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# Ibuprofen Affects Arylamine *N*-Acetyltransferase Activity in *Helicobacter pylori* from Peptic Ulcer Patients

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Key words: ibuprofen; *N*-acetyltransferase; 2-aminofluorene; *N*-acetyl-2-aminofluorene; *p*-aminobenzoic acid; *N*-acetyl-*p*-aminobenzoic acid.

**Arylamine *N*-acetyltransferase (NAT) activities with 2-aminofluorene and *p*-aminobenzoic acid were determined in the bacterium *Helicobacter pylori* collected from peptic ulcer patients. Cytosols or suspensions of *H. pylori* with or without specific concentrations of ibuprofen co-treatment showed different percentages of 2-aminofluorene and *p*-aminobenzoic acid acetylation. The data indicate that there was decreased NAT activity associated with increased levels of ibuprofen in *H. pylori* cytosols. Inhibition of growth studies on *H. pylori* demonstrated that ibuprofen elicited a dose-dependent bactericide effect in *H. pylori* cultures, i.e. the greater the concentration of ibuprofen, the greater the inhibition of growth to *H. pylori*. For the cytosol and intact bacteria examinations, the apparent values of $K_m$ and $V_{max}$ were decreased after co-treatment with 40 μM ibuprofen. This report is the first demonstration of ibuprofen inhibition of arylamine *N*-acetyltransferase activity and ibuprofen inhibition of growth in the bacterium *H. pylori*.** © **1998 John Wiley & Sons, Ltd.**

## INTRODUCTION

Exposure to environmental and occupational carcinogens is thought to be responsible for a large portion of human cancers. Arylamine carcinogens are one of the most potent carcinogens. Once in the body, arylamine carcinogens are metabolized either to detoxification products, which are excreted, or they may be further activated to induce toxicities in target organs and tissues. The major site of arylamine metabolism in the body is usually the liver. However, *N*-acetyltransferase (NAT), an enzyme mainly found in the liver involved in several steps of both arylamine activation and detoxification,[1] is also found in many types of tissues and in humans.[2–5] In fact, in mice the potential N-acetylation capacity of extrahepatic tissues exceeds that of the liver.[2] Attenuation of NAT activity in the liver has been associated with several disease processes, such as bladder and breast cancer.[1,6] Thus, there is much interest in the role of NAT in chemical carcinogenesis.

Importantly, NAT in the liver of humans and other mammals shows a genetic polymorphism resulting in rapid, slow and some intermediate acetylation phenotypes.[4,5,7] 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.[1,6] For humans, epidemiological studies suggest that the rapid acetylator phenotype has been linked to increased risks of colorectal cancer[8,9] and the slow acetylator phenotype has been linked to increased susceptibility to occupational bladder cancer.[10]

*Helicobacter pylori* has been reported to be present in the stomachs of patients.[11] It has also been reported that there is an aetiological correlation of *H. pylori* with active chronic gastritis.[12] *Helicobacter pylori* is a possible causative factor in patients with gastric cancer.[13] Based on reports, it has been indicated that chronic atrophic gastritis is a precursor lesion of gastric cancer, and chronic gastritis might be induced or exacerbated by *H. pylori*[14,15] because gastritis, peptic ulcer and gastric cancer are related to the presence of this bacterium.[16] The present author's previous studies have already demonstrated that *H. pylori* contains NAT activity in cytosols and in intact bacteria.[17] Therefore, the investigation of the relationship between *H. pylori* and 2-aminofluorene (2-AF) metabolism was undertaken.

Ibuprofen, derived from propionic acid, is one of the non-steroidal anti-inflammatory analgesics acting as a cyclooxygenase inhibitor.[18] It has been reported that ibuprofen is safe and effective for the treatment of pain, dysmenorrhoea, inflammation and fever.[19] Several lines of evidence suggest that ibuprofen may be effective in preventing cancer:

(i) ibuprofen can reduce tobacco-specific carcinogen 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone-induced pulmonary and gastric tumorigenesis in A/J mice,[20]
(ii) ibuprofen exhibits anti-proliferative effects against human colon cancer cells,[21]
(iii) ibuprofen is effective in suppressing aberrant crypt formation or the progression to foci of multiple aberrant crypts in the rat colon;[22]

