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Attachment of Polymyxin B Sulfate to Polystyrene Fiber

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Introduction

We previously reported the efficacy of PMX-F (1 – 2). It was observed that PMX-F could detoxify 0.5 – 5 mg LPS per fiber in a solution of 0.1 mg/ml LPS. Our interest is to evaluate the capacity of this material to neutralize low-density endotoxin with specific respect to the clinical treatment of endotoxemia. This study is designed to evaluate the process by which PMX-F is manufactured, its capacity to neutralize low-density of endotoxin, and its biocompatibility.

Materials

Endotoxin: E. coli; B4: LPS was obtained from Difco Laboratories; Polymyxin B Sulfate (PMX) was purchased from Pfizer Taito Co., Ltd.; PMX-F was produced by Toray Company; endotoxin assay was measured by the Pyrodick test (Teikoku Zoki Co., Ltd.) and the Toxicolor test (Seikagaku Kogyo).

Manufacturing Process of PMX-F

1) Active halogen: polystyrene fiber was converted into an α-chloracetamide-group containing fiber (A) trough a reaction with N-methylol — α-chloracetamine in sulfurice acid. A was animated with etylene diamine and succinate combined with succinic anhydride to obtain the carboxy-group-containing fiber B. PMX-F was prepared by inducing the reation of PMX with (A) in MgO (Fig. 1).

2) Condensation: The reaction of fiber (B) was induced in water-soluble carbodiimide. A much larger amount of PMX adhered to this type of fiber than did to the halogen type (Fig. 1). PMX-F obtained through a reaction with halogen has the advantage over condensation of being less expensive and involving a shorter manufacturing process.

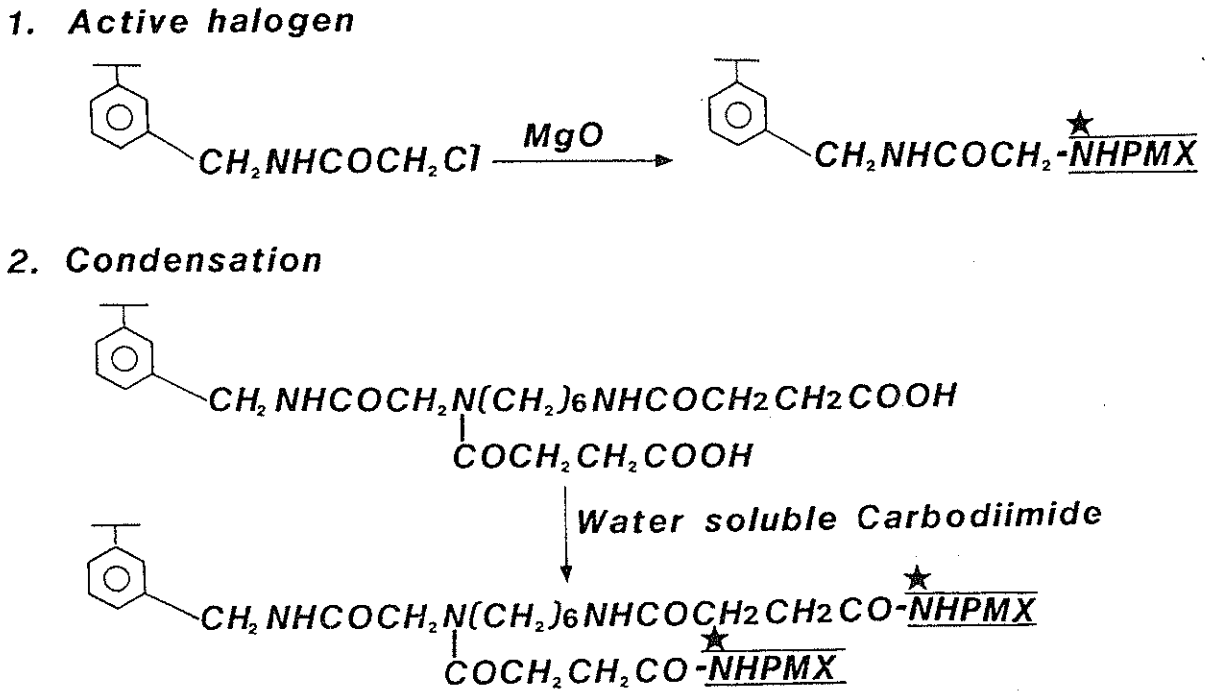

Fig. 1. Methods of immobilization. (MgO: Magnesium Oxidase, PMX: Polymyxin B.)

