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Effects of Ibuprofen on Arylamine *N*-Acetyltransferase Activity in Human Colon Tumor Cells

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The inhibition of arylamine *N*-acetyltransferase (NAT) activity by ibuprofen was determined in a human colon tumour (adenocarcinoma) cell line. Two assay systems were employed, one with cellular cytosols (9000 *g* supernatant) and the other with intact colon tumour cell suspensions. The NAT activity in a human colon tumour cell line was inhibited by ibuprofen in a dose-dependent manner in both systems, i.e. the greater the concentration of ibuprofen in the reaction, the greater the inhibition of NAT activities in both systems. The data also indicated that ibuprofen decreases the apparent K_m and V_{max} of NAT enzyme from human colon tumour cells in both systems examined. This report is the first demonstration to show that ibuprofen affects human colon tumour cell NAT activity. © **1998 John Wiley & Sons, Ltd.**

INTRODUCTION

Exposure to environmental and occupational carcinogens is thought to be responsible for a large proportion of human cancers. Arylamine carcinogens are well-known potent carcinogens[1] that require metabolic activation by host enzymes as a prerequisite to the initiation of carcinogenesis in target organs and tissues.[2] The major site of arylamine metabolism in the body is usually the liver, however *N*-acetyltransferase (NAT), an enzyme mainly in the liver involved in several steps of both arylamine activation and detoxification, is also found in many types of tissues and in humans.[3–6] In fact, in mice the potential N-acetylation capacity of extrahepatic tissue exceeds that of the liver.[5] *N*-Acetyltransferase localizes in the cytosol fraction of the liver and other organs or tissues and requires acetyl-coenzyme A (acetyl-CoA) for its activity. Attenuation of NAT activity in the liver has been associated with several disease processes.[7,8] *N*-Acetyltransferase in the liver of humans and other mammals shows a genetic polymorphism resulting in rapid, slow and some intermediate acetylation phenotypes.[6,9–10] In humans, the effect and toxicity of a number of drugs and carcinogens have been reported to differ markedly among individuals and to depend on the N-acetylation polymorphism. Extrahepatic expression of acetylator-genotype-dependent NAT activity has also been reported in tissues of human colon and bladder.[11] Thus, the genetically mediated variation in acetyltransferase activities within target organs for arylamine-induced neoplasm may indicate different risks among the human population.

Ibuprofen, derived from propionic acid, is a non-steroidal anti-inflammatory analgesic that is relatively safe and effective for the treatment of pain, dysmenorrhoea, inflammation and fever, acting as a cyclooxygenase inhibitor.[12,13] Numerous studies have indicated that ibuprofen may have anti-cancer properties. Ibuprofen inhibits the growth of some tumour cells,[14] reduces tobacco-specific carcinogen 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone-induced pulmonary and gastric tumorigenesis in A/J mice,[15] exhibits anti-proliferative effects against human colon cancer cells,[16,17] suppresses aberrant crypt formation or the progression to foci of multiple aberrant crypts in the rat colon,[18] affects the *in vitro* invasiveness of a human transitional bladder cell carcinoma,[19] and has chemopreventive potential against the development of breast cancer.[20]

Studies in our laboratory indicate that ibuprofen also inhhibits NAT activity in *Klebsiella pneumoniae*.[21] There are no reports that address whether ibuprofen alters the acetylation of arylamine carcinogens in human tumour cells, therefore the purpose of the present study was to elucidate the possible effects of ibuprofen on NAT activity in colon tumour cells. Our initial choice of 2-aminofluorene (AF) and *p*-aminobenzoic acid (PABA) as test substrates was based on previous studies in mice[5] and current interest in comparing the metabolism of a carcinogen (AF) to a non-

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carcinogen (PABA). The results presented in this report demonstrate that by using AF and PABA as substrates for NAT activity determinations, ibuprofen did decrease colon tumour cell NAT activity in cytosols and in intact cells.

