EVALUATION OF CHITOSAN AS A NEW HEMOSTATIC AGENT: IN VITRO AND IN VIVO EXPERIMENTS

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INTRODUCTION

Life-threatening bleeding through the pores of vascular grafts is a well-recognized complication in major cardiac and thoracic aortic surgery despite the use of low porosity dacron grafts (1, 2). There have been a number of investigations in which this situation is improved by the preclothing of the grafts, and Bethea and Reemtsmak (1) have described a process where-in the graft was autoclaved after immersion of the graft into heparinized blood. Cooley (3) has since modified the technique by immersion of the grafts in plasma rather than blood, and then autoclaving. He found that this improved the handling characteristics of the grafts. More recently, Malette et al. (4) described the use of a new hemostatic agent, chitosan. They replaced the abdominal aorta of dogs by grafts which had been treated in chitosan and found, at autopsy, that the grafts were encased in smooth muscle, with a living endothelial lining and an abundant vasa-vasorum. The control grafts showed the usual fibrous healing. They also found the chitosan-treated grafts to be impermeable to blood. Following these observations, we decided to investigate the use of chitosan as a hemostatic agent in critical situations, mimicking in vivo cardio-pulmonary bypass conditions where patients are given high doses of heparin systemically. We also looked at the effect of chitosan on graft healing and pseudointima formation. Several techniques for the treatment of the grafts with chitosan were used.

In this study we infiltrated and coated grafts with chitosan in several forms. The film-forming characteristics of chitosan served to seal the grafts, thus acting as a hemostatic agent. The in vitro and in vivo experiments indicate that this approach is promising.

MATERIALS AND METHODS

Graft Hemostasis: In Vitro Study

A perfusion system with systemic pressure maintained at 100 mm Hg by a roller pump (Med Science electronic pump) and a flow of 165 ml/min was used. Different groups of 6 cm long vascular low-porosity Cooley dacron vascular grafts (n = 5 per group) were perfused for 5 minutes with human packed red blood cells diluted in 200 ml of a 0.9% saline solution with 2100
u of heparin added per 500 ml of solution (equivalent of 300 \( \mu \)/kg in a 70 kg man). The blood losses were collected for the 5 minutes of the perfusion and weighed.

The chitosan used in these experiments was specially prepared from a single species of crab (blue crab) and had a molecular weight of about 900,000. A solution of chitosan acetate was prepared, at a concentration of 8 wt pct. The grafts were dipped into chitosan acetate solutions for fifteen minutes and allowed to dry in air. At this point, the grafts were coated with chitosan acetate. The coated grafts were then treated in 1 molar sodium hydroxide for 30 minutes, rinsed in distilled water, and then dried in air. This treatment converted chitosan acetate to chitosan. In one experiment, the grafts were dipped into chitosan acetate and then perfused wet. Since the blood was slightly alkaline, the acetate was probably converted to chitosan in situ, in the form of a gel.

Six different groups of grafts were perfused.

I. Control: grafts without preclotting.
II. Grafts preclotted with non-heparinized human blood.
III. Grafts autoclaved after immersion in heparinized human blood.
IV. Grafts treated with chitosan solution, used in the air-dried conditions.
V. Grafts treated with chitosan, air dried and then autoclaved at 130\(^\circ\)C.
VI. Grafts immersed in chitosan solution and perfused wet (chitosan gel).

Graft Hemostasis and Healing: In Vivo Experiment

![Diagram of graft hemostasis and healing: in vivo experiment.](image)

Figure 1. Graft hemostasis and healing: in vivo experiment.

Ten mongrel dogs weighing from 20 to 30 kg underwent a midline laparotomy; the infrarenal aorta was dissected free from the surrounding tissue and the collateral branches were tied. Two 3 cm. lengths of lowporosity Cooley vascular dacron grafts were inserted into place after resection of a suitable length of abdominal aorta. All anastomoses were end-to-end (Figure 1). The pulsating grafts were observed for 5 min in order to observe hemostasis after the clamps were removed, and finally, the retroperitoneum and the abdominal incision were closed. The dogs were divided into two groups: