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# COMMUNICATIONS

## Human liver and plasma aspirin esterase

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**Abstract**—The plasma, in addition to the liver, is a major site of hydrolysis of aspirin. Human plasma and liver aspirin esterase activities in samples from a group of patients varied over a two fold range and there was a significant correlation between individual plasma and liver activities. Human liver aspirin esterase was present in the cytosolic and microsomal fractions. Cytosolic and microsomal enzymes had different activities and apparent affinities for aspirin.

Following oral administration, aspirin (acetylsalicylic acid) undergoes hydrolysis by presystemic metabolism in the liver and the gut (Rowland et al 1972). Aspirin reaching the systemic circulation is then hydrolysed by the plasma, red blood cells and the liver. The enzymes involved in aspirin hydrolysis include hepatic carboxylesterases (Inoue et al 1980; White & Hope 1981; Heymann & Mentlein 1988), plasma cholinesterase and albumin (Morikawa et al 1986) and red blood cell arylesterases (Costello & Green 1983). We have shown considerable inter-individual variation in plasma aspirin esterase activity which correlates with circulating aspirin concentrations after oral administration and with analgesia following dental surgery (Seymour et al 1984; Williams et al 1986). Because of the importance of liver metabolism in the elimination of aspirin, these findings would suggest hepatic and plasma esterases might vary in parallel. We have therefore examined the correlation between the activities of aspirin esterase in plasma and liver. Liver carboxylesterases may be both cytosolic or microsomal, therefore we have also investigated the subcellular distribution of hepatic aspirin esterase activity in man.

### Methods

*Subjects.* Human liver samples (wedge biopsy) were obtained from 16 patients undergoing cholecystectomy or vagotomy and pyloroplasty. Liver tissue was immediately placed in liquid nitrogen before storage at −80°C until analysis. All liver samples were later shown to be histologically normal. Before surgery venous blood was obtained from 11 patients. Blood was centrifuged as soon as possible, the plasma separated and stored at −80°C. Patients gave informed consent for withdrawal of blood and use of excess liver, and the study received prior approval from the Newcastle Area Health Authority Ethics Committee. Patients details are shown in Table 1.

*Plasma aspirin esterase activity* was determined by a direct reading spectrophotometric assay (Sorensen 1983). A freshly prepared acetylsalicylic acid solution (1 mM) was incubated at 37°C in a spectrophotometric cuvette with 0·2 mL plasma in 3 mL pH 7·4 Tris calcium (200 mM) buffer. The salicylate liberated by hydrolysis was measured by monitoring at 300 nm. Enzyme activity was expressed as nmol salicylate formed mL plasma$^{-1}$ min$^{-1}$. Spontaneous hydrolysis of acetylsalicylic acid was less than 10% of that in the presence of plasma.

Table 1. Patient details.

| No | Sex | Age (years) | Smoking | Current Therapy |
|---|---|---|---|---|
| 1 | F | 65 | N.S. | Pentobarbitone |
| 2 | M | 62 | N.S. | Nil |
| 3 | M | 60 | N.S. | Nil |
| 4 | F | 42 | N.S. | Cimetidine |
| 5 | F | 59 | N.S. | Nil |
| 6 | F | 62 | S | Nil |
| 7 | M | 69 | N.S. | Cimetidine |
| 8 | F | 65 | N.S. | Carbamazepine |
| 9 | F | 78 | S | Dichloralphenazone Diazepam |
| 10 | M | 67 | S | Cimetidine |
| 11 | M | 39 | S | Ranitidine |
| 12 | F | 69 | N.S. | Ranitidine |
| 13 | F | 49 | S | Nifedipine |
| 14 | M | 53 | N.S. | Nil |
| 15 | M | 65 | N.S. | Bendrofluazide |
| 16 | F | 57 | N.S. | Thyroxine |

*Preparation of the liver homogenate and microsomal and cytosolic fractions.* Liver tissue (approx. 200 mg) was thawed at room temperature (20°C) and the weighed portion homogenized with 10 mL ice cold buffer pH 7·4 (0·25 M potassium phosphate, 0·15 M potassium chloride) using a glass to glass homogenizer. Aliquots of the homogenate were retained for incubation. Homogenates were centrifuged for 5 min at 1000 *g* followed by 10 min at 18 000 *g*. Microsomes were prepared by centrifugation of the 18 000 *g* supernatant for 60 min at 120 000 *g* and the cytosol fraction was retained. The microsomal pellets were resuspended in 0·25 M phosphate buffer (pH 7·4 containing 0·15 M potassium chloride) and recentrifuged for 60 min at 120 000 *g*. The pellet was finally resuspended in phosphate buffer as above.