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(iv) ibuprofen inhibits the growth of some tumour cells;[23]
(v) ibuprofen affects the *in vitro* invasiveness of a human transitional bladder cell carcinoma;[24]
(vi) ibuprofen has chemopreventive potential against the development of breast cancer[25] and is considered to be a potential cancer chemopreventive agent.[26]

There are no reports available that address whether ibuprofen would alter the acetylation of arylamine carcinogens. Therefore, the purpose of the present study was to elucidate the possible effects of ibuprofen on NAT activity and growth in the bacterium *H. pylori*. The initial choice of 2-AF and *p*-aminobenzoic acid (PABA) as test substrates was based on previous studies in mice,[6] bacteria[19] and the author's interest in comparing the metabolism of a carcinogen (2-AF) to a non-carcinogen (PABA). The results presented in this report demonstrated that by using 2-AF and PABA as substrates for NAT activity determinations, ibuprofen did decrease *H. pylori* NAT activity in cytosols and in intact bacteria.

## EXPERIMENTAL

### Chemicals and reagents

Ibuprofen, ethylenediaminetetraacetic acid (EDTA), *p*-aminobenzoic acid (PABA), *N*-acetyl-*p*-aminobenzoic acid (N-Ac-PABA), 2-aminofluorene (2-AF), 2-acetylaminofluorene (2-AAF), bovine serum albumin (BSA), phenylmethylsulphonylfluoride (PMSF), TRIS·HCl, leupeptin, acetyl carnitine, dithiothreitol (DTT), carnitine acetyltransferase and acetyl-coenzyme A (Ac-CoA) were obtained from Sigma Chemical Co. (St. Louis, MO). Acetic acid, acetonitrile, dimethyl sulphoxide (DMSO) and potassium phosphates were obtained from Merck Co. (Darmstadt, Germany). All of the chemicals used were reagent grade.

### *Helicobacter pylori*

*Helicobacter pylori* bacteria were clinically isolated from patients who visited the Department (China Medical College Hospital) for endoscopy as described in previous reports.[18]

### Preparation of bacterial cytosols

About $10 \times 10^{10}$ colony-forming units (CFU) were washed twice in cold phosphate-buffered saline, placed immediately in 1 ml of 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), disrupted by a sonicator and then centrifuged for 30 min at 10 000 *g*. The supernatant was kept on ice until assayed for NAT activity.

### NAT activity determination

The determination of Ac-CoA-dependent N-acetylation of PABA and 2-AF was performed as described by Chung *et al.*[17] Incubation mixtures in the assay system consisted of a total volume of 90 μl of bacterial cytosol, diluted as required in 50 μl of lysis buffer (20 mM TRIS·HCl, pH 7.5, 1 mM DTT, 1 mM EDTA and 1 mM acetylcarnitine), and 2-AF or PABA at a specific concentration for the substrate. The reactions were started by addition of 20 μl of Ac-CoA. The control reactions had 20 μl of distilled water in place of Ac-CoA. The final concentration of PABA or 2-AF was 0.1 mM, and that for Ac-CoA was 0.5 mM. The reaction mixtures were incubated at 37°C for 10 min and stopped with 50 μl of 20% trichloroacetic acid for PABA reactions and 100 μl of acetonitrile for 2-AF reactions. All of the reactions of the experiments and controls were run in triplicate. The amounts of acetylated product and remaining non-acetylated substrate were determined by HPLC.[6,19] An aliquot of the NAT incubation mixture was injected onto the C18 reversed-phase column (Spherisorb $4.6 \times 250$ 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 4.0 min and that of N-Ac-PABA was 6.5 min. For 2-AF and 2-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 was 6.5 min for 2-AAF and 9 min for 2-AF. All of the compounds were quantitated by comparison of the integrated area of the elution peak with that of known amounts of standards. The NAT activity is expressed as nmol acetylated substrate $min^{-1}$ $mg^{-1}$ cytosolic protein.

### Protein concentration

Protein concentration in the cytosols from *H. pylori* were determined by the method of Bradford,[27] with BSA as the standard. All of the samples were assayed in triplicate.

