NATIONAL DEFENSE MEDICAL CENTER
TRI-SERVICE GENERAL HOSPITAL

DETERMINATION OF THE EFFICACY OF A NEW HEMOSTATIC DRESSING IN A MODEL OF EXTREMITY ARTERIAL HEMORRHAGE IN SWINE

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ABSTRACT

Background: Chitosan containing dressings such as CELOX® Gauze have been used in the past few years for treating external compressible hemorrhage in combat casualties. A newly developed product, AnsCare® ChitoClot Gauze "Chito-SAM™ 100 Gauze" was produced mainly based on chitosan. In the study, the hemostatic effect of two distinct chitosan-contained dressing materials - AnsCare® "Chito-SAM™ 100" and CELOX® Gauze were evaluated and compared. The efficacy and acute safety of the dressings were tested in a standard arterial hemorrhage model.

Materials and Methods: The study design was based on an animal study of swine comprising two study groups, AnsCare® "Chito-SAM™ 100" (n=10) and CELOX® Gauze (n=10) dressing materials. 25 pigs in total were operated in this study, among which 5 pigs were excluded during the study due to failed procedure during intubation for general anesthesia and malposition of endotracheal tube which induced hypoxia while in operation. Anesthetized pigs (34-38 kg) were instrumented, and arterial blood was collected for hematological and coagulation assays. After splenectomy, the right femoral artery was isolated, injured (6 mm arteriotomy), and unrestricted bleeding allowed for 45 seconds. The hemostatic dressing was then applied over the wound randomly and compressed for 2 minutes. Fluid resuscitation was administered and titrated to maintain a mean arterial pressure of 65 mm Hg. Animals were observed for 180 minutes or until death.

Results: The baseline physiological and hematological measurements of the pigs including body weight, temperature, MAP, hemoglobin, platelet count, prothrombin time, activated partial prothrombin time, fibrinogen and arterial blood gas analysis, showed no significant differences between the two study groups (AnsCare® "Chito-SAM™ 100" vs. CELOX® Gauze) in the beginning of the operation. In the outcomes of treating a groin arterial hemorrhage with different dressings in swine, better initial hemostasis was achieved in the CELOX® Gauze group compared with the AnsCare® "Chito-SAM™ 100" group (4 vs. 2) and better survival rate of the AnsCare® "Chito-SAM™ 100" group to the CELOX® Gauze group (50% vs. 40%) was also observed; however, no other significant differences were observed in the parameters of total time of hemostasis, total blood loss, pretreatment blood loss, posttreatment blood loss, total resuscitation fluid amount, and survival time. In the final hematological analysis of these two groups, there were not any significant differences in hemoglobin, platelet count, prothrombin time, activated partial prothrombin time, fibrinogen and arterial blood gas analysis at the end of study. However, in the baseline and final hematological measurements in each group, a significant decline in hemoglobin was observed in both groups (AnsCare®

"Chito-SAM™ 100" vs. CELOX® Gauze: P=0.004 vs. 0.005), and a significant decrease in platelet count was measured in the AnsCare® group (P=0.004).
Conclusion: The AnsCare® "Chito-SAM™ 100" and CELOX® Gauze groups showed a similar hemostatic effect.

Key Words: Chitosan, CELOX, AnsCare, Chito-SAM™ 100, ChitoClot, Hemorrhage, Dressing, Swine, Coagulation.

BACKGROUND

Chitosan containing dressings such as CELOX® Gauze have been used in the past few years for treating external compressible hemorrhage in combat casualties. A newly developed product, AnsCare® ChitoClot Gauze "Chito-SAM™ 100 Gauze", was produced mainly based on chitosan. In the study, the hemostatic effect of two distinct chitosan-contained dressing materials - AnsCare® "Chito-SAM™ 100" and CELOX® Gauze were evaluated and compared. The efficacy and acute safety of the dressings were tested in a standard arterial hemorrhage model.

MATERIALS AND METHODS

This study was approved by the Animal Care and Use Committee of the National Defense Medical Center. All animals received care and were used in strict compliance with the Guide for the Care and Use of Laboratory Animals.

