

COMPARATIVE EFFICACY OF TOPICAL SODIUM HYALURONATE IN RENAL TRAUMA MODEL

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ABSTRACT

Objective: In this study, the efficacy of sodium hyaluronate (NaHA) was examined in comparison with other agents in topical hemostasis and prevention of adhesions in a rat renal injury model.

Material and Methods: A standardized traumatic renal injury was created in adult male Wistar rats. The hemostatic effect of NaHA, a viscoelastic compound, on bleeding site was compared with microfibrillar collagen powder (MCP) and oxidized regenerated cellulose (ORC). Animals that were treated only with surgical gauze served as controls. The time necessary to achieve complete hemostasis was recorded for each animal. Kidneys were removed 7, 15 and 30 days later to examine parenchymal effects of topical agents and the presence of perirenal adhesions.

Results: Complete hemostasis was achieved in all animals within several minutes, but MCP and NaHA were clearly superior to control group when compared with ORC ($p < 0.05$). Histological findings were most prominent with the MCP while NaHA constituted the least inflammatory reaction. NaHA treated kidneys were also showed less adhesion formation to surrounding organs.

Conclusion: NaHA was found effective for the control of parenchymal bleeding and to prevent perirenal adhesion formation in this experimental renal injury model.

Key words: kidney; trauma; wounds and injury; sodium hyaluronate; rats

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INTRODUCTION

Surface bleeding from kidney parenchyma is usually controlled by such standard means of hemostasis as pressure, suture ligating of bleeding arterial vessels or electrocoagulation. Venous bleeding generally stops after the parenchymal defect is closed (1). But the attainment of adequate hemostasis is often complicated by the presence of friable tissues, laceration and widespread oozing. In these cases, application of topical hemostatic agents to injured surface is an alternative method for the control of bleeding. These agents are generally expected to aid the patient's coagulation system via the rapid development of an occlusive clot (2). The reduction of the perirenal adhesions can also be of interest because of the future risk of perirenal sclerosis with hypertension when the renal injury is in the lower pole, near

to the ureter, or when the injury is very extensive (1). However, currently available topical hemostatic products have potential drawbacks such as infection and adhesion formation (3). These findings make more safe topical hemostatic agents desirable.

Viscoelastic compounds which have a high molecular weight polysaccharide have been found to have wide applications as biomaterials in the field of ophthalmic surgery to protect the corneal endothelium and to form a mechanical barrier in the case of hemorrhage (4). The antiadhesive barrier properties of hyaluronate (HA), a viscoelastic compound, have been well demonstrated in intra-abdominal (5-8) and orthopedic surgery (9). HA and its derivatives were also administered in applications toward the wound healing (10).

Based on this knowledge, we examined the hemostatic action and antiadhesive properties of

NaHA upon the injured rat kidneys in comparison with two other commercially available hemostatic agents microfibrillar collagen powder (MCP) and oxidized regenerated cellulose (ORC).

MATERIAL AND METHODS

This study was performed on adult male Wistar rats each weighing 250-285 g in the Adnan Menderes University, Faculty of Veterinary Surgical Research Laboratories. The animal ethics committee of the institution approved the experimental protocol. Four rats were used for pre-study experience procedures. Rats were fed with standard pellet diet and water ad libitum and housed in cages of 3 animals. Animals were divided randomly into 4 groups (9 animals per group).

Anesthesia was induced with an intraperitoneal injection of xylazine (5 mg/kg body weight) plus ketamine (25 mg/kg body weight) in room temperature. A 20-gauge angiocatheter was placed into the carotid artery for measurement of systemic arterial blood pressure throughout the procedure. The rats were then placed in the prone position, shaved in the lumbar area, prepared with povidone iodine solution and then draped in sterile fashion. The left kidney was exposed via dorsal flank incision by the same surgeon and surrounded by surgical gauze. Experimental kidney injury was created using the model described by Raccuia et al. (11). In summary, a lower pole segment of each kidney representing approximately 10% of renal volume was excised with a standardized pre measured template and active parenchymal bleeding occurred. Immediately after bleeding, the following materials were used topically to assess local hemostatic action: 1)- Control group: The standardized surgical gauze having 1.5 cm² surface area was applied to bleeding site; 2)- MCP group: (Colgen poudre®, Laboratoire Interphar, France). MCP was applied with a forceps, as approximately a 1 mm layer, to the bleeding surface; 3)- ORC group: (Surgicel®, Ethicon Ltd, UK). Adequate size of oxidized cellulose was cut and then applied to the bleeding surface; 4)- NaHA group: (Bialon®, Laboratoire Chauvin, France). NaHA, enough to cover the bleeding surface, was applied with its specific syringe injector.

