Effects of Local Delivery of Antibiotics from Chitosan Constructs on Hemostasis

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Statement of Purpose: In traumatic injury, local delivery of antibiotics has the advantage of increased efficacy against contaminating bacteria and reduced risk of toxicity compared to systemic administration. The lyophilized chitosan sponge, a biopolymer drug delivery device, is being used to deliver these compounds over an extended period of time maintain therapeutic levels of the drug. Chitosan is known to have hemostatic properties, owing to its positive charge in acidic solvent. However, neutralizing these constructs to improve biocompatibility and drug elution characteristics may decrease these hemostatic properties. In addition, the incorporated antibiotics may have an effect on hemostasis, particularly since they are in high concentrations within the drug delivery device. In this study, the hemostatic characteristics of drug delivery sponges were compared to those of antibiotics alone and to controls.

Methods: *Fabrication.* Chitosan powder (Primex, Iceland) with 71% DDA was dissolved to 1 w/v % in a 1 v/v % solution of lactic and acetic acid (75:25). Solutions were frozen at -80°C and then lyophilized in a freeze dryer (Labconco, Kansas City, MO) for 72 hours. Sponges were neutralized by immersion in 0.2 M sodium hydroxide solution and rinsing with distilled water. Neutralized sponges were frozen, relyophilized, and sterilized with gamma irradiation at 32 kGy.

Antibiotic Loading. Chitosan sponges were hydrated with 5 mg/mL antibiotics in phosphate buffered saline (PBS) solution at the following variations: (1) amikacin (MP Biomedicals, Solon OH), (2) vancomycin (MP Biomedicals), (3) daptomycin (Cubist Pharmaceuticals, Lexington, MA), (4) amikacin/vancomycin, and (5) amikacin/daptomycin.

Prothrombin Time Assay. Blood was collected from normal, healthy donors into 0.1 M buffered citrate anticoagulant (9:1 ratio). Platelet-rich plasma (PRP) was obtained by centrifugation of the anticoagulated blood. Platelet-poor plasma (PPP) was obtained by centrifuging the residual blood. Prothrombin time was determined with a ST4 Coagulation Instrument (Diagnostica Stago, Asnieres, France) using STA – Neoplastine Cl Plus reagent (Diagnostica Stago). Chitosan samples were placed in a microcentrifuge tube and saline or antibiotics were added for 1 min before PPP was added to the tube. For antibiotic-only assays, PPP was mixed with an aliquot of antibiotic solution of appropriate strength to yield the desired final antibiotic concentration. PPP plus saline, without sponge or antibiotic, served as the control.

Platelet Activation Assay. Blood was collected and platelet count in PRP was adjusted to 2.5×10^8 platelets/mL. Flow cytometry was preformed to quantify surface expression of platelet activation markers CD62P and CD63. Following incubation with samples, an aliquot of PRP was removed from each tube and incubated with anti-CD62P and anti-CD63 (BD Biosciences, San Jose, CA) antibodies. An aliquot of this solution was diluted in PBS and assayed on a BD FACSCalibur flow cytometer. Negative controls (saline) and positive controls (thrombin-receptor agonist peptide) were used to determine platelet activation.

Statistics. All data are presented as the mean ± standard deviation and analyzed initially by one-way ANOVA. For the prothrombin time and platelet activation assays, the Student Newman-Keuls or Dunnett's multiple comparison procedures were used to evaluate differences between specific treatments. No outlying data points were detected using Chauvenet's criterion.

Results: Daptomycin and Vancomycin increased prothrombin time at higher concentrations (above 400 µg/ml) (Fig. 1). At these higher concentrations, combination with chitosan sponges did not have a significant effect. Vancomycin increased platelet activation at high concentrations alone and in combination with other antibiotics (Fig. 2). Platelet activation was slightly reduced when antibiotics were combined with chitosan sponges (Fig.3).

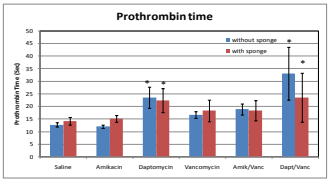

Figure 1. Prothrombin time for different combinations of 2000 µg/ml antibiotic with and without chitosan carrier. Asterisks denote statistical difference compared to saline controls.

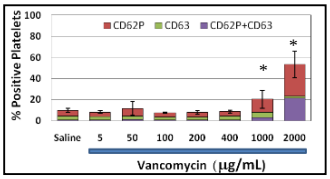

Figure 2. Platelet activation at varying concentrations of vancomycin (without chitosan carrier). Asterisks denote statistical difference compared to saline controls.

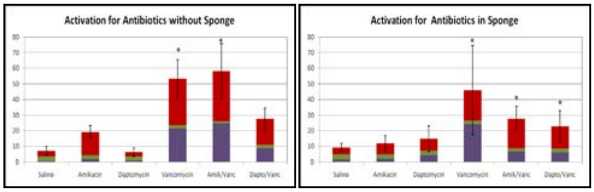

Figure 3. Platelet activation response to 2000 µg/ml of various antibiotics with and without chitosan carrier.

Conclusions: Neutralized chitosan sponges do not have a significant effect on prothrombin time or platelet activation. However, antibiotics delivered from these constructs could have an effect on hemostasis, since they can be found at high concentrations locally. Delivering vancomycin in a local wound site could result in increased platelet activity and coagulation.

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