Test Material

The study design was based on an animal study of swine comprising two study groups, AnsCare® "Chito-SAM™ 100" (n=10) and CELOX® Gauze (n=10) dressing materials. CELOX® Gauze, a product of Medtrade Products Ltd (Crewe, UK), is a 10 ft long, 3 inch wide roll of nonwoven medical gauze impregnated with small chitosan particles. The AnsCare® ChitoClot Gauze (also known as "Chito-SAM™ 100 Gauze", a registered trademark of SAM Medical Products, Wilsonville, Oregon, USA), a product of BenQ Materials Corp. (Taoyuan, Taiwan), is completely made of nonwoven chitosan and is a 6 ft long, 3 inch wide roll.

In Vivo Methods

Yorkshire cross-bred pigs (castrated males only) weighing 34 kg to 38 kg were purchased from Animal Technology Institutes Taiwan and used in this study. The study design was based on an animal study of swine comprising two study groups, AnsCare® "Chito-SAM™ 100" (n=10) and CELOX® Gauze (n=10) dressing materials. 25 pigs in total were operated in this study, among which 5 pigs were excluded during the study due to failed procedure during intubation for general anesthesia and malposition of endotracheal tube which induced hypoxia while in operation. Before the surgery date, venous blood samples were collected from pigs and complete blood count (CBC) and standard coagulation parameters (prothrombin

time, activated partial thromboplastin time, fibrinogen) were measured to ensure these values are within normal range before proceeding with experiments. Pigs were fasted for 12 hours to 18 hours before the surgery with free access to water. On the day of surgery, pigs were premedicated with buprenorphine (0.025 mg/kg, intramuscular [i.m.]) for analgesia and glycopyrrolate (0.01 mg/kg, i.m.) to reduce saliva secretion and block vagally mediated bradycardia during the surgical procedure. Animals were induced with an injection of tiletamine-zolazepam (Virbac Zoletil®, 4–6 mg/kg, i.m.) and anesthetized initially with 5% isoflurane in oxygen via face mask. They were then intubated and mechanically ventilated with 100% oxygen. Anesthesia was maintained with 1% to 2% isoflurane added to oxygen gas by the ventilator. Maintenance fluid, lactated Ringer's (LR), was administered at 5 mL/kg/hr through a venous line placed in an ear vein.

Surgical Procedures

The right carotid artery was cannulated for blood draws and direct recording of blood pressure (systolic, diastolic, and mean) and heart rate throughout the experiment. The right jugular vein was also catheterized for administering resuscitation fluid. A midline laparotomy was then performed, followed by a splenectomy to minimize blood changes that may occur as result of autotransfusion from the pig's contractile spleen. The blood loss from splenectomy was replaced by infusing LR at three times the weight of the spleen. A cystostomy was also performed to aid in the drainage of urine. The abdomen was then closed with suturing, and the skin was stapled. Preinjury (baseline) blood samples were collected from the arterial line for CBC, coagulation, and blood gas analysis. To create a severe hemorrhage in the groin area, ~5 cm of femoral artery was dissected free from surrounding tissues, and the overlying abductor muscle was removed. Injury to the adjacent femoral vein and nerve was avoided. The vessel was then bathed with a few milliliters of 2% lidocaine to relax vasospasm and dilate the artery to its normal size. Next, a 10-minute stabilization period was allowed (no manipulation) and baseline data including mean arterial pressure (MAP) and body temperature were recorded. A stable MAP of 60 mm Hg or higher was required before proceeding with the rest of the experiment. The maintenance fluid was discontinued at this point. The artery was clamped proximally and distally and a 6-mm diameter arteriotomy was made on the anterior surface of the vessel using a vascular punch (International Biophysics Corp., Austin, TX). The clamps were then released, and free bleeding was allowed for 45 seconds. The shed blood was collected by suction, weighed, and recorded as pretreatment blood loss.

