STUDIES ON ASPIRIN ESTERASE OF HUMAN SERUM

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Accepted March 19, 1979

Abstract—We studied the aspirin-hydrolyzing activity in human serum and found a difference between the sexes in the aspirin-hydrolyzing activity expressed in mU salicylic acid released from ml of serum. However, aspirin-hydrolyzing activity expressed in mU salicylic acid per ml albumin showed no such difference. In cases of chronic hepatitis, liver cirrhosis and nephrosis, the aspirin-hydrolyzing activity was much lower than that seen healthy persons. In disc electrophoretic experiments, the aspirin-hydrolyzing activity was found to be located in pseudocholinesterase and albumin and this activity in the cholinesterase region was inhibited by eserine sulfate. When the albumin from human serum was purified by chromatography on DEAE-cellulose and SephadeX G-100, the purified albumin hydrolyzed aspirin and this aspirin-hydrolyzing activity of purified albumin, although not inhibited by eserine sulfate and diisopropyl fluorophosphate, was inhibited by treatment with acetic anhydride. These results suggest that the aspirin-hydrolyzing activity from human serum included the pseudocholinesterase activity and acetylation effect of aspirin.

Aspirin is probably the most widely used drug in the world, either singly or in combination. Degradation of aspirin depends on enzyme systems present in the serum, liver and intestine (1, 2), however, detailed studies have apparently not been documented. Menguy et al. reported that sex difference was found in the enzyme activity of aspirin-hydrolyzing in human serum, and that the rate of hydrolysis affects the pharmacological action or toxicity of this drug (3). On the other hand, Okumura reported that the aspirin esterase activity decreased in the serum of liver damaged patients (4) and they suggested that the aspirin esterase activity could be an indicator of the pathological changes seen in cases of liver injury (4, 5). Aspirin esterase, an aspirin-hydrolyzing enzyme, is probably not a single enzyme, but belongs to a group of enzymes known as acetylene esterase. It has been differentiated from other esterase of serum, particularly from acetylcholinesterase (1). As there are a number of unclarified points regarding aspirin esterase, we investigated the aspirin-hydrolyzing activity in human serum.

MATERIALS AND METHODS

Human serum: Sera were obtained from 50 healthy Japanese, males (25) and females (25) volunteers (aged from 18 to 32) and from patients at Jikei Medical University Hospital. Assay of esterase activity: The esterase activity was assayed using acetylsalicylic acid
(aspirin) as a substrate according to the method described previously (6). The pseudocholinesterase activity was assayed using benzoyl choline chloride as a substrate according to the method of Augustinsson (7). One unit of enzyme was defined as the amount of product formed per min.

**Assay of protein:** Protein was determined according to the method of Lowry et al. (8), and albumin was determined according to the method of Doumas et al. with human serum albumin as the standard.

**Disc electrophoresis:** Disc electrophoresis was carried by the method of Davis using 7.5% polyacrylamide gel at pH 9.4 (10).

**Materials:** Pseudocholinesterase (Type VII) was purchased from Sigma Chem. Co., LTD., and human albumin (crystallized) was purchased from Miles Laboratories INC. Diethylaminoethyl (DEAE)-cellulose was purchased from Brown and Sephadex G-100 (Pharmacia) was purchased from Seikagaku Kogyo Co., Tokyo. Other chemicals were of reagent grade and were the best quality available.

**RESULTS**

**Aspirin hydrolyzing activity in human serum:** As shown in Fig. 1, the aspirin-hydrolyzing activity was measured in terms of the amount of salicylic acid released per ml serum, per mg protein and per mg albumin. We found that when the values per ml serum men and women were separated, the average values (37.8±3.1 and 29.7±4.8 respectively) were significantly different (p<0.01). However, the values per mg albumin showed no sex difference. Thus, we observed the content of albumin and cholinesterase activity to determine if there was a difference between males and females.

**Aspirin-hydrolyzing activity in the sera of patients:** Sera were obtained from patients with acute and chronic hepatitis, liver cirrhosis and nephrosis, and the aspirin-hydrolyzing activity of these sera was determined. The data are summarized in Table 1.