*Liver enzyme assays.* Aspirin esterase activity was measured by determining the liberation of salicylate by endpoint HPLC assay. Incubations were carried out at 37°C and contained liver homogenate, microsomal or cytosolic fractions (equivalent to 2–15 mg wet weight liver), plus phosphate buffer (pH 7·4) to 0·27 mL. For most incubations the reaction was initiated by the addition of 0·03 mL freshly prepared acetylsalicylic acid to give a

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final concentration of 1 mM. The reaction was stopped after 10 min incubation by the addition of 0·3 mL perchloric acid (6% containing *p*-toluic acid (1 $\mu$g mL$^{-1}$) followed by vortexing and placing on ice. Incubates were centrifuged and then stored at −80°C before HPLC analysis. Analysis was always on the same day as incubation. Incubations were carried out in duplicate and blanks were incubated in parallel to control for spontaneous hydrolysis of aspirin. The appropriate blank was injected onto the HPLC adjacent to the protein incubation. Incubation conditions had been checked by varying protein concentrations and incubation times. Kinetic parameters were determined using six substrate concentrations between 200 $\mu$M and 10 mM. Spontaneous aspirin hydrolysis was determined at each substrate concentration and subtracted from the activity observed in the presence of enzyme. $K_m$ and $V_{max}$ values were calculated from Direct Linear plots (Cornish-Bowden 1976) and enzyme activities were expressed as nmol salicylate formed g liver$^{-1}$ min$^{-1}$.

*HPLC conditions.* HPLC was carried out using a Waters Z module containing an ODS C18 column with C18 precolumn. The mobile phase was 50–50 methanol–orthophosphoric acid (0·072% w/v), at a flow rate of 4 mL min$^{-1}$ and UV detection was at 234 nm. Retention characteristics were as follows; acetylsalicylic acid 2·4 min, salicylic acid 3·4 min, and *p*-toluic acid (internal standard) 4·4 min.

## Results

Plasma aspirin esterase activity (mean 106 ± 7·0 nmol mL$^{-1}$ min$^{-1}$) and liver (homogenate) aspirin esterase activity (mean 372 ± 30 nmol g liver$^{-1}$ min$^{-1}$) varied twofold in the 11 patients studied (See Table 2). There was a significant correlation between individual liver and plasma activities (r = 0·62, n = 11, $P < 0·05$, Fig. 1). We did not find any influence of age, most patients were over 60 years, sex, smoking or drug consumption on individual plasma or liver aspirin esterase activities.

Table 2. Individual plasma and liver aspirin esterase activities. Activities were measured at 1 mM acetylsalicylic acid as substrate and results expressed as nmol salicylate formed mL(g)$^{-1}$ min$^{-1}$.

| | Aspirin esterase activity | |
|---|---|---|
| Patient No. | Plasma | Liver homogenate |
| 1 | 120 | 345 |
| 2 | 95 | 221 |
| 3 | 95 | 410 |
| 4 | 62 | 279 |
| 5 | 81 | 271 |
| 6 | 137 | 431 |
| 7 | 135 | 497 |
| 8 | 124 | 380 |
| 9 | 110 | 552 |
| 10 | 93 | 342 |
| 11 | 114 | 365 |
| mean ± s.e.m. | 106 ± 7·0 | 372 ± 30 |

*Kinetics.* The formation of salicylate from acetylsalicylate in the presence of liver tissue was linear for 20 min and proportional to the amount of tissue per incubation (2–15 mg wet weight liver) at 1 mM substrate concentration. Spontaneous aspirin hydrolysis represented 30% of total activity at 1 mM aspirin substrate concentration and was measured in parallel with all enzyme assays. Spontaneous hydrolysis increased to 50% of the total hydrolysis measured at higher substrate concentrations approaching the $V_{max}$.

