INFLUENCE OF β-LACTAM ANTIBIOTICS ON PLATELETS. I. IN VIVO EFFECTS OF LATAMOXEF AND RELATED β-LACTAM ANTIBIOTICS ON MEMBRANE PROTEINS AND GLYCOPROTEINS OF RAT PLATELETS

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The effects of latamoxef (moxalactam) and the other β-lactam antibiotics on platelet membrane were studied by intravenous administration of these drugs to rats for 8 d. Blood was taken from the abdominal aorta and washed platelets were prepared by centrifugation. Platelet membrane proteins labeled with 125I and the glycoproteins with 3H were used and analyzed by electrophoresis. Differences of membrane proteins and glycoproteins, compared with the control, were not observed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and two dimensional electrophoresis.

Keywords — latamoxef; platelet dysfunction; surface protein; glycoprotein; electrophoresis

INTRODUCTION

Latamoxef (moxalactam) (LMOX) is synthetic oxacephem antibiotic containing N-methyl-tetrazolethiol (NMTT) in the molecule and is widely used in the treatment of severe gram-negative infections. Occasional occurrences of bleeding diathesis in vitamin-K deficient patients treated with LMOX and the other NMTT-containing β-lactam antibiotics have been reported in several clinical studies, as well as in experimental animals. Although hypoprothrombinemia in the vitamin-K deficient state is prevented by administration of vitamin-K, some investigators indicate the LMOX impairs platelet functions. There have already been a number of reports of impaired platelet responses to aggregating and release-inducing agents when certain β-lactam antibiotics (ticarcillin, carbenicillin, etc.) have been added to platelet-rich plasma in vitro, or when patients, volunteers or animals received large doses of these antibiotics.

Recently, Iida and coworkers presented data that the electrophoretic mobility (E.P.M.) of platelets is increased by injection of LMOX in rabbit or by incubation of platelet-rich plasma with LMOX in vitro and they found adenosine diphosphate (ADP)-induced primary aggregation to be negatively correlated with E.P.M. The same results were observed in rat platelets treated with LMOX as well as other β-lactam antibiotics. Platelets carry an intrinsic net negative surface charge which has been shown to be a factor in platelet adhesion and aggregation. Therefore, changes in the surface charge would be expected to alter the electrostatic interactions between platelets and affect functional responses, as have already been exemplified on estrogen treated prostatic cancer patients or on rats administered oral contraceptives. The negative charge of platelets is mainly contributed by the surface exposed sialic acids of the membrane located glycoproteins, with minor contributions from phosphate groups and charged groups on polypeptides. To obtain insight into the effect of LMOX and related β-lactam antibiotics on the platelet membrane, we analyzed the electrophoretic characteristic of membrane proteins and glycoproteins of rat platelets by administration of high doses of drugs during certain periods.

MATERIALS AND METHODS

Drugs — LMOX, cefotaxime (CTX), ceftazidime (CMD) and carbenicillin (CBPC) were used.

Animals and Administration of Drugs — Sprague-Dawley rats (JCL, male, 8 weeks) were used and maintained on commercial rat chow (CA-1, Japan Clea, Tokyo) and water ad libitum. The dosage of drugs was 300 mg/kg/d, and the drugs were injected intravenously in the tail once a day for 8 d. Blood was taken from the abdominal aorta with an injection syringe containing 1/10
volume of acid citrate dextrose (2.5% w/v trisodium citrate, 1.5% w/v citric acid, 1.5% w/v dextrose) under sodium methylxanthal anesthesia.

Preparation of the Washed Platelets — Washed rat and human platelets were prepared mainly by the method of Phillips et al.18) Blood was centrifuged at 160 × g for 10 min to give platelet-rich plasma (PRP). PRP was further centrifuged at 2000 × g for 15 min and the supernatant fluid was discarded. The pellet was resuspended in a washing buffer (0.12 M NaCl, 0.0129 M trisodium citrate, 0.03 M dextrose). Contaminated red blood cells were removed by centrifugation at 120 × g for 15 min. This differential centrifugation was repeated more than twice and the washed platelets were finally suspended in an appropriate buffer for the following procedures.

Labeling of the Platelet Membrane Proteins and Glycoproteins — [125I] Iodination of platelet surface proteins of rat as well as human platelets was performed according to the method of Painter et al.19) Washed platelets (0.2 × 10⁹ cells) were suspended in 1 ml of Tris-buffered saline (30 mM Tris, 120 mM NaCl, pH 7.4) and 200 μCi of Na¹²⁵I (14.8 mCi/μg of iodine, Amersham) and 40 μg of lactoperoxidase (Sigma) were added. Ten μl of 1 mM hydrogen peroxide were added in 1 min intervals 10 times. Then, 1 ml of non-labeled platelets (0.8 × 10⁹ cells/ml in Tris-buffered saline, pH 7.4) were added, diluted with 10 ml of Tris-buffered saline (pH 6.4) and suspended in 100 μl of the same buffer. Labeling of respective rat and human platelet glycoproteins were performed by the method of McGowan et al.20) Washed platelets (1 × 10⁹ cells) were suspended in 1 ml of Hapes buffer (150 mM NaCl, 1 mM ethylenediaminetetraacetic acid (EDTA), 10 mM Hapes, pH 7.40) and 0.1 ml of 20 mM sodium metaperiodate was added. The suspension was kept for 10 min at 4 °C in the dark, washed three times with Hapes buffer and resuspended in 1 ml of the same buffer. Then, 1 mCi of NaB³H₄ (532 mCi/mmole, New England Nuclear) was added and the suspension kept for 30 min at room temperature, washed three times with Hapes buffer and suspended in 100 μl of Tris-buffered saline (pH 6.4).