Neutralization of Low-Density ET

Fig. 2 summarizes the experimental circuit. 10 mg of ET were added to 20 ml of isotonic saline and then perfused through a column containing 2 g PMX-F for 1 hr at a flow rate of 10 ml/min. After the perfusion of PMX-F, the ET concentration decreased from 0.5 mg to 0.1 mg as determined by the Pyrodick assay. Next, we evaluated the amount of PMX-F necessary to detoxify a low-concentration ET solution.

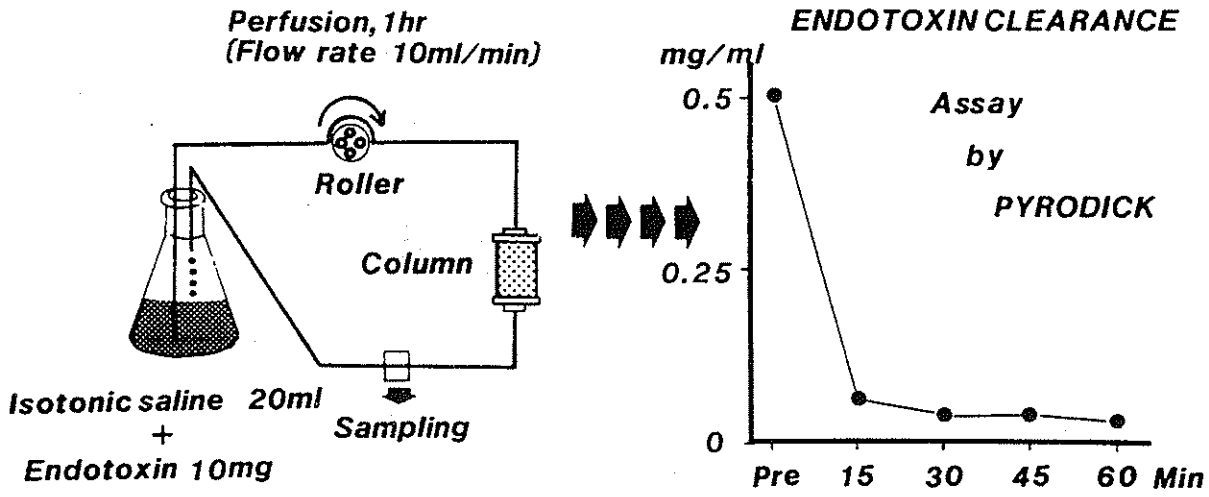

Fig. 2. Removal of endotoxin by PMX-F perfusion.

Table 1. Neutralizing endotoxin in the bovine serum.

. Endotoxin (g/ml) Prepared observed		Neutralized endotoxin 10 cm fiber	50 cm fiber	Neutralizing Ability/10 cm fiber
10 micro	12.6	2.1	2.1	10.5 micro
100 nano	37.8	9	12	28.8 nano
10 nano	1.2	0.3	0	0.9 nano

Table 1 summarizes the results of this study. Three low-concentration ET solutions were prepared. Either 10 cm or 50 cm of PMX-F were mixed with each ET solution. The neutralizing capacity per 10 cm of fiber was calculated. It was observed that PMX-F satisfactorily detoxified low-concentration ET solutions.

Biocompatibility of PMX-F

We performed direct hemoperfusion (DHP) on 5 dogs for 2 hrs using heparin as an anticoagulant. Table 2 presents the data regarding changes in protein during DHP by PMX-F. This newly invented fiber exhibited a satisfactory biocompatibility with protein in this study. Next, the biocompatibility of PMX-F with RBC, WBC, and platelets was examined. The results are presented in Fig. 3. Regarding its blood compatibility with WBC, PMX-F effected the same decrease in WBC as did DHP-1. On the other hand, with respect to the platelet count, PMX-F effected a slighter decrease than did the other materials (DHP-1, IONEX). This study on blood compatibility was carried

Table 2. Changes in protein during DHP using PMX-F. (DHP: Direct hemoperfusion, PMX-F: Polymyxin-B immobilized fiber.)