In cases of chronic hepatitis and nephrosis, the aspirin-hydrolyzing activity per ml serum was lower than normal, but that activity per mg albumin was higher than normal. In patients with a liver cirrhosis, the aspirin-hydrolyzing activity per ml serum and per mg albumin was lower than normal. The aspirin-hydrolyzing activity in the sera of these
Correlation among aspirin-hydrolyzing activity, albumin content and cholinesterase activity: The aspirin-hydrolyzing activity was determined by the method of electrophoresis. After the electrophoresis, the polyacrylamide block was cut into pieces of 0.5 cm in width, each of which was eluted with 5 ml of 1/15 M phosphate buffer, pH 7.4, and we used 1 ml of the eluate. The aspirin-hydrolyzing activity was located in the regions of pseudocholinesterase and albumin and that in the cholinesterase region was inhibited by eserine sulfate. Sera from the controls was subjected to ammonium sulfate fractionation. Here, most of the aspirin-hydrolyzing activity and albumin was found in the ammonium sulfate fraction, at 50–70% saturation. Correlation between the aspirin-hydrolyzing activity and albumin was then determined. The ammonium sulfate fraction (50–70% saturation) was dialyzed overnight against 10 mM Tris-HCl buffer, pH 7.3 and the preparation applied to a Sephadex G-100 column equilibrated with the same buffer containing 0.1 M NaCl. The elution was carried out as described in the legend to Fig. 2. The active fractions were pooled and concentrated to 20 ml using collodion bags (Sartorius). The concentrated enzyme was dialyzed overnight against 10 mM Tris-HCl buffer, pH 7.3, and was applied to a DEAE-cellulose column equilibrated with the same buffer. The elution was carried out with a linear NaCl gradient from 0 to 0.3 M. The aspirin-hydrolyzing activity was eluted as shown in Fig. 2.

TABLE 1. Serum esterase activity and protein contents

<table>
<thead>
<tr>
<th></th>
<th>Protein (mg/ml)</th>
<th>Albumin (mg/ml)</th>
<th>Aspirin (mU/ml)</th>
<th>ChE (U/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal (50)</td>
<td>84.2 ± 10.1</td>
<td>50.4 ± 5.4</td>
<td>33.8 ± 3.6</td>
<td>1.34 ± 0.18</td>
</tr>
<tr>
<td>Acute hepatitis (5)</td>
<td>79.9 ± 14.5</td>
<td>43.1 ± 6.6</td>
<td>39.2 ± 8.4</td>
<td>1.47 ± 0.51</td>
</tr>
<tr>
<td>Chronic hepatitis (10)</td>
<td>67.3 ± 5.0</td>
<td>25.0 ± 5.2*</td>
<td>24.3 ± 5.4*</td>
<td>1.04 ± 0.16*</td>
</tr>
<tr>
<td>Liver cirrhosis (8)</td>
<td>76.4 ± 9.4</td>
<td>37.8 ± 6.9*</td>
<td>18.9 ± 4.5*</td>
<td>0.83 ± 0.32*</td>
</tr>
<tr>
<td>Nephrosis (11)</td>
<td>78.0 ± 5.2</td>
<td>37.3 ± 7.7*</td>
<td>26.4 ± 3.2*</td>
<td>1.31 ± 0.25</td>
</tr>
</tbody>
</table>

Mean ± S.D., *p<0.01, significant difference, ChE: Benzoyl choline, Units: µ moles/min.
in Fig. 3. Fractions No. 43, 63 and 82 which showed the protein peak, were subjected to
disc-electrophoresis, and the results are shown in Fig. 4. The zymograms of the fraction
No. 63 and the fraction No. 82 showed a correlative mobility while that of the fraction
No. 43 did not. The esterase activity was determined in fractions No. 63 and 82 (Fig. 4).

The esterase activities of the DEAE-fraction No. 63, 4.5% albumin solution and 0.1%
pseudocholinesterase solution were determined using β-naphthyl acetate, β-naphthyl
butyrate, aspirin and benzoyl choline as substrates. The results are shown in Table 2. The
DEAE-fraction No. 63 and albumin solution hydrolyzed β-naphthyl esters and aspirin, but
did not hydrolyze benzoyl choline. The pseudocholinesterase solution hydrolyzed benzoyl
choline, β-naphthyl esters and aspirin. Effects of different chemicals on the serum, DEAE-
fraction No. 63, albumin solution and pseudocholinesterase solution were examined and
the results are shown in Table 3. Diisopropyl fluorophosphate (DFP) and eserine sulfate
had no effect on the esterase activity of the DEAE-fraction No. 63 and albumin solution.
The esterase activity of pseudocholinesterase solution was strongly inhibited by DFP and

