U937 cell line

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**ABSTRACT** The effects of *p*-aminobenzoic acid (PABA), procainamide (PA), anisidine (AN) and isoniazid (INH) on *N*-acetyltransferase (NAT) activities in cultured human cells were determined. PABA increased the specific activity of PABA NAT in the U937 cells but not in the Hep G<sub>2</sub> cells. The enzyme activity in the PABA-treated U937 cells was restored to normal within 4 d after removing PABA from medium. These results imply that the PABA NAT activity in the U937 cells can be induced by PABA and the PABA NAT in the U937 cells is different from that in the Hep G<sub>2</sub> cells. INH increased the INH NAT specific activity in the U937 cells but decreased the PABA NAT activity. AN decreased both the AN NAT and the PABA NAT specific activities in the U937 cells. PA did not affect the specific activities of PABA NAT or glucose-6-phosphate dehydrogenase (G-6-P DH) in the U937 cells. PABA also increased the specific activities of AN NAT and G-6-P DH. This implies that the induction effect of PABA on the PABA NAT activity is not specific. In this study the PABA NAT specific activity was increased only by PABA, and the INH NAT activity only by INH. However, the AN NAT activity could be induced by PABA but not by AN. These results indicate that induction of some but not all NAT activities has a limiting specificity.

**KEY WORDS** acetyltransferases; enzyme induction; cell lines; cultured cells

*N*-Acetyltransferase (NAT, EC 2.3.1.5) is the main enzyme that involves in the acetylation of drugs and endogenous substances that are arylamines or hydrazine derivatives. The reaction is an important metabolic route for inactivation of these compounds. Although the effects of the human hepatic NAT on the metabolism and toxicity of certain hydrazine derivative and arylamine drugs have

been well studied<sup>(1)</sup>, the effects of chemical on the enzyme have not been elucidated, and there is no information available on induction of the NAT at activity either *in vivo* or *in vitro*. For this reason, we studied the effects of isoniazid, a hydrazine derivative, and 3 arylamines (*p*-aminobenzoic acid, procainamide, and anisidine) on the NAT activity, in the cultured human cell lines U937 and Hep G<sub>2</sub> cells.

### MATERIALS AND METHODS

RPMI1640 medium, minimum essential medium (MEM), non-essential amino acids (NEAA), foetal calf serum, new-born calf serum, and penicillin-streptomycin were from Gibco (Paisley, UK). Acetyl coenzyme A (AcCoA), phosphotransacetylase, acetyl-phosphate, EDTA, *DL*-dithioerythritol (DTT), *L*-glutamine, glucose-6-phosphate (G-6-P), bovine serum albumin, nicotinamide-adenine dinucleotide phosphate (NADP), *p*-dimethylaminobenzaldehyde (DMAB), potassium borate, *p*-aminobenzoic acid (PABA), procainamide (PA), anisidine (AN), and isoniazid (INH) were purchased from Sigma Chemical Co.

**Cell culture** The U937 cells were seeded and grown as suspension cultures in 100 ml flasks in RPMI1640 medium supplemented with 10% (vol/vol) new-born calf serum, penicillin (100 units · ml<sup>-1</sup>) and streptomycin (100 μg · ml<sup>-1</sup>). The Hep G<sub>2</sub> cells were grown as monolayer or multilayer cultures in 100 ml flasks in MEM supplemented with 1% (vol/vol) NEAA and *L*-glutamine, 10% (vol/vol) foetal calf serum, penicillin (100 units · ml<sup>-1</sup>) and streptomycin (100 μg · ml<sup>-1</sup>).

Both the U937 cells and the Hep G2 cells were grown in a humidified atmosphere of 5% CO<sub>2</sub> in air at 37°C.

**Induction** For the U937 cells, inducing agents were added into medium to specified concentrations either at the same time as cells were seeded or 24 h after cells were seeded as indicated in the legend and footnote for Fig 1 and Tab 1, cells were then exposed to these chemicals for 24 or 48 h. PABA was added to the medium 72 h after the Hep G2 cells were seeded in MEM, and the cells were further cultured for 48 h.

**Harvesting and measurement** The U937 cells were counted from identical 100 ml flasks each initially containing approximately  $6 \times 10^6$  cells. At appropriate times, the cells were washed 3 times in 50 ml of phosphate buffered saline, pH 7.6, and for determination of the cells number and viability an aliquot (100  $\mu$ l) was added to 100  $\mu$ l of trypan blue saline.

Finally both the U937 and the Hep G2 cell pellets were resuspended in an equal volume (referring to the cell pellets) of lysis buffer<sup>(2)</sup>, then frozen at -70°C in liquid nitrogen for at least 12 h in order to assay the NAT activity and protein content in the cytosol.

The PABA NAT, the AN NAT, and the PA NAT activities were determined<sup>(2)</sup>.