Protein

	Flow Rate (ml/min)	Duration (mins)	Pre-perfusion (mg/dl)	Post-perfusion (mg/dl)	Decrease (%)
ALB	9	120	5.43	5.39	★ 0.7 ±0.4
α_1-Glob	9	120	0.80	0.77	3.7 ±1.1
α_2-Glob	9	120	1.03	1.02	0.01±0.01
β-Glob	9	120	0.70	0.66	5.7 ±1.1
γ-Glob	9	120	0.92	0.91	1.1 ±0.90

★ : (Mean±SE)

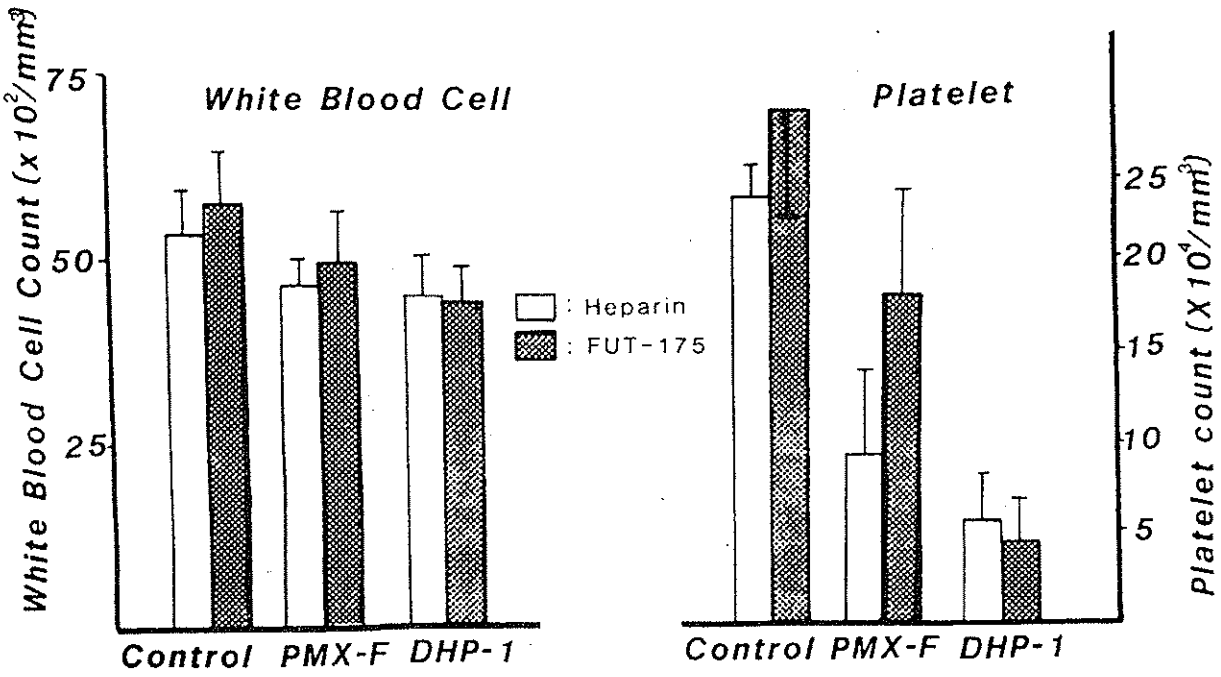

Fig. 3. Changes in white blood cell and platelet counts in ex vivo perfusion with human blood.

out via an ex vivo perfusion of human blood with FUT-175 or heparin used as an anticoagulant. A more detailed study on the blood compatibility of PMX-F will be undertaken.

Discussion

We were able to immobilize PMX through condensation or by inducing it to react with halogen. A much larger dose of PMX per 1 g of fiber adhered to the polystyrene fiber after the condensation reaction than it did following the halogen reaction. However, there were no differences in their capacity to neutralize ET per 1 g fiber. Perhaps the amount of PMX immobilized at the surface of the fiber must be measured in addition. Ineffective PMX may adhere to the internal surface of the fiber. PMX-F obtained through a reaction to halogen has the advantage over condensation of being less expensive and involving a shorter manufacturing process. We previously reported on the efficacy of PMX-F in high-concentration ET solutions. In the present report we evaluated this fiber in low-concentration ET solutions. PMX-F satisfactorily detoxified low-concentration ET solutions. Also, the biocompatibility of PMX-F with platelets was shown to be fairly good, and with RBC, WBC and protein, extremely good. Oka reported the effect of FUT-175 (6-amidino-2-naphtyl-4 guanidino bezoate-dimethanesulfonate), a new synthetic protease inhibitor, on PMX-F3 (3). In the future when DHP using PMX-F is performed in the treatment of endotoxemia etc., FUT-175 should be used as an anticoagulant. More detailed studies are presently under way regarding the blood compatibility of PMX-F, and we are now in the process of inventing a more safe and powerful fiber.

References

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