Effects of 1-S Replaced and/or Decarboxylated Latamoxef on Rabbit Platelet Aggregation In Vitro

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Abstract—Latamoxef, 1-S replaced and/or decarboxylated derivatives of latamoxef were examined for their effects on ADP-, collagen- and platelet activating factor (PAF)-induced rabbit platelet aggregation in vitro. The results were compared with those of cefotaxime, cefmetazole, carbenicillin and aspirin. Latamoxef produced a dose-dependent inhibition of platelet aggregation at concentrations over about 4 mM, and the potency was almost similar to that produced by the other β-lactam antibiotics, although the inhibiting effect on ADP-induced aggregation was more potent for latamoxef, whereas that on collagen-induced aggregation was stronger for cefmetazole and carbenicillin. The inhibitory effect of β-lactam antibiotics on collagen-induced aggregation was, however, much weaker than that of aspirin. With respect to drug potency, replacement of the oxygen atom in the oxacephem ring with a sulfur atom caused no significant change in ADP-induced aggregation or slightly stronger inhibition of collagen- and PAF-induced aggregations. The decarboxylated derivatives of latamoxef and the 1-S replaced analogue of latamoxef showed slightly weaker inhibition of ADP-induced aggregation, but much stronger inhibition of collagen- and PAF-induced aggregation than the parent compounds. These data suggest that 1) the oxygen atom in the oxacephem ring is not responsible for the inhibitory effect of latamoxef on platelet aggregation and 2) the carboxyl group in the amide side chain had no significant role in this inhibition.

Some β-lactam antibiotics, including carbenicillin (1–4), ticarcillin (5, 6) and latamoxef (7–9), produce platelet dysfunction. The carboxyl group in their side chain is postulated to be responsible for this adverse effect (10), but no direct evidence has been established.

In addition, latamoxef is an oxacephem antibiotic, in which the sulfur atom at position 1 of the cephem ring has been replaced by an oxygen atom; but the influence of this replacement on platelet aggregation has not yet been examined.

In the present experiments, we examined the effects of 1-S replaced and/or decarboxylated derivatives of latamoxef in order to clarify the influence of the oxygen replacement in the cephem ring and to determine the influence of the carboxyl group in the amide side chain of latamoxef on rabbit platelet aggregation in vitro.

Materials and Methods

Compounds and reagents: Compounds examined in the present experiments are shown in Fig. 1. They were synthesized by Narisada and his coworkers (11) of our laboratories. In addition, commercially available antibiotics, cefmetazole, cefotaxime and carbenicillin, were used for some experiments. The compounds were dissolved in saline before use. Adenosine diphosphate (ADP, Pharmacia, U.S.A.) was dissolved in saline, and collagen (Hormon-Chemie, West Germany) was suspended in saline. Platelet activating factor (PAF, Avanti Polar-Lipids, Inc.) was dissolved in ethanol and diluted with saline containing 2.5 mg/ml of bovine serum albumin (Sigma) and used within 3 hr after preparation.

Preparation of platelet-rich plasma (PRP): Mature male rabbits (NIJS-JW) weighing 2.4–2.6 kg were used. Blood was withdrawn
from the carotid artery by cannulation into centrifuge tubes containing 1/10 vol. of a 3.8% sodium citrate solution under sodium pentobarbital anesthesia (Somnopentyl, Pitman Moore; ca. 20 mg/kg, i.v.). After the mixture had been left standing for 20 min at room temperature, the blood samples were centrifuged at 22°C, 210xg for 10 min, and platelet-rich plasma (PRP) was obtained. The remaining blood samples were centrifuged at 3000 rpm for 10 min to obtain platelet-poor plasma (PPP). The PRP preparations were adjusted to 5-6 x 10^5 platelets per ul with the PPP.

Determination of platelet aggregation: Platelet aggregation was examined by the method of Born (12), using a Type AUTO-RAM 61 aggregometer (Rika-Denki Co., Ltd., Tokyo) as reported previously (13). Briefly, 230 ul of PRP was put into a cuvette, the mixture was warmed at 37°C for 1 min with stirring (1200 rpm), and 10 ul of a solution of test compounds was added. Exactly 2 min later, 10 ul of an agonist solution were added, and the changes in light transmission were recorded. The light transmissions of PRP and PPP were taken as 0% and 100% aggregation, respectively, and the maximum light transmission after addition of an agonist was designated as the maximum aggregation. The inhibition rate of a compound on platelet aggregation was expressed as the percentage of the maximum aggregation by a test compound to that by the saline control.