EXPERIMENTAL

Chemicals and reagents

Ibuprofen, ethylenediaminetetraacetic acid (EDTA), *p*-aminobenzoic acid (PABA), *N*-acetyl-*p*-aminobenzoic acid (N-Ac-PABA), acetylcarnitine, leupeptin, bovine serum albumin (BSA), phenylmethylsulphonylfluoride (PMSF). TRIS, dimethyl sulphoxide (DMSO), dithiothreitol (DTT) and carnitine acetyltransferase were obtained from Sigma Chemical Co. (St. Louis, MO). Acetyl-coenzyme A (acetyl-CoA) was obtained from P-L Biochemicals Inc. (Milwaukee, WI). 2-Aminofluorene (AF) and *N*-acetyl-2-aminofluorene (AAF) were obtained from K and K Laboratories (Plainview, NY). All the chemicals used were reagent grade.

Human colon tumour cell line

Human colon adenocarcinoma cell line (Colo 205 from a male 70-year-old Caucasian) were obtained from the Taipei Veterinary Hospital (Taipei, Taiwan). The cells were placed into 75-cm^2 tissue culture flasks and grown at 37°C under a humidified 5% CO_2 atmosphere in RPMI 1640 medium (Sigma Chemical Company, St. Louis, MO) supplemented with 10% fetal bovine serum (Gibco BRL, Grand Island, NY) and 2% penicillin–streptomycin (10,000 U ml^{-1} penicillin and 10 mg ml^{-1} streptomycin).

Preparation of human colon tumour cell cytosols

Approximately 5×10^7 cells were placed in 2 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 described previously.[4] The suspensions were centrifuged at 9000 *g* for 1 min in a Model 3200 Eppendorf/Brinkman centrifuge and the supernatant fraction was subsequently centrifuged at 10,000 *g* for 60 min. The supernatant was kept on ice for NAT activity and protein determinations.

N-Acetyltransferase activity determinations

The determination of acetyl-CoA-dependent N-acetylation of PABA and AF was performed as described by Chung *et al*.[5] Incubation mixtures in the assay system consisted of a total volume of 90 μl: cell cytosol in 50 μl of lysis buffer (20 mM TRIS·HCl, pH 7.5, 1 mM DTT and 1 mM EDTA); 20 μl of an acetyl-CoA recycling mixture of 50 mM TRIS·HCl (pH 7.5), 0.2 mM EDTA, 2 mM DTT, 15 mM acetylcarnitine, and 2 U ml^{-1} carnitine acetyltransferase and AF or PABA at the specified concentrations, and the reactions were started by the addition of 20 μl of acetyl-CoA. The control reactions had 20 μl of distilled water instead of acetyl-CoA. For the single-point activity measurements, the final concentrations were < 0.1 mM for AF or PABA and 0.5 mM for acetyl-CoA. The reaction mixtures with or without specific concentrations of ibuprofen were incubated at 37°C for 10 min and stopped with 50 μl of 20% trichloroacetic acid for the PABA reactions and with 100 μl of acetonitrile for the AF reactions. All the reactions (experiments and controls) were run in triplicate. The amounts of acetylated product and remaining non-acetylated substrate were determined by HPLC.[21,22] An aliquot of the reaction mixture was injected onto a C_{18} reversed-phase column (Spherisorb, 4.6×250 mm) of a Beckman HPLC (pump 168 and detector 126) and eluted at a flow rate of 1.2 ml min^{-1}. For PABA and N-Ac-PABA, the solvent system was 50 mM acetic acid/CH_3CN (86:14) with detection at 266 nm. The retention time for PABA was 8.0 min and that for N-Ac-PABA was 11.0 min. For AF and 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 for AAF was 6.5 min and that for AF was 9.0 min. All the compounds were quantitated by comparison of the integrated area of the elution peak with that of known amounts of standards. The NAT activity was expressed as nmol acetylated substrate min^{-1} mg^{-1} cytosolic protein.