### Effects of various concentrations of ibuprofen on growth of *H. pylori*

Twenty-two strains of *H. pylori*, cultured individually in *Brucella* anaerobic culture plates in an anaerobic jar for 5 days to obtain growth at the level of $10^8$ bacteria, were placed in individual tubes containing brain heart infusion media with or without different concentrations of ibuprofen (4, 8, 16, 32 and 64 mM). The culture tubes were incubated at 37°C in a microaerobic atmosphere (5% $O_2$, 10% $CO_2$ and 85% $N_2$) and checked for growth after 5 days. This bacterium grows slow and usually needs 4–5 days of incubation. The determination of the effects of ibuprofen on the growth of *H. pylori* was based on measurements of absorbance by an optical density method (OD at 650 nm). The control groups were prepared under the same conditions as the ibuprofen-treated groups except without the ibuprofen. Growth inhibition (%) was determined using the following equation:

$$1 - \left( \frac{\text{Original OD–Final OD(+ ibuprofen)}}{\text{Original OD}} \times 100 \right)$$

### Effects of various concentrations of ibuprofen on NAT activity in *H. pylori*

Ibuprofen dissolved in DMSO at specific concentrations ranging from 0.04 to 400 mM were prepared. The reaction mixtures consisted of 50 μl of cytosol, diluted as required, 20 μl of recycling mixture containing 2-AF or PABA at specific concentrations as substrates and 10 μl of ibuprofen (at a specific concentration). The reactions were started by the addition of Ac-CoA. The control reactions had 20 μl of distilled water in place of Ac-CoA. Following the NAT activity, determinations were made to assess the effect of ibuprofen on NAT activity in *H. pylori*.

### Effects of ibuprofen on kinetic constants of NAT from *H. pylori*

Cytosols of *H. pylori* were co-treated with or without specific concentrations of ibuprofen (4, 8, 16, 32 and 64 mM) and the NAT activity was determined as described above. All of the reactions were run in triplicate. For the intact bacteria studies, $3 \times 10^9$ bacterial cells in brain heart infusion broth were incubated with arylamine substrates (2-AF or PABA), with or without specific concentrations of ibuprofen, for 96 h at 37°C in a microaerobic atmosphere (5% $O_2$, 10% $CO_2$ and 85% $N_2$). At the conclusion of incubation, the cells and media were removed and centrifuged. For the experiments with 2-AF, the supernatant was immediately extracted with ethylacetate/methanol (95:5), the solvent evaporated and the residue redissolved in methanol and assayed by HPLC. All of the samples were run in triplicate. The kinetic constants were calculated by the Cleland HYPER Program[28] which performs linear regression using a least-squares method. The amounts of acetylated product and remaining non-acetylated substrates were assayed by HPLC as described above under NAT activity determination. The velocity ($1/V$) versus substrate ($1/S$) data were linearized by plotting $1/S$ versus $1/V$.

### Statistical treatment of data

Statistical analysis of the data was performed with an unpaired Student's *t*-test. The mean significant difference between ibuprofen-treated and control groups was $P < 0.05$.

## RESULTS

### Effects of various concentrations of ibuprofen on the growth of *H. pylori*

The rationale for the author's initial studies was based on two observations from other reports. First, earlier studies have already demonstrated that *H. pylori* contains NAT activity in cytosols and in intact bacteria with 2-AF and PABA used as substrates.[17] Second, ibuprofen has been shown to decrease *Klebsiella pneumoniae* NAT activity.[29] Therefore, the results of an examination for the effects of ibuprofen on the growth of *H. pylori* are given in Table 1. *Helicobacter pylori* was inhibited by ibuprofen, i.e. the higher the concentration of ibuprofen in the *H. pylori* culture, the higher the inhibition of *H. pylori*. When the concentration of ibuprofen reached 64 mM, the inhibition reached about 60%.

