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Effect of procainamide on ultrastructure of blood platelet in rabbits

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KEY WORDS procainamide; arachidonic acids; platelet aggregation; electron microscopy

AIM: To study the effect of procainamide (PA) on the ultrastructure of blood platelets. **METHODS:** Arachidonic acid was added to PA-treated platelet-rich plasma to induce platelet aggregation. The 50-nm sections were examined with a transmission electron microscope. **RESULTS:** PA 8.5 - 136 $\mu mol \cdot L^{-1}$ markedly inhibited changes of pseudopods, α-granules, dense granules, glycogens, open canalicular system, and dense tubular system. **CONCLUSION:** PA markedly inhibited the changes of ultrastructure of blood platelet and releasing response.

Procainamide (PA) not only inhibited the platelet aggregation induced by adenosine diphosphate (ADP), arachidonic acid (AA), thrombin, $CaCl_2$, calcimycin (A23187), and clonidine, but also inhibited TXB_2 production[1-4]. PA reduced platelet adhesion[5], and inhibited pulmonary thromboembolism and malondialdehyde (MDA) production[6]. To investigate the mechanism of PA-produced inhibition on platelet aggregation, we studied the effect of PA on platelet ultrastructure changes in AA-induced platelet aggregation.

MATERIALS AND METHODS

Reagents PA was bought from Beijing Pharmaceutical Factory (lot № 906048). AA was obtained from Fluka (lot № 65011/1) and dissolved in anhydrous ethanol before use. Glutaraldehyde was purchased from Nacalai Tesque, (lot № M_2K1854) and 2.5 % solution was prepared with phosphate buffered saline (PBS, pH 7.4). 1 % Osmic acid (Johnson Matthey, Material Technology, UK, Batch № OB12BO8) was prepared with PBS (pH 7.4). Spurr's resin[7] consisted of vinyl-cyclohexen-dioxid (VCD, product № 94956, Fluka), diglycidylether of polypropyleneglycol (DER736, analysis № 257322/11094, Fluka), nonenylbernsteinsäure-anhydrid (NSA, product № 74378, Fluka) and dimethylaminoethanol (DMAE, lot № 38990, Taab Laboratories, England).

Platelet aggregation experiment[2,3] New Zealand rabbits of either sex (n = 6, weighing 2.1 ± 0.3 kg) bred by the Experimental Animal Centre of First Military Medical University. Turbidimetric tubes containing 0.5 mL PRP was divided equally into 6 groups (6 tubes/group). A: normal group; B: treated with 0.85 % saline 50 μL; C: treated with indometacin (0.98 $\mu mol \cdot L^{-1}$) 50 μL, D, E, and F: treated with 50 μL PA (136, 34, and 8.5 $\mu mol \cdot L^{-1}$, respectively). Turbidimetric tubes of group B - F were placed in the aggregometre (Model BS631, Beijing Biopharmaceutical Factory, Beijing) and AA 10 μL (94 $\mu mol \cdot L^{-1}$) was added to induce platelet aggregation.

Electron microscopy Aggregated-PRP (platelet-rich plasma) was spun at 1100 × g at 4 ℃ for 15 min. Platelet pellets were fixed in 2.5 % glutaraldehyde for 2 h, and then washed with PBS, post-fixed in 1 % osimic acid for 2 h, dehydrated in graded acetone, and embedded in Spurr's resin. The embedded blocks were sliced into 50-nm section with the LKB-5 ultratome (LKBV Co, Switzerland). The sections were stained with uranyl acetate and lead citrate, and examined with JEM1200EX transmission electron microscope (Japan Electron Co, Tokyo).

RESULTS

Normal group (Fig 1A) The majority of platelets were circular or oval, demarcation membrane system (DMS) was clear, glucocalyx was distributed evenly on platelet surface, and platelet membrane did not show pseudopod-like