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Serum</th>
<th>DEAE-fraction</th>
<th>Albumin</th>
<th>Cholinesterase</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(mU/ml)</td>
<td>(relative activity)</td>
<td>(mU/ml)</td>
<td>(relative activity)</td>
</tr>
<tr>
<td>β-Naphthyl acetate</td>
<td>508.8</td>
<td>52.48 (100)</td>
<td>127.21</td>
<td>130.1 (100)</td>
</tr>
<tr>
<td>β-Naphthyl butyrate</td>
<td>791.7</td>
<td>11.49 (156)</td>
<td>34.88 (21.8)</td>
<td>43.0 (33.1)</td>
</tr>
<tr>
<td>Aspirin</td>
<td>33.4</td>
<td>1.01 (6.6)</td>
<td>2.06 (1.9)</td>
<td>7.4 (5.7)</td>
</tr>
<tr>
<td>Benzoyl choline</td>
<td>1352.9</td>
<td>N.D.</td>
<td>N.D.</td>
<td>1352.6 (266)</td>
</tr>
</tbody>
</table>

Table 2. Substrate specificity of serum, DEAE-fraction, albumin and cholinesterase

Albumin: human albumin crystallized.
eserine sulfate. Correlation between the aspirin-hydrolyzing activity and the acetylation effect of aspirin was also studied. The aspirin-hydrolyzing activity of DEAE-fraction and albumin was inhibited by the pre-treatment with acetic anhydride at 0 °C for 4 hr.

TABLE 3. Effect of various reagents on esterase activity of serum, DEAE-fraction, albumin and cholinesterase

<table>
<thead>
<tr>
<th>Reagent</th>
<th>Remaining activity (%)</th>
<th>Serum</th>
<th>DEAE-fraction</th>
<th>Albumin</th>
<th>Cholinesterase</th>
</tr>
</thead>
<tbody>
<tr>
<td>DFP</td>
<td>20</td>
<td>91</td>
<td>90</td>
<td>N.D.</td>
<td></td>
</tr>
<tr>
<td>Eserine sulfate</td>
<td>42</td>
<td>100</td>
<td>100</td>
<td>N.D.</td>
<td></td>
</tr>
<tr>
<td>Acetic anhydride</td>
<td>82</td>
<td>53</td>
<td>47</td>
<td>100</td>
<td>N.D.*</td>
</tr>
</tbody>
</table>

The concentration was 10⁻⁴ M except at the asterisk to be 10⁻³ M. Albumin: human albumin crystallized. Cholinesterase: human serum.

DISCUSSION

Aspirin absorbed into the blood stream is rapidly hydrolyzed by enzymes mainly in the liver; about 20% by serum aspirin esterase (1, 11, 12). The aspirin hydrolyzing activity may probably account for the different reactivity of individuals to the therapeutic and toxic effects of salicylates. Aspirin esterase, despite its specific name, is probably not a single enzyme.

In our experiment, sex difference was observed in the aspirin-hydrolyzing activity expressed by mU salicylic acid released from 1 ml of human serum. This is in good agreement with the results by Menguy et al. (3). However, the aspirin-hydrolyzing activity expressed by mU salicylic acid per mg albumin was the same in males and females (Fig. 1.). Chronic damage, i.e. chronic hepatitis and liver cirrhosis, decreased the aspirin-hydrolyzing activity, the cholinesterase activity and the amount of albumin. In the sera of nephritic patients, the cholinesterase activity showed no change, but the aspirin-hydrolyzing activity per ml serum and the amount of albumin was lower than normal (Table 1). In disc electrophoretic experiments, the aspirin-hydrolyzing activity was observed in the pseudocholinesterase region. Pseudocholinesterase obtained from Sigma Chem. Co. hydrolyzed aspirin. But both aspirin-hydrolyzing activities were inhibited by DFP and eserine sulfate. The aspirin-hydrolyzing activity from serum was inhibited moderately (50% inhibition) by eserine sulfate. These results suggest that pseudocholinesterase participates in the aspirin hydrolysis.

Albumin from human serum was purified by chromatography on DEAE-cellulose and Sephadex G-100. It was observed that the purified albumin solution hydrolyzed aspirin (Table 2), but that hydrolyzing activity was not inhibited by eserine sulfate and DFP (Table 3). There is a report that aspirin acetylates the lysin residue of human serum albumin (13). In our experiment, the aspirin-hydrolyzing activity of purified albumin was inhibited by treatment with acetic anhydride. All these results taken together suggest that there is a correlation between the aspirin-hydrolyzing activity of albumin and the acetylation effect.
of aspirin.

Thus, the aspirin-hydrolyzing activity from human serum probably includes pseudo-
cholinesterase activity and acetylation of aspirin.

**Acknowledgement:** We thank Prof. Yukio Ikawa for provision of serum samples from patients.

**REFERENCES**