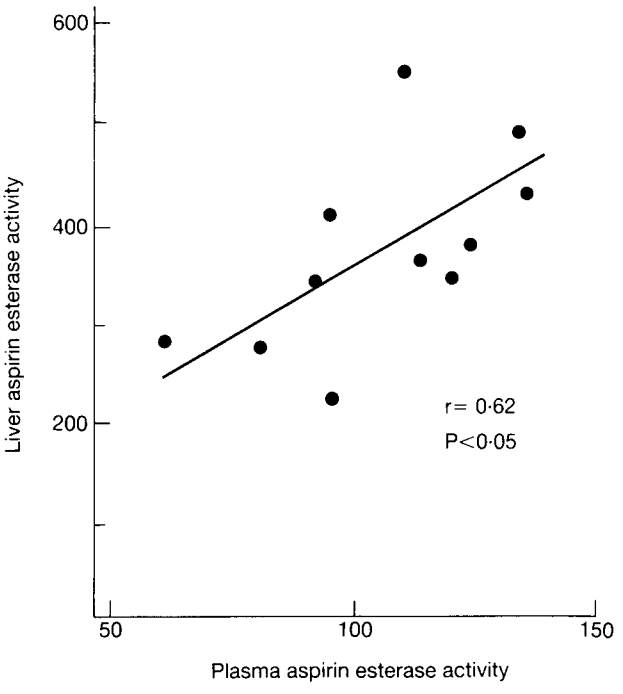


FIG. 1. The relationship between plasma aspirin esterase activity (nmol salicylate formed mL plasma$^{-1}$ min$^{-1}$) and liver aspirin esterase activity (nmol salicylate formed g liver$^{-1}$ min$^{-1}$) measured at 1 mM aspirin in samples from individual patients. (r = 0·62 $P < 0·05$).

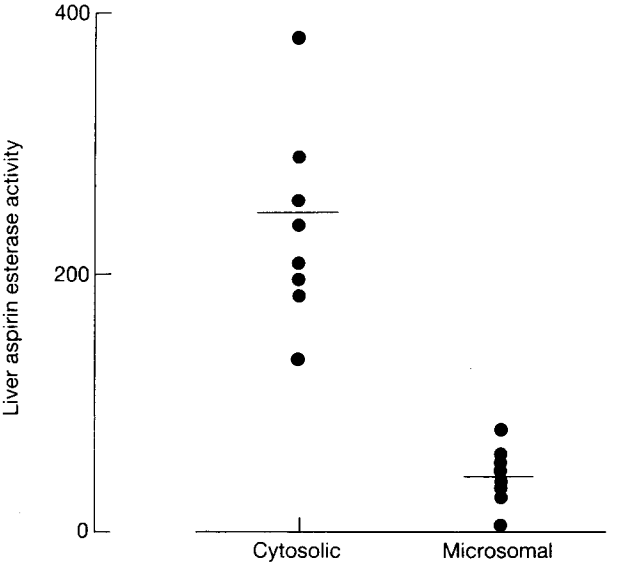


FIG. 2. Distribution of human liver aspirin esterase activity (nmol salicylate formed g liver$^{-1}$ min$^{-1}$) between the cytosolic and microsomal fractions. Incubations of 1 mM acetylsalicylic acid as substrate in the presence of cytosolic or microsomal fractions derived from 2–15 mg wet weight liver per incubation were for 10 min at 37°C.

Under the conditions used for the plasma aspirin esterase assay at 1 mM substrate concentration, spontaneous hydrolysis was less than 10% of the total activity.