Analysis of the Labeled Platelets — Sodium dodecylsulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was performed according to the

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FIG. 1. Comparison of Platelet Membrane Proteins between Rat and Human
Platelets were iodinated, solubilized in the presence of 2.5% 2-mercaptoethanol and subjected to electrophoresis on 7.5% acrylamide gel according to Laemmli.36) Dried gel was autoradiographed. (A) rat platelets and (B) human platelets.

FIG. 2. Membrane Glycoproteins of Rat Platelets
Platelets were labeled with tritium and electrophoresed as in Fig. 1. Dried gel was fluorographed. Roman numbers written on the right side of the figure indicate an approximate position of glycoproteins characterized in the human platelets.
method of Laemmli. Two-dimensional gel electrophoresis was performed according to the method of O'Farrel. Labeled proteins and glycoproteins were detected by autoradiography and fluorography respectively.

RESULTS
Comparison of the Membrane Proteins and Glycoproteins of the Platelets among Animal Species

Four major glycoproteins (Ia, Ib, IIa, and IIb) and a number of minor glycoproteins have been identified on human blood platelets depending on the electrophoretic separation, staining and labeling techniques used. The major glycoproteins in a wide variety of animal species have been illustrated to have marked interspecies similarities, although very little is yet known about the functional relationships. We compared membrane proteins and glycoproteins of rat platelets with those of humans by SDS-PAGE followed by autoradiography and fluorography. As shown in Fig. 1, species differences of membrane proteins between rat and human platelets were observed. Fluorography of tritium labeled glycoproteins of rat is shown in Fig. 2. The major band observed in rat seems to correspond with GPIb, which is a major glycoprotein of human platelets and is also known as the

![Figure 3](image)

**FIG. 3. Effect of β-Lactam Antibiotics on Rat Platelet Membrane Proteins**
Platelets from antibiotic-treated rats were iodinated. Autoradiograms of two-dimensional gel electrophoresis of the platelets are shown. (A) control, (B) CTX, (C) CMD, (D) LMOX and (E) CBPC. Control preparation was made by administration of saline in the same manner.
specific binding site for the von Willebrand factor as well as thrombin.

Other bands corresponding to GPIIb, GPIII and GIP IV of the human platelets were also seen.

**Effect of β-Lactam Antibiotics on the Rat Platelet Membrane**

After administration of β-lactam antibiotics (LMOX, CTX, CMD or CBPC) to rats intravenously for 8 d, the animals were sacrificed and washed platelets were immediately prepared and labeled as described. The controls were prepared by administration of saline to rats in the same manner. These preparations were analyzed by two-dimensional electrophoresis. As shown in Figs. 3 and 4, no differences were observed for platelets from rats administered antibiotics compared with those of control.

**DISCUSSION**

Many reports on the mechanism for platelet dysfunction have been presented over the past 10 years, although a precise mechanism for suppression of the platelets has not been well understood. Several hypotheses can be proposed for platelet dysfunctions by antibiotics: 1) impairment of agonist binding to the platelets or des-
abilization of the initial interactions between cells due to electrostatic alteration of the platelet membrane, 2) perturbation of platelet membrane lipid bilayer by the interaction of weakly lipophylic antibiotics, which may cause functional suppression of subsequent signal transduction within the cells.

Membrane components of rat platelets that bind chemical agonists (ADP, thrombin, etc.) has not yet been characterized. Thus, receptors for these agonists appear to include several proteins or glycoproteins that are presumably embedded within the membrane’s lipid bilayer as predicted from the results presented in human platelets. The approximate life span of rat platelets has been reported to be about 4 d, therefore 8 d administration of the drug might be sufficient to investigate the effects of drugs during platelet production from megakaryocytes. We could not detect any electrophoretic changes on membrane proteins or glycoproteins of the platelets and small changes could not be detected by our technique. Another possibility for the increased net negative charge of the platelets treated with antibiotics may be due to direct binding of drugs to the platelet surface, all of which generally contain carboxyl groups with the molecules and may interact hydrophobically with phospholipid bilayers of the platelets, as previously shown for penicillin on artificial phospholipid bilayer. This assumption agrees well with an in vitro observation that platelets incubated with antibiotics become more negatively charged and correlated with impaired responses to aggregating agents. Neither LMOX nor other related antibiotics affected bulk platelet membrane lipid fluidity as investigated by electron spin resonance (ESR) spin labeled technic. However, a negative result which reports on the average motion of membrane lipids does not totally exclude more localized yet significant interaction of the drugs with membrane lipids. We analyzed the phospholipid content of the platelets from control and drug injected rats. However, no differences were observed (data not shown).

Our data were obtained from rats and since functional differences are well known to exist for platelets among animals species, we cannot extrapolate our results with animals to effects in humans. We are now conducting investigations on the precise effects of β-lactam antibiotics on agonist binding, as well as on the signal transduction of the platelets. These results will be published elsewhere.

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