Intact cell NAT activity determination

Human colon tumour cells (in 1 ml of RPMI 1640 medium with glutamine and 10% fetal calf serum) were incubated with arylamine substrate at 1×10^6 cells ml^{-1} in individual wells of 24-well cell culture plates with or without ibuprofen co-treatment for the time indicated at 37°C in 95% air/5% CO_2. At the end of incubation, the cells and media were removed and centrifuged. For experiments with AF, the supernatant was immediately extracted with ethyl acetate/methanol (95:5), the solvent was evaporated and the residue was redissolved in methanol and assayed for AAF as described above. For experiments with PABA, aliquots of the supernatant were assayed directly for N-Ac-PABA.

Protein determination

Protein concentrations in the human colon tumour cell cytosols were determined by the method of Bradford[23] with bovine serum albumin as the standard. All the samples were assayed in triplicate.

Statistical treatment of data

Statistical analysis of the data was performed with an unpaired student's *t*-test. The kinetic constants were calculated with the Cleland HYPER Program,[24] which performs linear regression using a least-squares method.

RESULTS

Effects of various concentrations of ibuprofen on human colon tumour cell NAT activity in cytosol

The possible effects of ibuprofen on NAT activity in human colon tumour cells, both in cytosols and in

intact cells, were examined by HPLC, assessing the percentage of acetylation of AF and PABA. The means ± SD of NAT activity co-treated with or without ibuprofen with both substrates are given in Table 1. The data indicate that there is decreased NAT activity associated with increased ibuprofen both in cytosols and in intact cells. For the cytosol examinations in the presence of 0.02, 0.2, 2 and 20 mM ibuprofen, the NAT activities were inhibited by 6.2, 34.3, 53.1 and 67.1% for AF and by 6.6, 28.3, 53.8 and 71.7% for PABA, respectively. For the intact cell examinations in the presence of 0.02, 0.2, 2 and 20 mM ibuprofen, the NAT activities were inhibited by 4.2, 31.6, 54.7 and 76.1% for AF and by 4.1, 25.0, 52.0 and 80.2% for PABA, respectively.

Effects of 2 mM ibuprofen on human colon tumour cell NAT activity in intact cells

The NAT activities for the acetylation of AF (15, 30, 60 and 100 μM) measured from intact cells were 1.68± 0.08, 3.10 ± 0.34, 6.48 ± 0.69 and 5.89 ± 0.48 nmol 10^{-6} cells for acetylation of AF without co-treatment with ibuprofen. In the presence of 2 mM ibuprofen, the NAT activities were decreased by about 43–55% for AF (Fig. 1a). The NAT activities for the acetylation of PABA (15, 30, 60 and 100 μM) measured from intact cells were 1.18 ± 0.07, 2.46 ± 0.30, 5.12 ± 0.43 and 5.06 ± 0.46 nmol 10^{-6} cells, respectively, for acetylation of PABA without co-treatment with ibuprofen. In the presence of 2 mM ibuprofen, the NAT activities were decreased by about 48–59% (Fig. 1b).

Time course effect of 2 mM ibuprofen on NAT activity in human colon tumour intact cells

To determine the time course effect of 2 mM ibuprofen on NAT activity in human colon tumour cells, the cells were incubated at 37°C and harvested at 6, 12, 18 and 24 h, respectively. Increased time of incubation led to increased AAF and N-Ac-PABA production up to 24 h (Fig. 2). Figure 2 illustrates the time course of AAF and N-Ac-PABA production from human colon tumour cells at both 30 and 60 μM AF and PABA.

