Plasminogen Free Fibrin Sealant

Israel Nur; Lilliana Bar; Roberto Meidler, Anne J Gorman;
Omrix Biopharmaceuticals and Johnson & Johnson Wound Management

Statement of Purpose: A plasmin(ogen) free fibrin sealant has been developed such that the sealant is inherently stable. Plasmin(ogen) is removed from the fibrinogen component by a novel affinity chromatographic resin containing a tranexamic acid ligand. The resulting sealant does not require addition of fibrinolytic inhibitors and demonstrates an in vivo degradation profile similar to that of other fibrin sealants containing inhibitors. Safety and hemostatic efficacy has been demonstrated in rabbit liver resection model of moderate to severe bleeding. No neurotoxic effects were found in a rabbit craniotomy study.

Methods: Plasminogen removal: The BAC component of Crosseal (a virally inactivated human fibrinogen sealant) was purified free of plasminogen by passing through a column of Tranexamic acid sepharose**. Safety and Efficacy**: Three groups of 10 rabbits received one application (at least 1 ml) of fibrin sealant on a cut liver surface after partial hepatectomy. One group received standard formulation and the other two received one batch each of plasminogen free material. Time to hemostasis and volume of sealant used were recorded. Animals were observed for 13-14 days post surgery, after which time they were sacrificed and a full necropsy performed Hematological parameters were measured at three time points, before surgery, immediately following skin closure, and immediately prior to sacrifice. In a separate study neurological safety was demonstrated. Two groups of 10 rabbits were treated with 0.5ml of plasminogen free fibrin sealant applied subdurally following craniotomy. Each group received one batch of material. A third group of 10 animals were given sham operations with no application of product.

Evaluation of adhesion formation/prevention: In a rabbit uterine horn model of adhesion, the uterine horns were exteriorized and traumatized by abrasion of the serosal surface with gauze until punctate bleeding developed. Ischemia of both uterine horns was induced by removal of the collateral blood supply. Fibrin glue was applied to the abraded surface and the horns returned to their normal anatomic position Control animals received no treatment of the abraded surface. After 7 days, the rabbits were sacrificed and the percentage of the area of the horns adherent to various organs determined. Adhesion formation was evaluated on a numerical scale according to extent and type of adhesions formed.

Clot Stability(in vitro): Clots were formed from four batches of plasminogen free fibrin sealant. Each clot was placed in a tube containing buffered saline with urokinase and incubated at 37^{o}C. The amount of clot remaining after 3 days was measured by solubilizing the residual clot and determining the protein concentration. **In Vivo:** An abdominal wall defect was made by cutting a 2 cm x 1 cm flap in the abdominal wall of Sprague-Dawley male rats that penetrated the wall muscles. The wounds were sprayed with test material and the incision and the skin were closed. Five groups of 10 animals were used. Two groups received plasminogen free fibrin sealant, and one group each received Quixil/Crosseal (Omrix/Johnson and Johnson Wound Management, Tissucol (Baxter in the EU) or Tisseel (Baxter in the US and UK). Two animals from each group were sacrificed at various time points (1, 3, 5, 7 and 12±2 days - last time point were determined according quantity of clot left on day 7). Any remaining clot was extracted , washed to remove attached tissue, weighed and tested for total protein.

Results: Plasminogen removal and clot stability: The plasminogen content of the BAC was reduced from ~300 µg/ml to 4-11 µg/ml. The in vitro degradation studies demonstrated that clot stability was a function of plasminogen concentration, with 50-80% remaining after 3 days in the 4-11µg/ml samples vs. 4.5% in the 300µg/ml preparations. The in vivo degradation kinetics showed no significant difference between the plasminogen free preparation and other fibrin sealants. The clot in all products showed exponential decay, with less than 10% remaining at day 12.

Safety and Efficacy No significant difference was seen in the time to hemostasis or the volume of sealant used for the plasminogen free vs. standard formulation fibrin sealant. No macroscopic signs of local intolerance or systemic toxicity were found. No abnormal clinical or neurobehavioral signs were seen during the 14-day observation period. No macroscopic signs of local intolerance related to treatment were seen. Analysis of cerebrospinal fluid did not show any major signs of inflammation or any difference between the treated and sham operated groups. A local inflammatory reaction associated with implantation of foreign material was seen in 2/10 animals in each of the treated groups No treatment specific neurotoxic effects were observed

Evaluation of adhesion formation/prevention: Both fibrin glues received a lower adhesion score than the untreated controls, indicating that they were effective in reducing the extent of adhesion formation.

Conclusions: An affinity chromatographic resin using a tranexamic ligand for plasminogen removal effectively removed 96-99% of the plasminogen from the BAC component of Quixil/Crosseal. The resulting plasminogen free fibrin sealant was proven to be safe and effective in animal models of hemostasis. No neurotoxic effects were found in a rabbit craniotomy study. Clots formed from this fibrin sealant were more stable in vitro in the presence of urokinase than clots prepared from other fibrin sealants containing plasminogen plus a fibrinolytic inhibitor. The degradation kinetics of the fibrin sealant in vivo was not significantly different from three other marketed sealants (Quixil, Tisseel and Tissucol). In addition, the sealant reduced adhesion formation in a rabbit uterine horn model of adhesion prevention, where treated animals exhibited fewer adhesions than untreated controls.