

Research Article

Investigation of the Effectiveness of Algan Hemostatic Agent in Renal Venous Bleeding Model in Rats

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Abstract

Objectives: Major vascular injury is one of the primary causes of death after trauma, and effective and rapid control of bleeding is important to avoid deaths. Today, many products are available for this purpose, but an ideal product to control life-threatening hemorrhage has not yet been produced and more effective hemostatic products are needed. The purpose of this study was to evaluate the hemostatic efficacy of the novel, plant-based blood-stopper Algan Hemostatic Agent (AHA) in an uncontrolled renal venous hemorrhage model in rats. This is the first application of this model in the literature.

Methods: A total of 32 rats aged between 5 and 7 weeks were used in the study. The rats were randomly divided into 4 groups, each consisting of 8 rats. An experimental renal vein hemorrhage was established in all groups. A saline-impregnated sponge was applied to the control group to manage the hemorrhage. AHA was applied topically to the study groups in 3 different formulations: AHA-impregnated gauze, a gel, and lyophilized powder were employed to evaluate the hemorrhage control effect.

Results: The duration of bleeding was significantly shorter in the AHA groups compared with the control group.

Conclusion: This study demonstrated that AHA appears to be a highly effective hemostatic agent when applied locally in lyophilized, gel, or liquid form, and is beneficial in controlling hemorrhage.

Keywords: Algan Hemostatic Agent, bleeding, hemostasis, rat

Major vascular injury is one of the most important causes of death after trauma.^[1] Direct pressure is applied to the bleeding site to stop circulation in trauma patients.^[1] In this process, we need fast-acting ingredients to stop the bleeding. Some of the locally available products are; N-acetyl glucosamine polymer (Chitin[®]), poly-N-acetylglucosamine and oxidized cellulose (Bloodcare[®]), microporous hydrogel-forming polyacrylamide (BioHemostat[®]),

chitosan linear polymer (Celox[®]), Ankaferd Blood Stopper[®] (ABS), fibrin glue, fibrin glue, microporous polysaccharide Hemosphere (TraumaDEX[®]).^[2-8]

Bleeding after uncontrolled trauma is a major problem, accounting for approximately 40% of trauma-related deaths, and therefore uncontrolled bleeding is the leading cause of potentially preventable death in patients. In trauma, most hemorrhagic deaths occur within the first 6 hours. There-

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fore, effective and rapid control of the bleeding is important in reducing deaths. However, in spite of all the major improvements and many products available for this purpose, an ideal product to be used in the control of the severe hemorrhage has not yet been produced and more effective hemostatic products are expected to be produced.

The Algan Hemostatic Agent, an herbal extract derived from standardized blend of six different plants^[9] (Table 1). As far as we informed, it is the first and only patented product made solely of herbs, with no additives in the world. (Patent application publication no. TR2015 0018 A2).

Each of the plants that form AHA has a content which is effective in hemostasis by alone or in combination. All biocompatibility tests such as sensitization, cytotoxicity, irritation and hemodynamic tests of the AHA were performed, and the results supported its safety and efficacy as a hemostatic agent. It is easily applied locally. Further, it has low cost, and does not require special storage conditions.

Currently, there are products that have clinical trials and have been affected by similar mechanisms that have been granted for internal and external use.^[10-14] When AHA used in moist environment, it quickly polymerizes into a thin elastic film which has high tensile strength and firmly adheres to the anatomy of the tissue on which it is applied. Here, we aimed to evaluate the hemostatic effect of AHA in renal vein uncontrolled hemorrhage in rats.

Methods

For this study, approval for animal experimentation was obtained from KU Animal Experiments Local Ethics Committee (Decision no 2018/08). The experiment is described for the first time in the literature. In the study, 180-210 grams of weight, 5-7 week-old 32 rats were used. Rats were fed ad libitum and examined under standard laboratory conditions according to a 12-hour dark-light period. The rats were randomly divided into 4 groups, each of which was composed of 8 rats.

The groups were assigned as follows. 1st group (control group), 2nd group (AHA powder group), 3rd group (AHA gel

group), 4th group (AHA liquid impregnated sponge group). The procedures were performed under general anesthesia with ketamine hydrochloride (100 mg/kg) and xylazine hydrochloride (10 mg/kg).