### Effects of various concentrations of ibuprofen on the NAT activity in *H. pylori*

The possible effects of ibuprofen on the NAT activity in *H. pylori* in cytosols and in intact bacteria were examined by HPLP assessment of the percentage acetylation of 2-AF and PABA. Cytosols of *H. pylori* with or without specific concentrations of ibuprofen co-treatment showed different percentages of 2-AF and PABA acetylation. A comparison of the relative cytosolic NAT activity with or without specific concentrations of ibuprofen is presented in Table 2. Percentage acetylation of 2-AF and PABA by *H. pylori* with or without specific concentrations of ibuprofen co-treatment in intact cells is shown in Fig. 1. The data indicate that there was decreased NAT activity associated with increased ibuprofen levels in *H. pylori* in intact bacteria, i.e the higher the concentration of ibuprofen in the reaction mixtures, the higher the inhibition of NAT activity. Because 32 mM of ibuprofen showed inhibition of the NAT activity both in cytosol and in intact bacteria (inhibition ratios were 51% and 53%

**Table 1. Effect of ibuprofen on the growth of *H. pylori*[a]**

| | Concentration of ibuprofen (mM) | | | | | |
|---|---|---|---|---|---|---|
| | 0 | 4 | 8 | 16 | 32 | 64 |
| Strain | Percentage inhibition | | | | | |
| 1 | 0 | 16 ± 6 | 34 ± 9 | 47 ± 9 | 56 ± 12 | 68 ± 14 |
| 2 | 0 | 14 ± 6 | 31 ± 8 | 41 ± 9 | 54 ± 10 | 62 ± 12 |
| 3 | 0 | 12 ± 4 | 28 ± 6 | 34 ± 6 | 46 ± 8 | 61 ± 12 |
| 4 | 0 | 11 ± 4 | 21 ± 4 | 27 ± 5 | 40 ± 8 | 51 ± 10 |
| 5 | 0 | 15 ± 4 | 28 ± 4 | 34 ± 5 | 46 ± 8 | 58 ± 12 |
| 6 | 0 | 17 ± 6 | 32 ± 6 | 40 ± 8 | 59 ± 12 | 70 ± 14 |
| 7 | 0 | 12 ± 4 | 24 ± 6 | 30 ± 6 | 47 ± 8 | 60 ± 12 |
| 8 | 0 | 14 ± 6 | 20 ± 6 | 31 ± 6 | 47 ± 8 | 61 ± 12 |
| 9 | 0 | 12 ± 4 | 21 ± 6 | 22 ± 6 | 40 ± 8 | 52 ± 10 |
| 10 | 0 | 16 ± 6 | 28 ± 6 | 40 ± 4 | 59 ± 10 | 68 ± 12 |
| 11 | 0 | 9 ± 2 | 17 ± 4 | 24 ± 4 | 32 ± 6 | 46 ± 8 |
| 12 | 0 | 12 ± 4 | 24 ± 6 | 32 ± 5 | 48 ± 8 | 59 ± 12 |
| 13 | 0 | 16 ± 6 | 28 ± 6 | 36 ± 8 | 54 ± 10 | 66 ± 14 |
| 14 | 0 | 10 ± 3 | 20 ± 6 | 31 ± 8 | 42 ± 8 | 53 ± 10 |
| 15 | 0 | 13 ± 4 | 23 ± 6 | 32 ± 6 | 46 ± 8 | 58 ± 12 |
| 16 | 0 | 14 ± 4 | 24 ± 6 | 35 ± 6 | 48 ± 8 | 64 ± 14 |
| 17 | 0 | 9 ± 2 | 16 ± 4 | 26 ± 6 | 34 ± 6 | 49 ± 10 |
| 18 | 0 | 12 ± 3 | 19 ± 4 | 34 ± 6 | 43 ± 8 | 61 ± 12 |
| 19 | 0 | 14 ± 4 | 22 ± 4 | 36 ± 6 | 46 ± 8 | 58 ± 10 |
| 20 | 0 | 11 ± 3 | 19 ± 6 | 31 ± 8 | 43 ± 8 | 56 ± 8 |
| 21 | 0 | 12 ± 3 | 21 ± 6 | 36 ± 6 | 45 ± 8 | 57 ± 8 |
| 22 | 0 | 15 ± 4 | 26 ± 4 | 37 ± 6 | 49 ± 8 | 64 ± 12 |

[a] *Helicobacter pylori* was incubated in the presence of various concentrations of ibuprofen (4, 8, 16, 32 and 64 mM) as described in the experimental section. The percentage inhibition was determined by using a spectrophotometer and then calculating the percentage inhibition. All experiments and controls were run in triplicate.