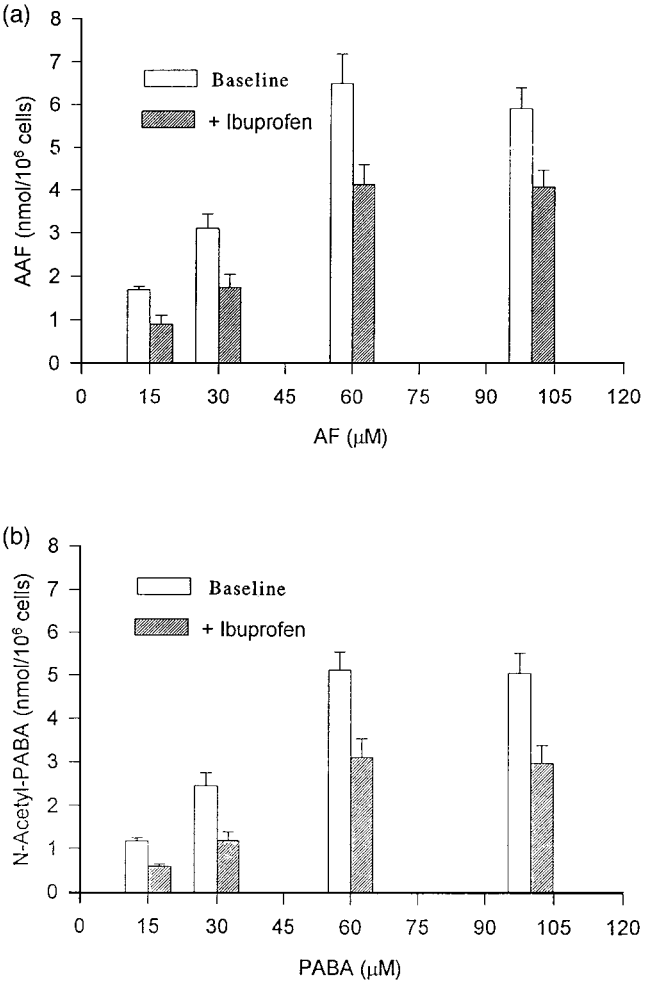

Figure 1. Effects of ibuprofen on the production of AAF and N-Ac-PABA by human colon tumour cells. Tumour cells were incubated as described for 18 h with AF (a) and PABA (b) and with 2 mM ibuprofen co-treatment. Both AAF and N-Ac-PABA were measured by HPLC assay. Each point represents the mean of triplicate assays of three incubations of cells.

Effect of 2 mM ibuprofen on kinetic constants of NAT activity in human colon tumour cells

In the presence or absence of ibuprofen, specific concentrations of AF and PABA (0.373, 0.435, 0.543, 0.745, 1.102 and 2.205 mM, respectively) were added to the recycling mixtures for determining human colon

Table 1. Effects of ibuprofen on human colon tumour cell NAT activity in cytosol and in intact cells[a]

Concentration of ibuprofen	In cytosol		In intact cells	
	AAF	N-Ac-PABA	AAF	N-Ac-PABA
Control	1.28 ± 0.23	1.06 ± 0.22	1.17 ± 0.26	0.96 ± 0.20
0.02 mM	1.20 ± 0.18	0.99 ± 0.14	1.12 ± 0.18	0.92 ± 0.12
0.2 mM	0.84 ± 0.12*	0.76 ± 0.10*	0.80 ± 0.16*	0.72 ± 0.10*
2 mM	0.60 ± 0.08**	0.49 ± 0.08**	0.53 ± 0.08**	0.46 ± 0.06**
20 mM	0.42 ± 0.04***	0.30 ± 0.04***	0.28 ± 0.04***	0.19 ± 0.03***

[a]Values are the mean ± SD of determinations on three experiments. The NAT activity is expressed as nmol/min^{-1} mg^{-1} protein in cytosol and as nmol/min^{-1} 10^{-6} cells in intact cells. Significant differences between 0.2 mM ibuprofen and control: *$P<0.05$, **$P<0.01$ and ***$P<0.001$.