In addition to the maximum aggregation, the inhibitory effect on collagen-induced aggregation was examined by other parameters: lag time, 50% aggregation time (AT50, time to 50% aggregation of the maximum) and slope (tan θ) of aggregation (Fig. 2). The lag time should be defined as the time after addition of the agonist until the start of aggregation, but in practice, it was difficult to determine the exact time when the aggregation started. Rabbit platelets show a decrease in light transmission soon after the addition of collagen due to their shape change and then show an increase in light transmission as aggregation progresses. Therefore, we designated the lag time as the time after addition of the agonist until the light transmission returned to the initial level (Fig. 2).

Results
The effects of latamoxef, cefotaxime, cefmetazole and carbenicillin on ADP- and
collagen-induced platelet aggregation are given in Table 1. These antibiotics inhibited platelet aggregation at high concentrations, but the inhibiting effect on ADP-induced aggregation was more potent for latamoxef, whereas that on collagen-induced aggregation was stronger for cefmetazole and carbenicillin.

Figure 3 shows the effects of the 1-S analogue (II) of latamoxef (panel A) and those of the corresponding decarboxylated derivatives (III, IV) (panel B) on ADP-induced platelet aggregation. The 1-S analogue (II) of latamoxef showed almost the same inhibitory effect as latamoxef (I), but the decarboxylated derivatives (III, IV) caused somewhat weaker inhibition than the parent compounds (I and II).

The effects of these derivatives on collagen-induced platelet aggregation are shown in Fig. 4. Either the 1-S replacement or decarboxylation significantly increased the inhibitory potency compared with latamoxef. The inhibitory effects on collagen-induced aggregation were examined by not only the maximum aggregation but also examined by the lag time, AT50 and slope (tan θ) of the aggregation (Tables 2 and 3). They changed almost in parallel, but the lag time and AT50 seemed to be more sensitive. On the other hand, shape change occurred in most cases to almost the same extent even when aggregation was inhibited.

Figure 5 shows the effects of these derivatives on PAF-induced platelet aggregation. The 1-S analogue (II) showed a slightly stronger inhibition than latamoxef (I), and the decarboxylated derivatives (III, IV) caused much stronger inhibition than their parent compounds (I, II). The potency of the decarboxylated 1-S analogue (IV) of latamoxef was about 8-fold that of latamoxef (I). Similar data were obtained with arachidonic acid (final concentration, 0.2 mM).

<table>
<thead>
<tr>
<th>Compound</th>
<th>ADP (1 μM)</th>
<th>Collagen (4 μg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>(% Inhibition)</td>
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<td></td>
</tr>
<tr>
<td>Latamoxef</td>
<td>0.8</td>
<td>6.7</td>
</tr>
<tr>
<td>Cefotaxime</td>
<td>3.1</td>
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<tr>
<td>Cefmetazole</td>
<td>0</td>
<td>6.3</td>
</tr>
<tr>
<td>Carbenicillin</td>
<td>1.9</td>
<td>9.9</td>
</tr>
</tbody>
</table>

a % Inhibition: The percentage of the maximum aggregation in a test compound added to PRP to that in a saline control.

**Table 1. Effects of some β-lactam antibiotics on rabbit platelet aggregation in vitro**

**Fig. 3.** Inhibiting effects of 1-S replaced and/or decarboxylated derivatives of latamoxef on ADP (1 μM)-induced rabbit platelet aggregation in vitro.
Latamoxef (I), the 1-S replaced derivative (II) and the decarboxylated derivative (III) caused no remarkable inhibition even at the highest concentration (16 mM), but the decarboxylated 1-S derivative (IV) completely inhibited arachidonic acid-induced aggregation at 8 mM (data not shown).