**Table 2. Effects of ibuprofen on *H. pylori N*-acetyltransferase activity in cytosols[9]**

| Concentration of ibuprofen | AAF (nmol min$^{-1}$ mg$^{-1}$ protein) | N-Acetyl-PABA (nmol min$^{-1}$ mg$^{-1}$ protein) |
|---|---|---|
| Control | 0.86 ± 0.12 | 0.64 ± 0.08 |
| 4 mM | 0.73 ± 0.12[b] | 0.53 ± 0.11[a] |
| 8 mM | 0.66 ± 0.10[c] | 0.42 ± 0.10[b] |
| 16 mM | 0.53 ± 0.09[d] | 0.38 ± 0.08[c] |
| 32 mM | 0.44 ± 0.08[e] | 0.31 ± 0.06[d] |
| 64 mM | 0.36 ± 0.06[f] | 0.26 ± 0.04[e] |

[a]*Helicobacter pylori* cytosols were incubated in the presence of various concentrations of ibuprofen (4, 8, 16, 32 and 64 $\mu$M) as described in the experimental section. All experiments and controls were run in triplicate. Values are means ± SD ($n = 3$). Statistical analysis of the data was performed with an unpaired Student's *t*-test.
[b]Differs between 4 mM ibuprofen and control ($P < 0.05$).
[c]Differs between 8 mM ibuprofen and control ($P < 0.02$).
[d]Differs between 16 mM ibuprofen and control ($P < 0.01$).
[e]Differs between 32 mM ibuprofen and control ($P < 0.005$).
[f]Differs between 64 mM ibuprofen and control ($P < 0.001$).

($P < 0.001$), respectively, for 2-AF and PABA in cytosols, and 41% and 46%, respectively, for both substrates in intact bacteria), we chose this concentration to perform the following studies. The inhibition by ibuprofen on the acetylation of PABA was slightly higher than that seen for 2-AF.

## Effects of various concentrations of ibuprofen on the kinetic constants of *H. pylori* NAT

The kinetic constants determined for *H. pylori* NAT using 2-AF and PABA as substrates with or without 32 mM ibuprofen are shown in Fig. 2 for the cytosol examination and Fig. 3 for the intact bacteria examination. For the cytosol examinations, the apparent values of $K_m$ and $V_{max}$ were 3.72 ± 0.49 mM and 16.67 ± 4.30 nmol min$^{-1}$ mg$^{-1}$ protein, respectively, for 2-AF (Table 3) and 3.71 ± 0.49 mM and 20.46 ± 4.48 nmol min$^{-1}$ mg$^{-1}$ protein, respectively, for PABA (Table 4). However, when ibuprofen was added to the reaction mixtures, the apparent values of $K_m$ and $V_{max}$ were 2.38 ± 0.28 mM and 7.69 ± 1.24 nmol min$^{-1}$ mg$^{-1}$ protein, respectively, for 2-AF (Table 3) and 1.85 ± 0.28 mM and 5.57 ± 0.45 nmol min$^{-1}$ mg$^{-1}$ protein, respectively, for PABA (Table 4). For the intact