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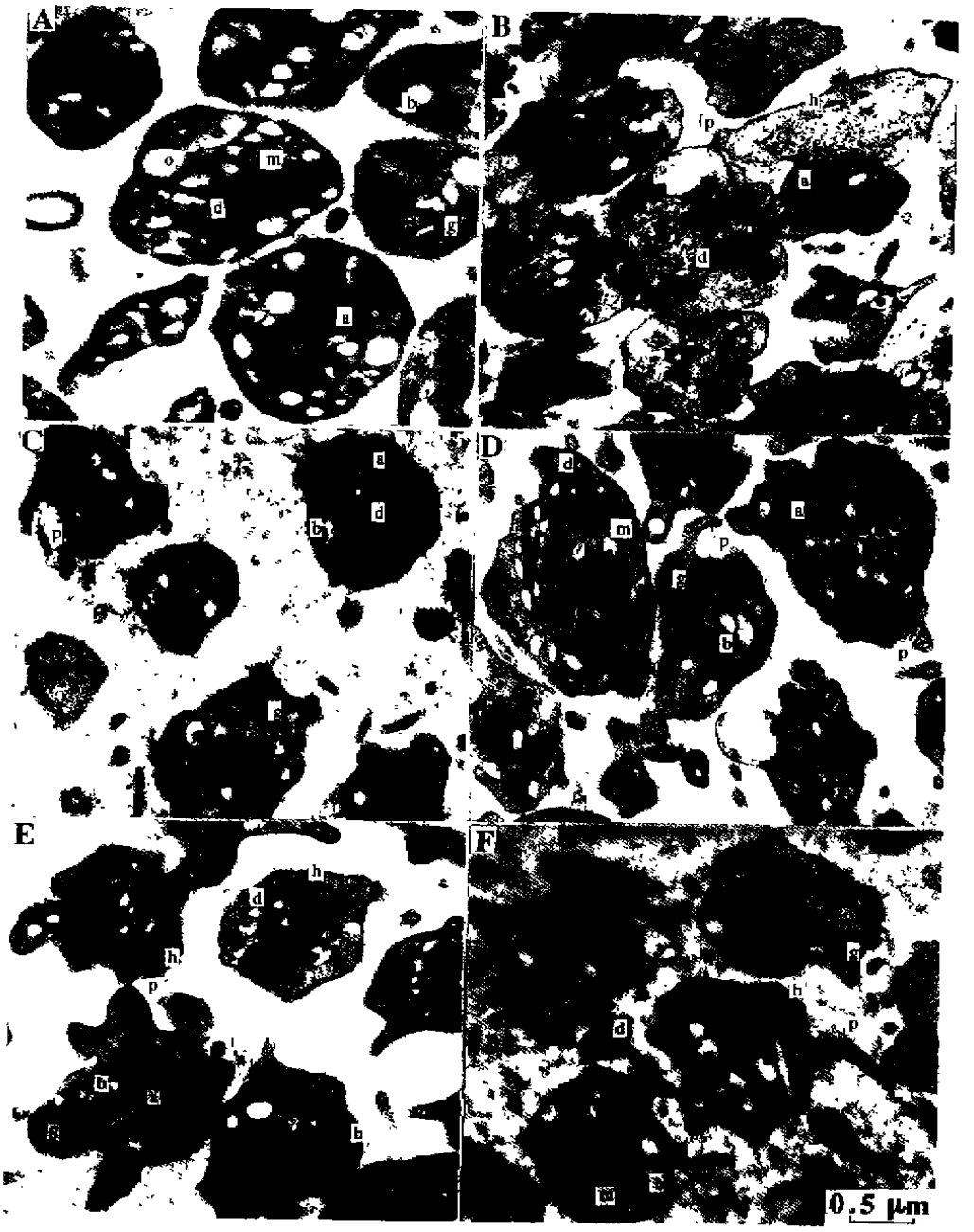

Fig 1. Electron micrographs of effect on blood platelet ultrastructure by procainamide (PA) in rabbits. A) × 19 600, B) × 19 400, C) × 19 500, D) × 19 500, E) × 19 100, and F) × 19 100. a = α-granule, b = dense granule, d = dense tubular system, g = glycogen, h = hyaloplasm, m = mitochondrion, o = open canalicular system, p = pseudopod.

process. Open canalicular system (OCS) distributed separably in the form of many, vesicle-like structures in cytoplasm. There were less dense tubular systems (DTS) in cytosol, and the electron density of the DTS was similar to that of cytoplasm. In dense granule, electron density was very high, and the space between DMS and cytoplasmic inclusion was a transparent interval. The number of α-granule increased. There was moderate electron density in α-granules, their surfaces were surrounded by demarcation membrane, and high electron density nuclei

existed in the centre of partial granules. Abundant glycogens distributed separably or tuftedly in cytoplasm. Mitochondrial cristae were obscure, and there was no clear demarcation line between hyalomere and granulomere.

NS group (Fig 1B) Platelet surface extended rough pseudopods. Most platelets adhered together and demarcation membranes blended each other. α-Granules, dense granules and glycogens reduced or disappeared obviously, and hyaloplasm expanded strikingly. The number of OCS reduced and its cavity narrowed down. The number of DTS increased, its cavity expanded, and its electron density enhanced.

Indometacin group (Fig 1C) The platelets manifested circular and their surfaces extended and formed slender pseudopod-like processes. There were abundant α-granules, dense granules, and glycogens in cytoplasm. The number of OCS reduced and its cavity narrowed down. The number of DTS increased mildly. The demarcation line between hyalomere and granulomere was not clear. The morphology of blood platelet was similar to that of normal group.

PA groups In 136 $\mu mol \cdot L^{-1}$ group, most of the platelets were oval, DMS was lucid, and platelet surface extended to form thin pseudopod-like saddle-backing. There were plentiful α-granules, dense granules, and glycogens which distributed diffusely with mitochondria, and lysosomes in cytoplasm. Demarcation line between hyalomere and granulomere was obscure. The number of OCS increased and its cavity narrowed down. DTS existed mixedly in cytoplasm with OCS. Electron density was slightly higher than that of the normal group. In comparison with NS group, the ultrastructure change was inhibited (Fig 1D).