The time required to achieve total hemostasis was recorded in each group. Compression on the bleeding surface, kidney parenchyma or hilar vessels to augment hemostasis was not used. After bleeding ceased, kidneys were returned to abdominal cavity. All materials were left in place except surgical gauze in control group. Local or systemic antibiotics were not administered in any animals. The animals were sacrificed by cervical dislocation and kidneys were removed in batches of three on day 7, 15 and 30 to evaluate the effects of hemostatic agents on kidney surface.

Before removing the specimens, we examined kidneys macroscopically for the presence of hematoma and perirenal adhesion formation to surrounding organs. The person detecting the adhesions was blinded as to the treatment-group assignment of the animals. For histological analysis, all kidneys were fixed in 10% neutral buffered formalin solution. The samples of both injured and treated sites of kidneys were processed by routine tissue processing techniques and embedded in paraffin. Sections of 5 µm thick were cut using a standard rotary microtome and stained with hematoxylin-eosin. The presence of residual material or foreign body reaction and the degree of inflammation and fibrosis were examined blindly under the light microscope (Olympus B X50).

Data are expressed as means ± SEM. Statistical analyses were carried out using two-tailed Student's t-test for comparison between groups and p values < 0.05 were considered statistically significant.

RESULTS

None of the animals died during the study period. The experimental procedure resulted in no statistically significant changes in average blood pressure (128.7 ± 4.5 mm Hg) in all animal treatment groups ($p > 0.05$). NaHA covered completely the bleeding surface while others adhered firmly. Complete hemostasis was achieved in all animals within several minutes. The time required to achieve hemostasis in each group is shown in Table. There was no statistically significant difference between the controls and ORC group ($p = 0.069$). Both MCP and NaHA were significantly more effective than surgi-

Table - Results overview of topical hemostasis by various agents in renal injury model.

Groups	No. of Animals	Hemostatic Time (Mean ± SEM)	Re-bleeding Hematoma	Adhesion Formation (%)
Oxidized cellulose	9	4.90 ± 1.03	ND	9/9 (100)
Microfibriller collagen	9	3.83 ± 0.66*	ND	9/9 (100)
Sodium hyaluronate	9	3.94 ± 0.73*	ND	9/9 (22)
Control	9	5.28 ± 1.68	ND	9/9 (100)

**p* < 0.05; ND = not determined.

cal gauze (*p* = 0.029 and *p* = 0.044, respectively). MCP and NaHA were also found more effective than ORC for the control of bleeding in injured renal surface (*p* < 0.05). However, no significant difference was found in NaHA group when compared to MCP group (*p* = 0.739). At macroscopic examination, none of the animals showed re-bleeding or hematoma formation and evidence of peritonitis. Splenic and/or omental adhesions to the injured surface were detected in all animals except in seven out of nine rats in NaHA group, which was statistically significant (*p* < 0.05). On examination of the kidney injury site after removal on day 7 and 15, the hemostatic materials were detected on wound surface and surrounded by an inflammatory mass except in NaHA group. By 30 days, the inflammatory mass disappeared in resting groups. Histological examination indicated progressive changes from acute inflammation to healing with varying degrees of chronic inflammation and even

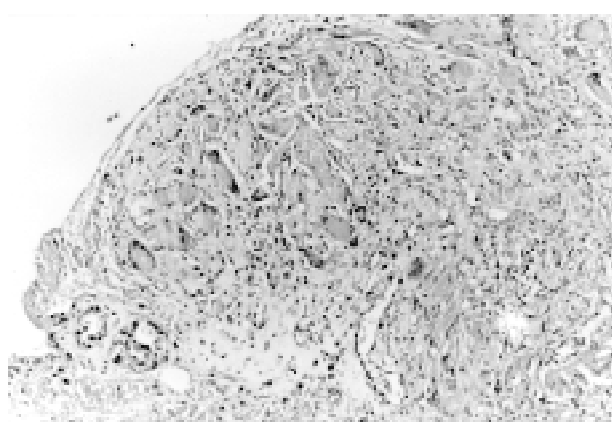


Figure 2 - Section of the injury site showing chronic inflammation with foreign body giant cells in MCP applied group on day 7 (Hematoxylin-eosin; magnification, X250).

scar formation. NaHA constituted the least inflammatory reaction and it was completely absorbed within 7 days from injured surface (Figure-1). MCP consti-