Wound Treatment and Resuscitation

The surgeons were blinded to the identity of test materials until the time of agent application. To the extent that was possible, the products were applied according to the manufacturers' instruction. After the 45-second free bleed, while bleeding continued, a package of each product was opened, and the material was packed in the wound. The material was covered immediately with gauze 4x4s and pressed against the wound with sufficient and equal pressure to occlude the artery and stop the bleeding. The arterial injuries, dressing applications, and compressions were done by the same investigator (Dai) for all the experiments to minimize variability. After 30 seconds compression, fluid resuscitation was started by infusing 500 mL of Hextend (6% hetastarch in balanced electrolytes plus glucose) to compensate for pretreatment blood loss. The colloid fluid was administered at 100 mL/min intravenously, and targeted to raise the MAP to 65 mm Hg, the average baseline blood pressure of anesthetized pigs. Compression was stopped after 2 minutes and hemostasis observed for 3 minutes without removing the gauze 4x4s. If rebleeding occurred during this period, the laparotomy gauze was removed and the failed agent taken out and replaced with fresh material. The 2-minute compression was then repeated with new laparotomy gauze. Wounds were treated at most twice with each product regardless of hemostatic outcome. Hemostasis was then observed for the next 3 hours with laparotomy gauze left in place. Any shed blood during this period was collected and measured as posttreatment blood loss.

After the infusion of Hextend, fluid administration was continued with LR (100 mL/min, maximum of 10 L) as needed to raise and maintain the MAP at 65 mm Hg throughout the experiment. The MAP of 65 mm Hg approximates systolic pressure of 90 mm Hg, which is in agreement with the level of permissive hypotensive resuscitation regimen. Animals were monitored up to 3 hours or until death as determined by end tidal P_{CO2}<15 mm Hg and MAP<20 mm Hg. Final blood samples (arterial) were collected for hematological measurements before euthanizing the animals.

The treated legs of surviving pigs were flexed and stretched five times simulating walking condition to test the stability of the hemostasis provided by the test agents. At the conclusion of experiments, the product was removed from the wound to check the status of injury and the patency of the vessel. Animals were then sacrificed after finishing all above procedures.

Data Analysis

Data are expressed as mean standard of error of the mean (SEM) and analyzed by analysis of variance, Fisher's exact, and Log rank for statistical comparisons. p values were adjusted according to False Discovery Rate method for bigroup comparison. The data with high variance were log transformed for analysis of variance. The nonparametric data were analyzed using Newman-Keuls multiple comparison test, and bigroup comparison was done using Dunnett's test. A $p<0.05$ was considered statistically significant.

RESULTS

The baseline physiological and hematological measurements of the pigs including body weight, temperature, MAP, hemoglobin, platelet count, prothrombin time, activated partial prothrombin time, fibrinogen and arterial blood gas analysis, showed no significant differences between the two study groups (AnsCare® "Chito-SAM™ 100" vs. CELOX® Gauze) in the beginning of the operation (Table 1).

Measure	AnsCare® "Chito-SAM™ 100", n=10	CELOX®, n=10	Overall *p*
Body weight (kg)	34.60±0.40	34.40±0.16	0.649
Temperature (℃)	37.81±0.18	37.60±0.22	0.477
MAP (mmHg)	93.10±3.48	86.30±3.11	0.162
HGB (hemoglobin)(g/dL)	8.20±0.48	9.22±0.39	0.118
PLT(platelets)(1,000/μL)	38.45±5.79	37.69±3.11	0.909
PT(prothrombin time)(s)	12.08±0.28	11.58±0.25	0.201
aPTT(activated partialprothromboplastin time)(s)	66.52±24.7	132.32±24.29	0.074
Fibrinogen (mg/dL)	143.31±23.87	110.62±17.86	0.297
pH	7.25±0.05	7.33±0.04	0.181
HCO_3 (mM)	34.96±1.32	35.30±0.70	0.815
pCO2 (mM)	73.20±4.86	63.36±6.06	0.229
pO2 (mM)	330.12±46.11	394.79±33.08	0.269

TABLE 1. Baseline Physiological and Hematological Measurements in the Pigs (mean±Std. Err)

In the outcomes of treating a groin arterial hemorrhage with different dressings in swine, better initial hemostasis was achieved in the CELOX® Gauze group compared with the AnsCare® "Chito-SAM™ 100" group (4 vs. 2) and better survival rate of the AnsCare® "Chito-SAM™ 100" group to the CELOX® Gauze group (50% vs. 40%) was also observed; however, no other significant differences were observed in the parameters of total time of hemostasis, total blood loss, pretreatment blood loss,

posttreatment blood loss, total resuscitation fluid amount, and survival time (Table 2).