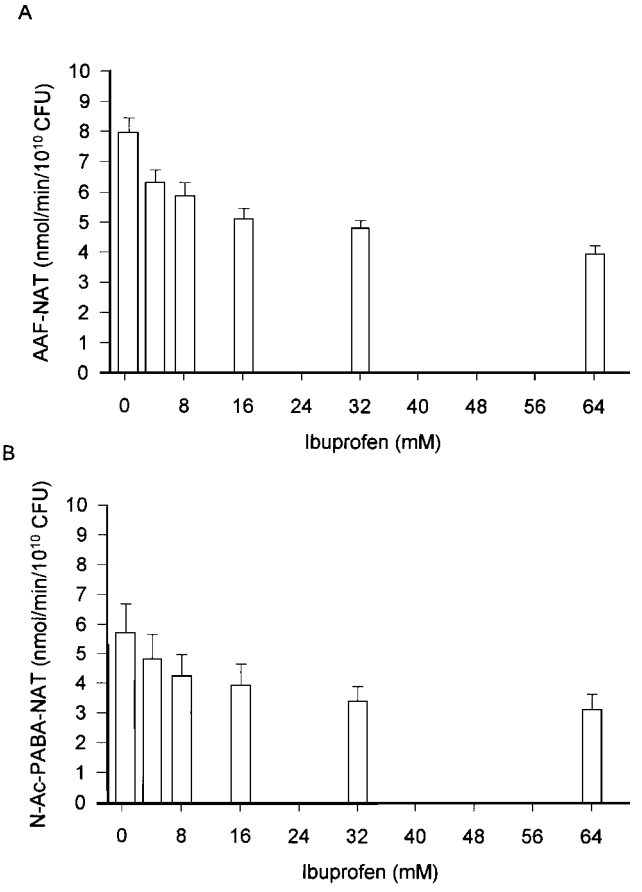


**Figure 1.** Percentage acetylation of 2-aminofluorene and *p*-aminobenzoic acid by *H. pylori* with or without specific concentrations of ibuprofen. The intact bacteria were prepared as described in the experimental section. The Ac-CoA concentration was 0.1 mM. Values are means ± SD ($n = 3$).

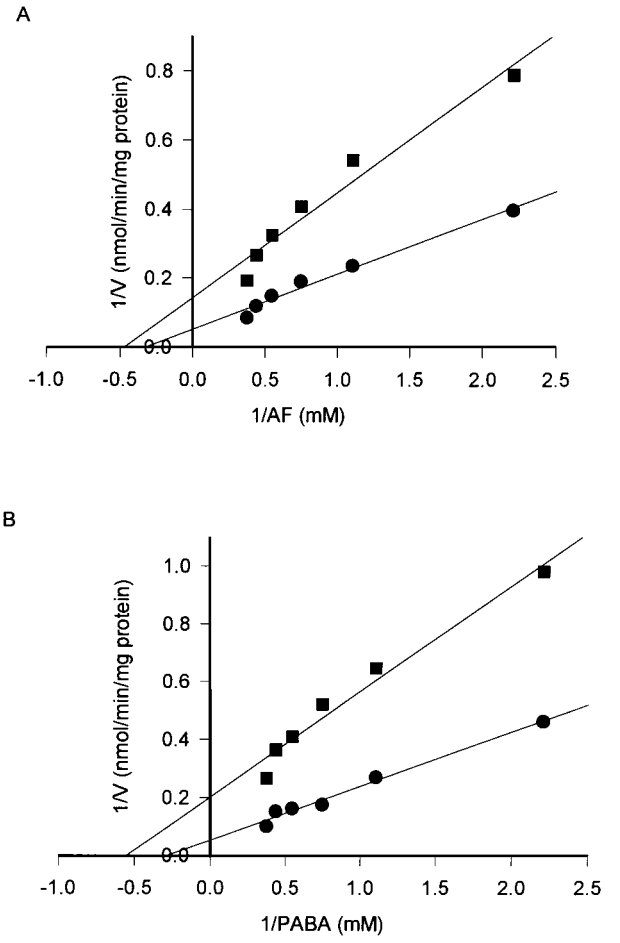


**Figure 2.** Lineweaver-Burk double-reciprocal plot of *H. pylori N*-acetyltransferase activity as a function of 2-aminofluorene and *p*-aminobenzoic acid concentrations in cytosols. (●) normal; (■) with ibuprofen. The cytosols were prepared as described in the experimental section. The Ac-CoA and ibuprofen concentrations were 0.1 mM and 32 mM, respectively. Values are means ± SD ($n = 3$).