Bleeding Test

The womb region of the rats was opened to expose the left renal vein. A renal venous incision was made with a green injector tip, while bleeding heads did not start. The injured area was treated either with AHA powder, gel, sponge, or saline impregnated buffer (as control). In AHA powder and gel forms, bleeding area was left unpressed after application. Bleeding state was checked 1 minute after the time of treatment. If bleeding stopped, it was noted as 'bleeding stopped'. In the first minute, if the bleeding did not stop, the treatment was repeated with a same amount of material for up to 2 min, or higher amount, i.e. 2 cc powder or gel form. Two minutes later the bleeding was checked and if the bleeding stopped, it was noted as 'second minute standing bleeding'; the same procedure was applied for the third time and was waited for 2 minutes. This additional 2 minutes later, the bleeding was checked and noted as 'stopped bleeding at the fourth minute' if the bleeding stopped. After stopping the bleeding (at the earliest 10 minutes after the end of the experiment) rats were euthanized by high intra-abdominal bleeding. The AHA application in the bleeding area is shown in figure 1.

Statistical Evaluation

SPSS software version 22.0 (SPSS Inc., Chicago, IL) was used to analyze the data of this study. Weight, bleeding time and adherence scores were calculated and mean values were compared among the four groups using analysis of variance (ANOVA). When differences were found, the difference group was determined by Duncan's multiple range test. The results were assessed at a 95% confidence interval and a significance level of $p < 0.05$.

Results

There was no difference in body weight between the groups. The shortest duration of bleeding was observed in AHA powder group. This was followed by the gel form and the liquid form groups. The duration of bleeding in the control group was longer than the experimental groups (Table 2 and 3).

Discussion

In this study, 3 different forms of AHA were studied; lyophilized powder, gel and liquid (sponge) and all were found very effective in local control of the bleeding. Although the hemorrhage controlled the powder and gel form more rap-

Table 1. Composition of algan hemostatic agent product

The name of the plant	English name	Used part
Achillea millefolium L.	Yarrow	Flower
Juglans regia L.	Walnut	Leaf
Lycopodium clavatum L.	Club moss	Whole plant
Rubus caesius L., R. fruticosus G.N. Jones	Blackberry	Leaf
Viscum album L.	European Mistletoe	Whole plant
Vitis vinifera L.	Vine	Leaf