Outcomes	AnsCare® "Chito-SAM™ 100", n=10	CELOX®, n=10	Overall *p*
Initial Hemostasis achieved	2/10	4/10	NS
Total time bleeding stopped (min)	40.10±9.90	34.00±11.47	0.69
Total bleeding amount (g)	1779.00±436.04	1765.00±482.47	0.96
Pretreatment blood loss (g/kg)	7.77±1.20	8.44±1.22	0.70
Posttreatment blood loss (g/kg)	43.15±11.67	42.55±14.20	0.97
Rate of first period bleeding (g/sec)	6.00±0.94	6.44±0.93	0.74
Rate of second period bleeding (g/min)	37.24±6.65	26.40±6.26	0.25
Total resuscitation fluid (L/kg)	0.11±0.02	0.08±0.03	0.45
Survival rate	50%	40%	NS
Survival time (min)	121.90±20.02	106.20±22.26	0.61

TABLE 2. Outcomes of Treating a Groin Arterial Hemorrhage with Different Hemostatic Dressing in Swine.

In the final hematological analysis of these two groups, there are not any significant differences in hemoglobin, platelet count, prothrombin time, activated partial prothrombin time, fibrinogen and arterial blood gas analysis at the end of study (Table 3). However, in the baseline and final hematological measurements in each group, a significant decline in hemoglobin was observed in both groups (AnsCare® "Chito-SAM™ 100" vs. CELOX® Gauze: P=0.004 vs. 0.005), and a significant decrease in platelet count was measured in the AnsCare® "Chito-SAM™ 100" group (P=0.004) (Table 4).

Measure	AnsCare® "Chito-SAM™ 100", n=10	CELOX®, n=10	Overall *p*
HGB (hemoglobin) (g/dL)	3.10±1.02	3.81±1.12	0.645
PLT (platelets) (1,000/μL)	12.19±3.65	22.27±8.15	0.276
PT (prothrombin time) (s)	25.24±6.70	27.14±6.90	0.846
aPTT (activated partial prothromboplastin time) (s)	107.21±28.77	145.41±22.89	0.314
Fibrinogen (mg/dL)	79.09±22.28	64.13±9.67	0.547
pH	7.38±0.09	7.26±0.06	0.435
HCO_3 (mM)	34.33±1.41	28.25±9.85	0.396
pCO_2 (mM)	61.50±14.03	53.25±7.22	0.619
pO_2 (mM)	296.20±65.24	381.00±12.92	0.295

TABLE 3. Final Hematological Measurements in the Operated Pigs (mean±Std. Err)

Measure	AnsCare® "Chito-SAM™ 100", *p*	CELOX®, *p*

HGB (hemoglobin) (g/dL)	0.004	0.005
PLT (platelets) (1,000/μL)	0.004	0.177
PT (prothrombin time) (s)	0.095	0.056
aPTT (activated partial prothromboplastin time) (s)	0.462	0.666
Fibrinogen (mg/dL)	0.104	0.127
pH	0.060	0.437
HCO_3 (mM)	0.869	0.504
pCO_2 (mM)	0.143	0.171
pO_2 (mM)	0.937	0.244

TABLE 4. Baseline and Final Hematological Measurements in the Operated Pigs

In the test of treating legs of surviving pigs by flexing and stretching five times to simulate the walking condition and test the stability of the hemostasis, the results showed that active bleeding appeared in all survival pigs of CELOX® group and 3 in 5 pigs of the AnsCare® "Chito-SAM™ 100" group (Table 5). In addition, no any arterial disruption was observed in both groups after removal of the dressings.

Outcomes	AnsCare® "Chito-SAM™ 100", n=5	CELOX®, n=4
Bleeding	3	4
No bleeding	2	0

TABLE 5. Outcomes of treating legs of survival pigs with flexing and stretching after 3 hours.

CONCLUSION

The AnsCare® "Chito-SAM™ 100" and CELOX® Gauze groups showed a similar hemostatic effect based on the swine groin arterial hemorrhage model in the study.