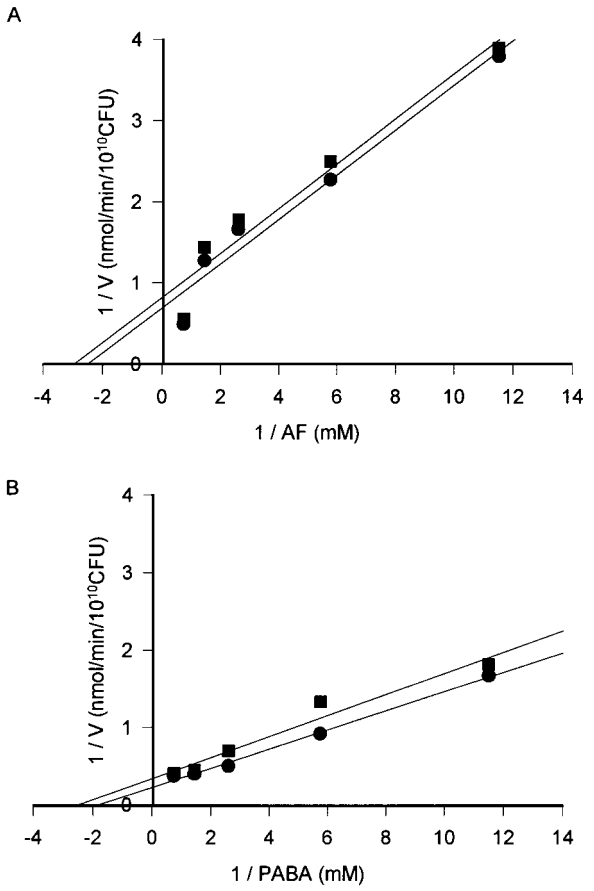


**Figure 3.** Lineweaver-Burk double-reciprocal plot of *H. pylori* *N*-acetyltransferase activity as a function of 2-aminofluorene and *p*-aminobenzoic acid concentration in intact bacteria: (●) normal; (■) with ibuprofen. The suspensions were prepared as described in the experimental section. The Ac-CoA and ibuprofen concentrations were 0.1 mM and 32 mM, respectively. Values are means ± SD ($n = 3$).

bacteria examination, the apparent values of $K_m$ and $V_{max}$ were 0.46 ± 0.09 mM and 1.63 ± 0.18 nmol $min^{-1}$ $(10 \times 10^{10})^{-1}$ colony forming units CFU, respectively, for 2-AF (Table 3) and 0.56 ± 0.11 mM and 6.66 ± 0.69 nmol $min^{-1}$ $(10 \times 10^{10})^{-1}$ CFU, respectively, for PABA (Table 4). However, when ibuprofen was added to the reaction mixture, the apparent values of $K_m$ and $V_{max}$ were 0.25 ± 0.06 mM and 1.29 ± 0.11 nmol $min^{-1}$ $(10 \times 10^{10})^{-1}$ CFU, respectively, for AF (Table 3) and 0.46 ± 0.18 nM and 4.00 ± 0.48 nmol $min^{-1}$ $(10 \times 10^{10})^{-1}$ CFU, respectively, for PABA (Table 4).

## DISCUSSION

There are many observations that are likely to be prerequisites for the observed effects of ibuprofen on NAT activity:

(i) the NAT enzyme exists in many kinds of experimental animals, including humans,[1,2,4,5] and NAT activity has been shown to be involved in some chemical carcinogenesis,[30,31]
(ii) rapid and slow acetylations have been demonstrated as a predisposing factor for the sensitivity of individuals to toxicity during exposure to many arylamines;[8–10]
(iii) some enzymes of enteric bacteria are known to contribute to the metabolic activation of chemical carcinogens in animal studies;[32,33]
(iv) the present author's previous studies have already shown that ibuprofen induces the inhibition of growth and decreases NAT activity in *Klebsiella pneumoniae*[29]
(v) according to the present author's preliminary studies, many kinds of enteric bacteria, such as *K. pneumoniae*, *Salmonella* group B and *E. coli*, exhibit NAT activity (manuscript in preparation).

It was found that *H. pylori* cytosols contained NAT activity.[17] Therefore, the present studies were focused on the effects of ibuprofen on NAT activity in *H. pylori*.

The data presented in this report clearly demonstrate that ibuprofen does affect *H. pylori* NAT activity and growth. The results clearly indicate that ibuprofen, in concentrations from 4 mM to 64 mM for cytosol tests, decreases the acetylated product of 2-AF and PABA by *H. pylori*. The results also show that when ibuprofen decreases the NAT activity in *H. pylori* it is a dose-dependent affect, i.e. the higher the concentration of ibuprofen, the higher the inhibition of NAT activity. The data presented from the intact bacteria tests also show that ibuprofen decreases the percentage of acetylated products of 2-AF and PABA. The concentrations (4–64 mM) of ibuprofen used in this study are reason-