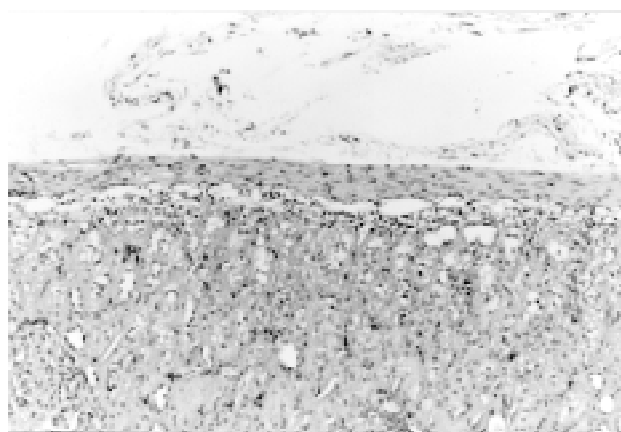


Figure 1 - Minimal scarring on surface and mild infiltration with chronic inflammatory cells in interstitium followed by application of NaHA on day 7 (Hematoxylin-eosin; magnification, X250).

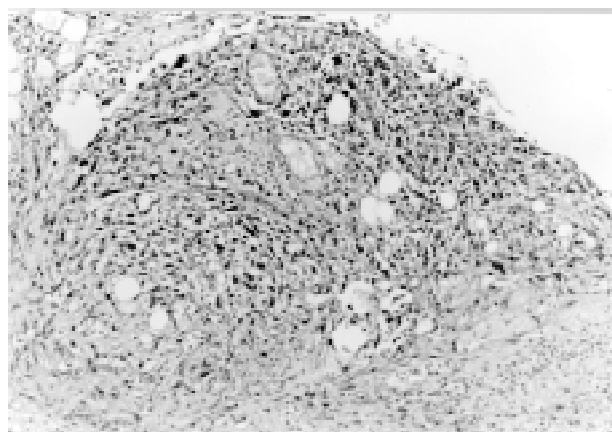


Figure 3 - Section of the injury site showing chronic inflammatory infiltration with granuloma formation in MCP applied group on day 7 (Hematoxylin-eosin; magnification, X250).

tuted the most inflammatory reaction (Figure-2) and even granuloma formation with foreign reactive cells in two rats on day 7 (Figure-3) which regressed to slight scarring on day 30. All agents left minimal scar tissue and complete healing with little evidence of past injury on day 30.

DISCUSSION

Adequate use of conventional surgical techniques with ligatures and sutures is the major requirement for the control of hemorrhage following renal injury. Nevertheless, minor bleeding or oozing from renal parenchyma can also be stopped by the topical application of hemostatic agents (1).

Several materials have been used to promote surgical hemostasis. Surgical gauze swab is used for contact activation alone in hemostatic procedure. The action of surgical gauze mainly depends on its physical characteristics on the bleeding surface, which traps blood elements within its fibers and facilitates platelet and blood coagulation. ORC is formed by passing surgical gauze over fuming nitric acid and thus oxidizing the cellulose and creating cellulosic acid. The hemostatic action of ORC is based on its physical matrix that collects blood into interstices and concentrates the coagulation factors (12). MCP is a fluffy, off-white, cross-linked collagen substance and is made from bovine dermis. The material is difficult to handle due to its high electrostatic charge. Topical hemostasis is facilitated by virtue of its fibrillar structure forming a sticky matrix for platelet aggregation allowing a clot to form (13). However, MCP and ORC may have potential drawbacks such as infection and scar formation (3,14). These findings make the development of a more effective topical hemostatic agent desirable.

Hyaluronan (HA), formerly known as hyaluronic acid, is a main glycosaminoglycan ubiquitously distributed in the extracellular matrix (ECM). It plays a multi-task role, having many structural, physiological and biological functions in the body space, particularly in the ECM. There is a specific binding interaction between fibrin, the major clot protein, and HA in the early stages of the wound healing. Trombin-induced formation of fibrin clots is affected by HA,

which increases the rate of clot formation (15,16). The HA-fibrin matrix also plays a major role in the subsequent tissue reconstruction process. Both wound vascularization and healing showed faster improvement in HA-treated animals. This may be partly explained by the known effects of HA degradation products on endothelial cell proliferation and angiogenesis (17).

Viscoelastic compounds act as an excellent lubricant, coat tissue surface and protect tissues from mechanical trauma (4). NaHA, the first commercially available viscoelastic agent, has been shown to be nontoxic, non-antigenic and non-inflammatory (4,6,15). NaHA is structurally similar to heparin but it does not possess anticoagulant activity (18), although it has been used to form an occlusive compound for percutaneous embolization of arteries in rats (19).