The INH NAT activity assays were carried out by a modification of Weber's procedure<sup>(3)</sup>.

**Protein determination** The protein content in supernatants of the cell lysates was determined by Bio-Rad protein assay or microassay procedure as described by the manufacturer.

**Statistical analyses** The *t* test was used to compare the enzyme specific activities and protein content in supernatants of cell lysates with the control.

RESULTS

The inhibitory effects of PA, AN, and

INH on the U937 cell growth were very strong and increased sharply with concentrations in the medium. The U937 cells were all killed at concentrations of PA 3 mmol · L<sup>-1</sup>, AN 5 mmol · L<sup>-1</sup>, and INH 10 mmol · L<sup>-1</sup>, respectively, after 48 h. PABA was comparatively weakly toxic and caused loss of approximately 30%, and 30-40% of the U937 cells respectively, as the cells were cultured in medium containing PABA 20 mmol · L<sup>-1</sup> for 24 h and 48 h (Fig 1).

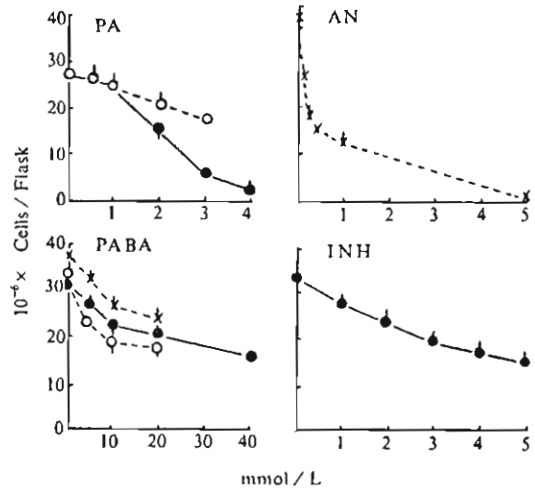


Fig 1. Inhibitory effects of PA, AN, INH and PABA on U937 cell growth. Chemicals were added 24 h after cells were seeded (○) for the cells treated for 24 h; Chemicals were added at the same time as (●) or 24 h after (×) the cells were seeded in the case of the cells treated for 48 h.  $\bar{x} \pm SD$ , from 2 to 15 flasks.

PA did not obviously affect the PABA NAT and the G-6-P DH activities (data not shown). No PA NAT activity was found in the U937 cells whether or not they were exposed to PA (Tab 1).

AN, even at 0.1 mmol · L<sup>-1</sup> in medium, decreased significantly both the AN NAT and the PABA NAT specific activities in the U937 cell cytosol (Tab 1).

INH significantly decreased the PABA NAT activity, but significantly increased INH NAT activity, although the absolute quantitative increase of the INH NAT activity was

**Tab 1. Effects of PA, AN, INH and PABA on NAT in U937 cells for 48 h. PA, INH and PABA were added at the same time as cells were seeded respectively; AN was added 24 h after cells seeded. For PA  $n=5$ , for AN, INH, and PABA,  $n=3$ .  $\bar{x} \pm SD$ . \*\* $P < 0.05$ , \*\*\* $P < 0.01$ .**

Chemical (mmol · L <sup>-1</sup> )		PABA NAT	PA NAT	AN NAT	INH NAT (× 10 <sup>-3</sup> )	Total cytosol protein / flask
PA	0	23.1 ± 1.7	0	—	—	3.16 ± 0.20
	0.5	20.9 ± 1.4	0	—	—	3.10 ± 0.27
	1	21.1 ± 2.5	0	—	—	3.22 ± 0.05
	2	19.8 ± 1.2	0	—	—	1.79 ± 0.02***
AN	0	17.3 ± 3.8	—	12.6 ± 2.9	—	4.08 ± 0.21
	0.1	10.3 ± 0.8**	—	6.8 ± 0.5**	—	3.13 ± 0.02**
	1	11.6 ± 0.7**	—	8.4 ± 0.5**	—	2.09 ± 0.01***
INH	0	24.6 ± 1.0	—	—	0.16 ± 0.02	2.34 ± 0.13
	2	23.0 ± 0.5	—	—	0.27 ± 0.02**	2.16 ± 0.03**
	4	18.4 ± 0.8**	—	—	0.46 ± 0.03**	1.56 ± 0.12**
PABA	0	16.2 ± 2.9	—	6.5 ± 0.3	—	3.8 ± 0.4
	5	20.9 ± 2.0	—	7.3 ± 0.3	—	3.5 ± 0.2
	10	22.4 ± 2.0	—	8.1 ± 0.1	—	3.0 ± 0.3***
	20	23.5 ± 2.7	—	10.6 ± 0.4***	—	2.4 ± 0.1***
	40	43.2 ± 9.9	—	10.7 ± 1.2**	—	1.1 ± 0.1***

NAT specific activity: nmol · L<sup>-1</sup> · min<sup>-1</sup> · mg<sup>-1</sup> protein.

very low (Tab 1).