**Table 3. Kinetic data for acetylation of 2-aminofluorene in *H. pylori*[a]**

| | In cytosol | | In intact bacteria | |
|---|---|---|---|---|
| | $K_m$ (mM) | $V_{max}$ (nmol $min^{-1}$ $mg^{-1}$) | $K_m$ (mM) | $V_{max}$ (nmol $10 \times 10^{10})^{-1}$ CFU) |
| Control | 3.72 ± 0.49 | 16.67 ± 4.30 | 0.46 ± 0.09 | 1.63 ± 0.18 |
| Ibuprofen | 2.38 ± 0.28[b] | 7.69 ± 1.24[c] | 0.25 ± 0.06[d] | 1.29 ± 0.11[e] |

[a]Values are means ± SD ($n = 3$). The acetyl-CoA and ibuprofen concentrations were 0.1 mM and 32 mM, and the kinetic constants were calculated from the modified HYPER Program of Cleland. All experiments and controls were run in triplicate. Statistical analysis of the data was performed with an unpaired Student's *t*-test. [b]Differs between 32 mM ibuprofen and control ($P < 0.05$).
[c]Differs between 32 mM ibuprofen and control ($P < 0.001$).
[d]Differs between 32 mM ibuprofen and control ($P < 0.01$).
[e]Differs between 32 mM ibuprofen and control ($P < 0.005$).

**Table 4. Kinetic data for acetylation of *p*-aminobenzoic acid in *H. pylori*[a]**

| | In cytosol | | In intact bacteria | |
|---|---|---|---|---|
| | $K_m$ (mM) | $V_{max}$ (nmol min$^{-1}$ mg$^{-1}$) | $K_m$ (mM) | $V_{max}$ (nmol $10 \times 10^{10})^{-1}$ CFU) |
| Control | $3.71 \pm 0.49$ | $20.46 \pm 4.48$ | $0.65 \pm 0.11$ | $6.66 \pm 0.69$ |
| Ibuprofen | $1.85 \pm 0.28$[b] | $5.57 \pm 0.45$[c] | $0.46 \pm 0.08$[d] | $4.00 \pm 0.48$[e] |

[a]Values are mean ± SD ($n = 3$). The acetyl-CoA and ibuprofen concentrations were 0.1 mM and 32 mM, and the kinetic constants were calculated from the modified HYPER Program of Cleland. All experiments and controls were run in triplicate. Statistical analysis of the data was performed with an unpaired Student's *t*-test.
[b]Differs between 32 mM ibuprofen and control ($P < 0.005$).
[c]Differs between 32 mM ibuprofen and control ($P < 0.0001$).
[d]Differs between 32 mM ibuprofen and control ($P < 0.01$).
[e]Differs between 32 mM ibuprofen and control ($P < 0.001$).

able because they are based on literature indicating that the analgesic dose of ibuprofen is 0.97–.3.88 mM and the antirheumatic dose is 3.88–15.51 mM for human daily used.[34] The data also demonstrate that ibuprofen induces the inhibition of growth in *H. pylori* culture, i.e. the higher the concentration of ibuprofen, the higher the inhibition of *H. pylori* growth. Based on the observations, if there is no inhibition of growth, then there is no decreased NAT activity by ibuprofen.

Because ibuprofen did inhibit the NAT activity of *H. pylori*, the kinetic constants were also affected. The reason for selecting 32 mM of ibuprofen for kinetic constant studies is that this concentration has already reduced acetylation by 50%. For the cytosol examinations, the apparent values of $K_m$ and $V_{max}$ decreased 0.36- and 0.53-fold for acetylation of 2-AF, and 0.50- and 0.72-fold for acetylation of PABA. For the intact bacteria examinations, the apparent values of $K_m$ and $V_{max}$ decreased 0.45- and 0.20-fold for acetylation of 2-AF, and 0.30- and 0.40-fold for acetylation of PABA. Based on the kinetic constant decreases, it was suggested that ibuprofen may act like a non-competitive inhibitor, a result that is consistent with that reported for arylamine NAT from pigeon liver.[35] While the nature of the interaction and the NAT protein domains involved in this interaction remain unclear, ibuprofen inhibition may be a useful tool to distinguish between different aromatic amine NATs. This point needs further investigation and is very important for the possibility of decreasing arylamine carcinogens in induced carcinogenesis, because other reports have demonstrated that elevated levels of NAT activity may be associated with increased sensitivity to the mutagenic affects of many arylamines[36] and attenuation of NAT activity has been reported to be associated with several disease processes.[1,2,4,5]

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