Assessment of the Efficacy of New Hemostatic Agents in a Lethal Model of Extremity Hemorrhage in Swine

Study Abstract

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Introduction: Three promising hemostatic agents were selected from screening tests among a number of new products and evaluated for efficacy and initial safety in a swine model of extremity hemorrhage that is 100% fatal with standard gauze treatment. These products included WoundStat (WS), a second mineral-based powdered hemostat, and a chitosan-based powdered product. WS and the chitosan-based powder are approved by the FDA for emergency treatment of bleeding in external wounds. The second mineral-based product is not approved. The three new agents were compared to two hemostatic products, QuikClot Advanced Clotting Sponge Plus (ACS^+) and a chitosan-based dressing (as controls), that have been used to treat combat casualties in US Armed Forces.

Methods: Anesthetized pigs (n= 46, ~37 kg) were instrumented and blood samples were collected for hematological measurements, including clotting activity using the thrombelastography (TEG) method. The *in vitro* effects of the new agents were compared to kaolin and celite, known clotting activators. For *in vivo* evaluation of these products, pigs were first splenectomized and hydrated, then the abdominal incision was closed. The right femoral artery was isolated non-traumatically and injured with a 6 mm aortic punch. Unrestricted bleeding was allowed for 45 seconds (blood loss was collected and measured as pre-treatment blood loss), and then controlled by applying an agent through a pool of blood. Each hemostatic agent was applied twice (if necessary) with the aid of a laparotomy sponge and 2 minute compressions. The sponges along with agents were left in place for the duration of the experiments. Fluid resuscitation (500 ml Hextend and up to 12 liter LR at 100 ml/min) was started with the first compression and titrated to achieve and maintain a mean arterial pressure of 65 mmHg (baseline pressure). Animals were monitored for 180 minutes or until death. Survival animals were CT scanned and arterial circulation of treated legs was assessed. Following euthanasia, tissue samples including femoral vessels, nerve, and muscle were collected from the wounds for histology. End points measured included survival (rate and time), total time bleeding was controlled, post-treatment blood loss, resuscitation volume, and wound temperature.

Results: The TEG analysis (*in vitro*) of blood treated with WS and the other mineral-based product indicated a significant increase in clotting activity of the samples (reduced reaction time, increased rate of clot formation, and increased clot strength) compared to untreated blood. The opposite effects were found in ACS^+-treated blood. No change in clotting activity was observed in blood treated with the chitosan-based powder.

Each agent was tested *in vivo* in 10 pigs except for ACS$^+$,which failed to produce hemostasis in 6 consecutive experiments and therefore was eliminated from the study. There were no differences in hemodynamic and hematological baseline measurements, pre-treatment blood loss (average 17.4 ml/kg), or resuscitation volume (average125 ml/kg) among groups.

Treatment with test agents resulted in 100% survival in the WS group compared with 70% and 60% survival in the other powdered hemostat groups. The survival rates with WS and the other mineral-based hemostat were significantly ($P<0.05$) higher than the survival rate in chitosan-based dressing group (10%). There were no statistical differences in survival between groups treated with chitosan, whether as a powder or a dressing. These survival rates corresponded to post-treatment blood losses, which were on average 9.5, 34.5, 40 ml/kg in WS, and the other powder hemostat groups, respectively, as compared with 85.6 ml/kg in chitosan dressing group ($p<0.05$). There were no statistical differences in post-treatment blood loss among the test groups. The average survival times, 180 min in the WS group, 164 and 138 min in the other powdered hemostat groups, and 83 min in the chitosan dressing group, also corresponded to the duration that bleeding was controlled by each agent, 166, 126, 108, and 25 minutes, respectively. Survival times and hemostasis duration of test agents were significantly longer than the chitosan dressing group ($P<0.001$). The survival time of WS was also higher than the powdered chitosan-based hemostat group ($p<0.05$). CT images of femoral vessels in successful experiments showed that blood flow to distal tissues was obstructed by all the agents. Histological evidence indicated least tissue damage with the chitosan dressing and ACS$^+$; mild to moderate and equal damage with WS and the chitosan-based powdered hemostat; while the most injury, including neural necrosis, was observed after treatment with the other mineral-based hemostatic powder. Traces of test agents were detected in the lumen of treated vessels and few thromboses were found in the adjacent tissues.

Conclusion: These results indicate that the new hemostatic granules/powders are significantly more effective in treating arterial hemorrhage than a chitosan-based dressing and QuikClot ACS$^+$. Among the new agents, WS granules appeared to be the most efficacious agent based on blood loss and survival time, followed by the other powdered hemostatic agents, when compared to chitosan-based bandages. A chitosan-based hemostatic powder also appeared somewhat efficacious and safe (non-thrombogenic) with mild to moderate tissue damage, but was inconsistent in hemostatic function. WS exhibited high efficacy with strong tissue adhesion and blood clotting properties. WS treatment also produced mild to moderate tissue damage. The clinical significance of tissue damage caused by the test agents and any potential risk of embolism with granular/powdered hemostatic products with procoagulant properties are unknown and warrant survival studies.