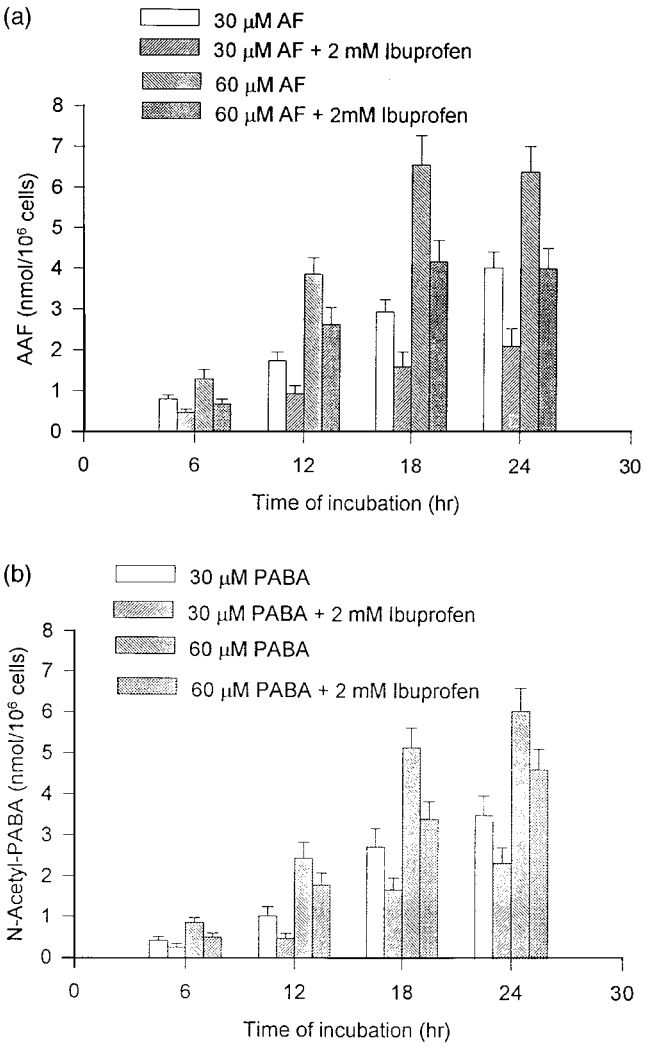

Figure 2. Effects of incubation time on AAF and N-Ac-PABA production by human colon tumour cells. Tumour cells were incubated with AF (a) and PABA (b) at 30 μM and 60 μM and with 2 mM ibuprofen co-treatment for the time shown. Both AAF and N-Ac-PABA were measured by HPLC assay. Each point represents the mean of triplicate assays of three incubations of cells.

tumour cell NAT kinetic constants. For the cytosol examinations, the apparent K_m and V_{max} values were 7.04 ± 1.26 mM and 28.57 ± 6.18 nmol min^{-1} mg^{-1} protein for AF and 2.21 ± 0.18 mM and 13.16 ± 1.99 nmol min^{-1} mg^{-1} protein for PABA, respectively (Tables 2 and 3). When 2 mM ibuprofen was added to the reaction mixtures, the apparent K_m and V_{max} values were decreased by 54.2% and 49.6% for acetylation of AF and by 11.3% and 13.6% for acetylation of PABA, respectively. For the intact cell examinations, the apparent K_m and V_{max} values were 6.99 ± 0.72 mM and 40.00 ± 7.89 nmol min^{-1} mg^{-1} protein for AF and 3.37 ± 0.48 mM and 14.70 ± 2.25 nmol min^{-1} mg^{-1} protein for PABA, respectively (Tables 2 and 3). When 2 mM ibuprofen was added to the reaction mixtures, the apparent K_m and V_{max} values were decreased by 33.0% and 36.8% for acetylation of AF and by 15.1% and 24.7% for acetylation of PABA, respectively. Clearly, the K_m and V_{max} values for the human colon tumour cell NAT activity were decreased in the presence of ibuprofen both in cytosol and in intact cell examinations.

DISCUSSION

The NAT enzyme has been reported to exist in many kinds of experimental animals, including humans, and NAT has been shown to be involved in some chemical carcinogenesis.[25,26] The genetically mediated variation in NAT activities within target tissues for arylamine-induced neoplasm may indicate differential risks among the human population. It was reported that colorectal adenoma and cancer risks were in relation to meat intake and acetylator status.[27] Arylamine NAT activity towards AF and PABA was detected in human colon tumour cells.[28] Ibuprofen decreased the NAT activity of *K. pneumoniae* in cytosol and in intact bacteria.[22] It is reasonable to hypothesize that ibuprofen may also alter the acetylation of arylamine carcinogens such as AF, therefore the present studies were focused on the ibuprofen effects on NAT activity in human colon tumour cells.