**Discussion**

Bang et al. (7) demonstrated that many $\beta$-lactam antibiotics inhibited ADP-induced human platelet aggregation in vitro at high concentrations such as 3000-4000 $\mu$g/ml, which roughly corresponded to 6-8 mM. In the present experiments with rabbit platelets, the antibiotics so far examined also inhibited the ADP-induced aggregation at 4 to 8 mM or more. Bang et al. (7) stated in their report that the antibiotics caused no significant inhibition of collagen- or epinephrine-induced aggregation.
aggregation, but our previous experiment with human platelets (14) demonstrated that the antibiotics inhibited not only ADP-induced aggregation but also collagen-induced aggregation at high concentrations over 8 mM. A similar result showing the inhibition of collagen-induced aggregation was obtained with the present rabbit platelets, but the potencies of the antibiotics were much weaker (about 1/400) than that of aspirin.

The mechanism for the inhibition of platelet aggregation by β-lactam antibiotics is the inhibition of fibrinogen binding to platelets, probably at the site of the glycoprotein Iib/IIa complex (15), but not by the interaction with the ADP-receptor (7). Nakano et al. (15) demonstrated that β-lactam antibiotics did not impair the ADP binding to rat platelets and subsequent reactions in platelets such as phospholipase A2 activation, myosin light chain phosphorylation, and 20K and 40K protein phosphorylation, but inhibited the fibrinogen binding to platelets.

PAF, on binding to the receptor, was re-
ported to unmask the glycoprotein IIb/IIIa complex (16). Therefore, if β-lactam antibiotics exert their inhibitory effect on platelet aggregation by inhibition of the fibrinogen binding to the glycoprotein IIb/IIIa complex, they will also inhibit collagen-, PAF- and even arachidonic acid-induced platelet aggregation. In the present experiments, we intended to clarify the effect of replacing the sulfur atom in the cephem ring with an oxygen atom and the effect of a carboxyl group in the amide side chain on platelet aggregation; we found that the replacement of the sulfur atom by an oxygen atom caused no significant change or rather a decrease in the potency of inhibiting platelet aggregation, and the carboxyl group in the amide side chain had no significant role in this inhibition.

The 1-S replaced analogue (II) of latamoxef showed almost the same potency as latamoxef in inhibiting ADP-induced platelet aggregation, but showed slightly stronger inhibitions of collagen-induced and PAF-induced aggregations. On the other hand, the decarboxylated derivatives, III and IV, showed slightly weaker inhibitions on ADP-induced aggregation, but caused much stronger inhibition of collagen-induced and PAF-induced aggregations than their mother compounds. Among them, the decarboxylated 1-S analogue (IV) showed the strongest inhibition, and this compound inhibited even arachidonic acid-induced platelet aggregation, on which the other compounds (I-III) showed no inhibition even at the highest concentration (16 mM).

ADP produces full aggregation, showing secondary aggregation in human or guinea pig platelets, but only primary aggregation in rabbit platelets. Latamoxef and other β-lactam antibiotics inhibit not only the primary aggregation, but also the secondary aggregation in vitro. When the effects of the decarboxylated derivatives (III, IV) of latamoxef on ADP-induced aggregation were examined in human platelets, they caused stronger inhibitions of the secondary aggregation and slightly weaker inhibitions of the primary aggregation, as compared with latamoxef (14). Therefore, the decarboxylated derivatives seem to increase the inhibiting potency on the secondary aggregation, but seem to decrease the potency on the ADP-induced primary aggregation. This would be the reason why these compounds (III, IV) showed slightly weaker inhibitions on ADP-induced platelet aggregation in rabbits.

Although it is postulated that the carboxyl group in the amide side chain of carbenicillin is related to the inhibitory effect of this compound on platelet aggregation (10), to the best of our knowledge, no direct evidence is available to support this speculation. At least with latamoxef, we can say that the carboxyl group in the amide side chain is not responsible for the inhibition of platelet aggregation caused by this antibiotic. Platelet dysfunction has been reported even for many antibiotics possessing no carboxy group in the amide side chain such as penicillin G, ampicillin, methicillin (17, 18), nafcillin (19), piperacillin (20), sulbenicillin (21) and the others (7). Bang et al. (7) reported that many β-lactam antibiotics including those of the third generation inhibited ADP-induced platelet aggregation in vitro without regard to the presence or absence of the carboxyl group. These data seem to support our conclusion that the carboxyl group in the amide side chain is not responsible for the inhibition of platelet aggregation.

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