*Subcellular localization of aspirin esterase activity in the liver.* For eight livers, microsomal and cytosolic fractions were separated. Aspirin esterase activity in the cytosolic fraction (mean 244 ± 26

nmol salicylate g liver$^{-1}$ min$^{-1}$) was five times that in the microsomal fraction (44·6 ± 7·5 nmol salicylate g liver$^{-1}$ min$^{-1}$) (Fig. 2). The values of the apparent kinetic constants for hepatic microsomal and cytosolic aspirin esterase for two patients are shown in Table 3. The cytosolic enzyme had higher affinity for aspirin (lower $K_m$) and higher $V_{max}$ than the microsomal enzyme.

Table 3. Apparent kinetic constants ($K_m$ and $V_{max}$) for aspirin esterase in human liver microsomes and cytosol.

| | $K_m$ (mM) | $V_{max}$ (nmol g liver$^{-1}$ min$^{-1}$) |
|---|---|---|
| Microsomes | | |
| Liver No. | | |
| 10 | 2·7 | 144 |
| 15 | 2·4 | 258 |
| Cytosol | | |
| Liver No. | | |
| 10 | 0·8 | 390 |
| 15 | 1·4 | 620 |

## Discussion

Two proteins exhibiting aspirin esterase activity have been isolated from human plasma; cholinesterase and albumin (acting as an esterase) (Rainsford et al 1980; Morikawa et al 1986), whereas aspirin esterase activity in human liver is due to carboxylesterases (Inoue et al 1980). Those authors have identified two esterases in human liver (on basis of molecular weight) which are capable of hydrolysing aspirin but further identification has not been carried out. Other species have been investigated in more detail; Heymann & Mentlein (1988) have isolated four carboxylesterases from rat liver microsomes characterized on the basis of their isoelectric points and substrate specificity, one of which was capable of hydrolysing aspirin. White & Hope (1981) have characterized a carboxylesterase from guinea-pig microsomes capable of hydrolysing aspirin, and an aspirin hydrolase of differing specificity to inhibition by organophosphorous compounds, from guinea pig cytosol (White & Hope 1984). However, the guinea-pig differs from man in having a plasma carboxylesterase which hydrolyses aspirin (White et al 1987).

In our study of human liver samples we have found aspirin esterase activity in both the cytosol and the microsomes. The capacity of human liver cytosol to metabolise aspirin was greater than the microsomal fraction. Limited kinetic studies on two livers confirmed higher $V_{max}$ values in the cytosol. Estimates of $K_m$ values in cytosolic and microsomal fractions for these two livers suggested a higher affinity of the cytosolic enzymes for aspirin, however investigation of larger numbers of livers is required to confirm that this is a real difference.

For comparison of hepatic and plasma esterase activity liver homogenate was studied. We have observed considerable inter-individual variation in plasma aspirin esterase activity which was paralleled by the liver (homogenate) aspirin esterase activity with a significant correlation between the two tissues. This observation is of interest as it indicates parallel variation in plasma cholinesterases and liver carboxylesterases. We have previously shown a negative correlation between plasma aspirin esterase activity and both the amount of aspirin in the systemic circulation and its analgesic effects, suggesting that the plasma activity reflects the body's overall ability to hydrolyse aspirin (Seymour et al 1984). Rowland et al (1972) calculated that only 14% of the whole body clearance of aspirin was due to hydrolysis in the blood, the rest being mainly due to liver and gut. However, the in-vitro activities that we have observed suggest that human plasma and liver have a similar capacity for aspirin metabolism when measured in-vitro and at 1 mM aspirin a higher concentration than would be encountered in-vivo. The livers studied were from patients aged 50–60 years and would have been about 1200 g in weight (Wynne et al 1989), therefore the mean total liver hydrolytic capacity was approximately 440 μmol aspirin hydrolysed min$^{-1}$. Plasma aspirin esterases and red blood cell aspirin esterases contribute 40 and 60%, respectively, to the total whole blood activity (Williams unpublished data), therefore the maximum possible overall contribution to hydrolysis by the blood could be 450 μmol min$^{-1}$ (assuming a blood volume of 4500 mL). These calculations suggests that, following the first pass through the liver, hydrolysis in the blood may be a major site for aspirin hydrolysis in man.

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