PABA increased the PABA NAT, the AN NAT specific activities, but increased the PABA NAT activity to a greater extent than the AN NAT (Tab 1). The PABA NAT and the AN NAT specific activities were increased by PABA, but total activities in the U937 cell population per flask were decreased in 48 h-PABA-treated groups as the toxicity of PABA to the cell growth and protein synthesis (Tab 1).

Tab 2 summarizes the restoration process of the PABA NAT activities after PABA was removed from the medium. The activity of the PABA-treated group decreased with time after PABA was removed from the medium, and decreased to a similar extent to that in the control group during the 4 d after PABA was removed (Tab 2).

PABA significantly increased the PABA NAT specific activity in the U937 cells, and decreased the PABA NAT specific activity in the Hep G2 cells, the PABA NAT activity of the U937 cells was higher (8-fold) than the

Hep G2 cells (data not shown).

### DISCUSSION

PABA increased the PABA NAT, the AN NAT, and the G-6-P DH specific activities.

**Tab 2. Restoration of PABA NAT in PABA-treated U937 cells after removal of PABA from medium. After the cells were exposed to PABA 15 mmol · L<sup>-1</sup> in medium for 48 h, the cells were washed 3 times using medium without PABA, and then cultured continuously and passaged in medium without PABA.  $n=2$ ,  $\bar{x} \pm SD$ . \* $P > 0.05$ , \*\* $P < 0.05$ , \*\*\* $P < 0.01$ .**

Days after PABA removed	Generation after PABA removed	Control	PABA-treated	NAT ratio (%)
0	1st	15.8 ± 1.1	19.6 ± 0.7*	124
2	2nd	14.7 ± 0.5	20.0 ± 1.0**	136
4	3rd	12.2 ± 1.5	9.3 ± 1.2*	77
9	5th	13.9 ± 1.6	12.3 ± 1.0*	88
14	9th	14.0 ± 0.6	14.5 ± 0.0*	103
24	14th	14.0 ± 0.4	15.2 ± 1.1*	109

NAT ratio = (NAT of PABA-treated cells) / (NAT of control cells).

This indicated that PABA was not a special inducing agent to the PABA NAT activity. However, the results also indicate that the PABA NAT specific activity can be induced only by PABA and not by PA, AN, or INH. The INH NAT activity can be induced only by INH. The AN NAT activity can be induced by PABA, but not by AN. These results suggest that the induction of some NAT activities needs a special inducing agent and has a limiting specificity, but some NAT activities do not.

PABA increased both the PABA NAT and the AN NAT specific activities, namely the two activities per cell in the U937 cell culture were increased by PABA, but their total activities in the cell population per flask were not increased and even decreased because of the inhibitory effects of PABA on the cell division. Further *in vivo* studies will be necessary to determine the biological significance of this induction of NAT activity.

The results indicate that there seems to be a relationship between the induction of the NAT activity and the toxicity of inducing agents to the cells. The U937 cells all died as they were cultured in medium containing 3 mmol · L<sup>-1</sup>, 5 mmol · L<sup>-1</sup>, 10 mmol · L<sup>-1</sup>, and >40 mmol · L<sup>-1</sup> of PA, AN, INH and PABA, respectively. In this study, PA and AN did not induce the NAT specific activity, INH decreased the PABA NAT activity but increased the INH NAT activity, PABA increased the PABA NAT, the AN NAT and

G-6-P DH specific activities. These results imply that PA and AN are too toxic to the cells to be an inducer.

**ACKNOWLEDGMENTS** I would like to thank Drs Shirley McCreedy and David Fell, Oxford Polytechnic, for their advices and discussions.

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**四种化学物质对人 U937 细胞株 *N*-乙酰转移酶活性的影响**

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**提要** 本文研究了对-氨基苯甲酸(PABA), 普鲁卡因酰胺(PA), 甲氧基苯胺(AN), 及异烟肼(INH)对人类培养细胞中 *N*-乙酰转移酶(NAT)活性的效应. 实验结果表明, 在 U937 细胞中, PABA NAT 比活性只能被 PABA 所诱导, INH NAT 比活性也只能被 INH 所诱导; 然而, AN NAT 可被 PABA 所诱导, 但不能被 AN 所诱导. 这意味着某些 NAT 活性的诱导没有底物特异性, 某些 NAT 活性的诱导仅具有有限的底物特异性. 结果还指出, PABA 可使 U937 细胞中的 PABA NAT 和 AN NAT 的活性增高, 但不能使 Hep G2 细胞中 PABA NAT 的活性增高.

**关键词** 乙酰基转移酶类; 酶诱导; 细胞株; 培养细胞

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