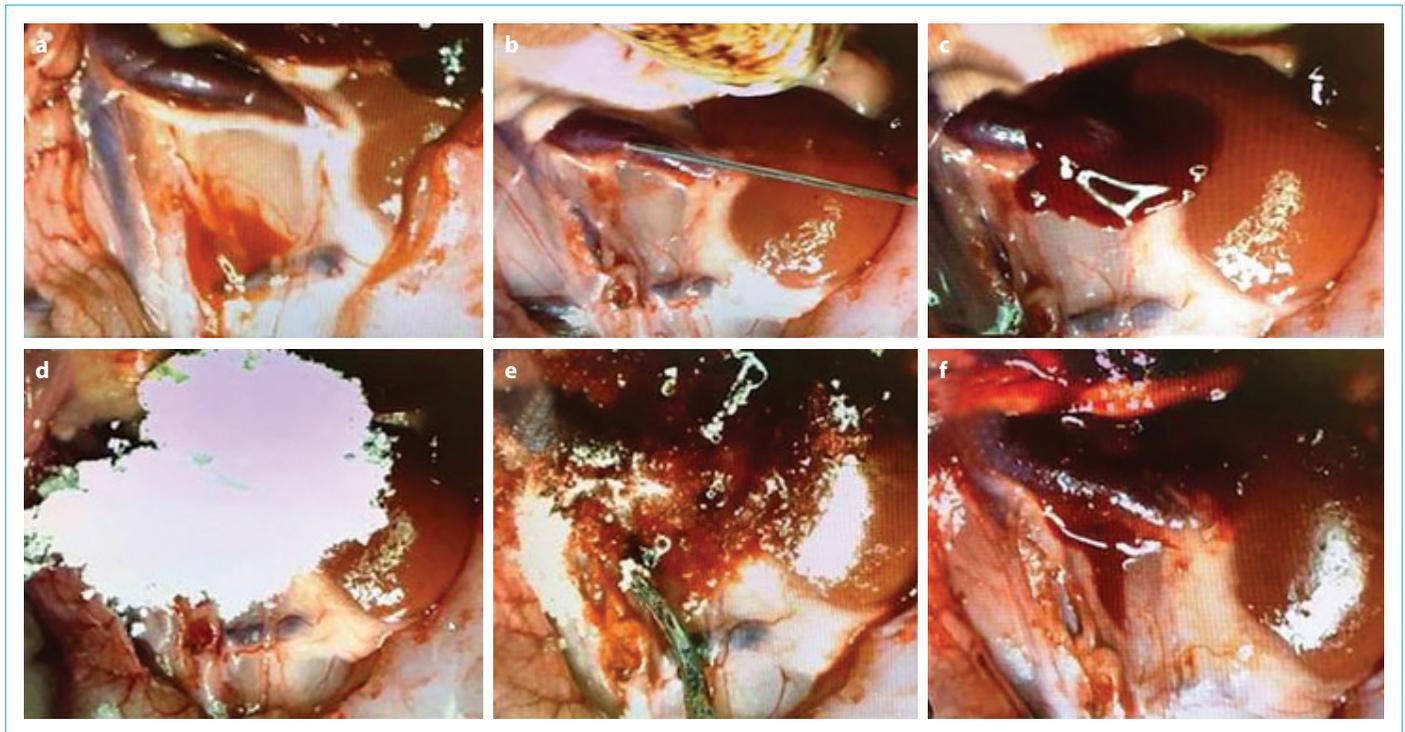


Figure 1. (a) revealing renal vein, (b) renal vein destruction. (c) hemorrhage formation, (d) AHA powder application, (e) hemostatic plug appearance in damaged vessel after dust removal, (f) resumption of bleeding after the hemostatic plug has been cleaned with gauze.

Table 2. Mean body weight distribution of the groups and homeostasis times of AHA Gel Groups

	Group 1 (C)	Group 2 (AHAP)	Group 3 (AHAG)	Group 4 (AHAL)	p
Body weight (grams)	180.4±9.5	185.5±8.3	179.2±10.6	188.9±6.7	p>0.05
BT (sec)		75 sec. (45-120)			

Table 3. Homeostasis time of control and fluid groups

	Bleeding controlled at 1 minute	Bleeding controlled at 2 minutes	Bleeding controlled at 4 minutes	Unsuccessful
Control	0 (0%)	0 (0%)	0 (0%)	8 (100%)
AHA Powder	5 (62.5%)	3 (37.5%)	0 (0%)	0 (0%)
AHA Gel	4 (50%)	4 (50%)	0 (0%)	0 (0%)
AHA Liquide	0 (0%)	7 (87.5%)	1 (12.5%)	0 (0%)

idly, the liquid form of the AHA was also found to be effective and no statistical difference in bleeding control efficacy was observed between the test groups. While, for the control group, statistically significant differences were found in the bleeding time comparing to each of test groups.

The highest efficacy was provided by the AHA powder

form, which was able to control the bleeding in shortest time, while the gel form was also found to be effective, the bleeding area was not compressed. In the inaccessible internal bleeding, which cannot be compressed, gel form has given hope as an effective hemostat. The bleeding time in the control group was much longer than in the experimental groups.

In our study, in the control group animals bleeding in the injured area treated with tampon solution was not stopped within 4 minutes. Although the renal vein model practiced in the present study was not previously reported in the literature, the mean duration of bleeding may differ according to the studies depending upon various factors such as animal weight, experience of the practitioner, technical differences, vessel variations, laboratory conditions, etc.

Limitations

These are the limitations of this study. The renal vein model has been performed in this study for the first time in the literature. There is no data in the literature on efficacy and reliability of this model. Another limitation of this study is that it is not compared with similar products.

Conclusion

According to the experimental results of this study, although the AHA formulations are found to be an effective

hemostatic agent, further experimentation should be carried out with different bleeding models and by comparatively evaluating its efficiency with other available hemostatic agents.

Disclosures

Acknowledgments: The authors do not have any relationship with the commercial company of the product tested (AHA). The authors have indicated that they have no other conflicts of interest regarding the content of this article.

Ethics Committee Approval: For this study, approval for animal experimentation was obtained from KU Animal Experiments Local Ethics Committee (Decision no 2018/08).

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