In 34 $\mu mol \cdot L^{-1}$ group, the morphology of part of platelets was irregular and their surface extended to form rough pseudopods. Plasmacules came together towards platelet center. There was clear demarcation line between hyalomere and granulomere. The number of DTS and its electron density increased (Fig 1E).

In 8.5 $\mu mol \cdot L^{-1}$ group, the morphological changes of platelets were potentiated, granule and glycogen in part of platelet were decreased, and the cavity in a small amount of DTS was expanded. In comparision with NS group, PA had stronger inhibitory effect on the morphological changes of these platelets (Fig 1F).

DISCUSSION

Blood platelets are multifunctional, sensitive, anuclear cells. Transformation, releasing and aggregating reaction appeared immediately in blood platelets when they were stimulated by some factors, such as aggregating-inducers, ADP, AA, thrombin and other. In the present paper, PA inhibited ultrastructure changes in AA-induced platelet aggregation strikingly, and the inhibitory effects of PA were dose-dependent. These results are uniform with the reported inhibition of PA on AA, ADP, thrombin and other-induced platelet aggregation[1-4].

There is Ca^{2+}-pump on DTS membrane in platelet, which transfers the Ca^{2+} from cytoplasm to DTS when blood platelet stands in static condition, which decreases free calcium concentration in cytoplasm. When blood platelet is activated, Ca^{2+} is released to cytoplasm from DTS, and free calcium activates constringent protein, promoting platelet transformation, extending-pseudopod, granule centralization and releasing reaction and other. In the present paper, PA inhibited the changes of DTS, speculating on the reduction of free calcium in cytoplasm and reaction as mentioned above. Also, the result was accordant with our report that PA reduced cytosolic free Ca^{2+} level in blood platelet[4,8].

Blood platelet needs energy when it maintains normal morphology and is activated. Platelet energy comes from glycogenolysis in which Ca^{2+} is necessary. In the present study, in PA groups, the amount of glycogen was higher than that of NS group, indicating the reduction of free-Ca^{2+} in cytoplasm. Therefore free-Ca^{2+} reduction is one of main mechanisms that PA inhibits platelet aggregation probably.

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普鲁卡因胺对兔血小板超微结构的影响

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关键词 普鲁卡因胺；花生四烯酸类；血小板聚集；电子显微镜检查

目的：研究普鲁卡因胺(PA)对血小板超微结构的影响。方法：将花生四烯酸(AA)加入经 PA (8.5 – 136 μmol·L^{-1})处理的富含血小板血浆(PRP)中，诱导血小板聚集，制备超薄切片，用电镜观察各组血小板超微结构变化。结果：PA 8.5 – 136 μmol·L^{-1}显著地抑制血小板伪足，α 颗粒，致密颗粒，糖原，开放管道及致密管道系统结构的改变。并存在着剂量依赖关系。结论：PA 对血小板超微结构和释放反应均有显著的抑制作用。

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Platelet-released ADP stabilizes PAF-induced rabbit platelet aggregation by stabilizing intracellular calcium[1]

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KEY WORDS platelet aggregation; platelet activating factor; apyrase; adenosine diphosphate; calcium

AIM: To examine whether platelet-released adenosine diphosphate (ADP) would contribute to the stabilization of rabbit platelet aggregation induced by platelet activating factor (PAF). **METHODS**: Rabbit platelet aggregation induced by PAF was measured turbimetrically. ADP release from rabbit platelets stimulated by PAF was determined by HPLC. Intracellular Ca^{2+} was measured using Ca^{2+}-sensitive fluorescent indicator Fura 2-AM. **RESULTS**: PAF ≥ 1 nmol · L^{-1} induced full platelet aggregation, which did not deaggregate over 5 min after aggregation reached peak. Platelet aggregation was deaggregated in a concentration-dependent manner by subsequent addition of ADP scavenger ATP-diphosphohydrolase (apyrase) at 5 – 100 mg ·L^{-1}. PAF 3 nmol ·L^{-1} stimulated release of ADP (29 % *vs* 6 % of control), and elicited a rapid rise in intracellular calcium ($[Ca^{2+}]_i$) which peaked at approximately 15 s. Then the $[Ca^{2+}]_i$ gradually decayed from 585 ± 80 nmol ·L^{-1} within 100 s to a low level (364 ± 82 nmol ·L^{-1}). Apyrase 100 mg·L^{-1}, added 2 min after PAF, reduced $[Ca^{2+}]_i$ to a lower level (171 ± 29 nmol · L^{-1}). **CONCLUSION**: Platelet-released ADP stabilizes PAF-induced rabbit platelet aggregation by stabilizing $[Ca^{2+}]_i$ at elevated level.

Platelet aggregation plays an important role in the pathogenesis of thrombosis. Deaggregation appears to take place most readily when the release reaction has not occurred[1]. The effects of the release reaction on the ability of platelets to

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