Ibuprofen, clinically used as an anti-inflammatory drug, has been considered as a possible chemopreventive drug. We were interested in studying whether or not ibuprofen would affect the NAT activity of human colon tumour cells both in cytosol and in intact cells. We found that the NAT activities decreased as the ibuprofen concentration increased, i.e. the higher the concentration of ibuprofen, the higher the inhibition of NAT activity. In order to simulate the conditions of the human large intestine, a pH of 7.5 was selected for all the experiments. This pH is close to that of the normal large intestine. The data from the cytosol and intact human colon tumour cell studies showed that

Table 2. Kinetic data for acetylation of 2-AF in human colon tumour cells[a]

	In cytosol		In intact cells	
	K_m (mM)	V_{max}(nmol/min^{-1} mg^{-1} protein)	K_m(mM)	V_{max}(nmol) 10^{-6} cells)
Control	7.04 ± 1.26	28.57 ± 6.18	6.99 ± 0.72	40.00 ± 7.89
Ibuprofen	3.22 ± 0.32*	14.38 ± 2.40***	4.68 ± 0.64†	25.26 ± 4.92***

[a]Values are means ± SD; $n = 3$. The acetyl-CoA and ibuprofen concentrations were 0.1 mM and 2 mM, and the kinetic constants were calculated from the modified HYPER Program of Cleland.[24] Significant differences between 2 mM ibuprofen and control: $P<0.05$, ***$P<0.001$ and †$P<0.005$.

Table 3. Kinetic data for acetylation of PABA in human colon tumour cells[a]

	In cytosol		In intact cells	
	K_m (mM)	V_{max}(nmol/min^{-1} mg^{-1} protein)	K_m(mM)	V_{max}(nmol) 10^{-6} cells)
Control	2.21 ± 0.18	13.16 ± 1.99	3.37 ± 0.48	14.70 ± 2.25
Ibuprofen	1.96 ± 0.16*	11.44 ± 1.52***	2.86 ± 0.44†	11.06 ± 1.42***

[a]Values are means ± SD; $n = 3$. The Acetyl CoA and ibuprofen concentrations were 0.1 mM and 2 mM, and the kinetic constants were calculated from the modified HYPER Program of Cleland.[24] Significant differences between 2 mM ibuprofen and control: * $P<0.05$, *** $P<0.001$ and † $P<0.005$.

there were significant differences of NAT activity between the control and ibuprofen treatment groups. The present studies demonstrate that ibuprofen can markedly inhibit the NAT activity of human colon tumour cells in both examined systems and that the inhibition is dose dependent. The data also indicate that ibuprofen decreases the NAT kinetic constants in the cytosols and in intact cells. Although it is not known whether a decrease of NAT activity would decrease tumour production or whether ibuprofen could prevent the development of colon cancer, other investigators have demonstrated that elevated levels of NAT activity are associated with increased sensitivity to the mutagenic effects of many arylamines.[29] It has reported that attenuation of liver NAT activity is related to bladder and breast cancer.[7] Further investigation is needed.

Flammang and colleagues have found high levels of acetyltransferase activity in human colonic mucosa.[30] They have also found that the mucosa itself catalyses the O-acetylation of the N-hydroxy metabolites to DNA-reactive metabolites,[31,32] which could initiate large bowel cancer. Recently, it has been demonstrated that the combination of fast-acetylator phenotypes with a high consumption of meat increased the risk of colorectal cancer.[27] The present study may offer some information about ibuprofen effects on NAT activity, because NATs are expressed in human ileum and colon and are able to metabolize arylamine compounds.[33,34]

In conclusion, this study indicates that ibuprofen inhibits NAT activity in human colon tumour cells *in vitro* and in intact cells. This report is also the first demonstration to show that ibuprofen can inhibit the NAT activity of a human colon tumour cell line.

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