To our knowledge, the efficacy of NaHA as a topical hemostatic agent on bleeding surface of injured kidney has not been previously reported. The time for achieving hemostasis were found to be statistically significant in MCP and NaHA groups when compared with control group. MCP and NaHA were also found more effective than ORC for the control of bleeding in injured renal surface. Collagen materials were found previously to be more hemostatic than ORC (2,14). Our findings are in agreement with these reports. Although easy to work with, ORC showed no superiority to surgical gauze alone in producing hemostasis, similar to study of Raccuia et al. (11).

The agents used in our study were also compared with regard to presence of perirenal hematoma and/or adhesion formation. We did not detect late bleeding or hematoma formation in any rats even in the control group. This may be explained by the early adhesion of spleen and omentum to the bleeding surface. However, most of the animals in the NaHA group neither demonstrated adhesion formation to surrounding organs nor showed re-bleeding or hematoma formation suggesting the good adaptation of the NaHA with injury site. Our macroscopic findings were also consistent with the studies regarding anti-adhesive effect of HA (5-8).

Histological findings were most prominent with the MCP while NaHA constituted the least in-

flammatory reaction. Foreign body reaction even with granuloma formation were demonstrated in early phase of healing in MCP group which regressed at day 30 to level of other groups. These findings were similar to other studies (11,14). The application of NaHA on the traumatic site was also found to be easier due to its special syringe and it did not adhere to gloves or instruments.

In conclusion, NaHA was found as effective as ORC and MCP for the control of parenchymal bleeding in this renal injury model. The major advantage of this compound over MCP and ORC seems to reduce the inflammatory reaction and to protect the formation of perirenal adhesions. Viscoelastic agents may also be useful in the clinical practice if further studies confirm these hemostatic and antiadhesive actions upon injured kidney surface.

REFERENCES

1. Brandes MD, McAnich JW: Reconstructive Surgery for Trauma of the Upper Urinary Tract. In: Atala A (ed.). Reconstructive Urology. Urol Clin North Am, 26: 183-199, 1999.
2. Wagner WR, Pachence JM, Rishtich J, Johnson PC: Comparative in vitro analysis of topical hemostatic agents. J Surg Res, 66: 100-108, 1996.
3. Scher KS, Coil JA Jr: Effects of oxidized cellulose and microfibrillar collagen on infection. Surgery, 91: 301-304, 1982.
4. Lane SS, Lindstrom RL: Viscoelastic Agents: Formulation, Clinical Applications, and Complications. In: Steinert RF (ed.), Cataract Surgery: Technique, Complications & Management. Philadelphia, WB Saunders, Chapt 4, pp. 37-45, 1995.
5. Shushan A, Mor-Yosef S, Avgar A, Laufer N: Hyaluronic acid for preventing experimental postoperative intraperitoneal adhesions. J Reprod Med, 39: 398-402, 1994.
6. Backer JM, Dayton MT, Fazio VW: Prevention of postoperative abdominal adhesions by a sodium hyaluronate-based bioresorbable membrane: a prospective, randomized, double blind multicenter study. J Am Coll Surg, 183: 297-306, 1996.
7. Rodgers KE, Johns DB, Girgis W, Campeau J, di Zerega GS: Reduction of adhesion formation with hyaluronic acid after intraperitoneal surgery in rabbits. Fertil Steril, 67: 553-558, 1997.
8. Koçak I, Unlü C, Akçan Y, Yakin K: Reduction of adhesion formation with cross-linked hyaluronic acid after peritoneal surgery in rats. Fertil Steril, 72: 873-878, 1999.
9. Hagberg L, Gerdin B: Sodium hyaluronate as an adjunct in adhesion prevention after flexor tendon surgery in rabbits. J Hand Surg, 17: 935-941, 1992.
10. Davidson JM, Nanney LB, Broadley KN: Hyaluronic acid derivatives and their application to wound healing: preliminary observations. Clin Mater, 8: 171-177, 1991.
11. Raccuia JS, Simonian G, Dardik M, Hallac D, Raccuia SV, Dardik H: Comparative efficacy of topical hemostatic agents in a rat kidney model. Am J Surg, 163: 234-238, 1992.
12. Dineen P: Antibacterial activity of oxidized regenerated cellulose. Surg Gynecol Obstet, 142: 481-487, 1976.
13. Cameron WJ: A new hemostatic agent in gynecologic surgery. Obstet Gynecol, 51: 118-122, 1978.
14. Blair SD, Backhouse CM, Harper R, Matthews J, Mc Collum CN: Comparison of absorbable materials for surgical hemostasis. Br J Surg, 75: 969-971, 1988.
15. Weigel PH, Fuller GM, LeBoeuf RD: A model for the role of hyaluronic acid and fibrin in the early events during the inflammatory response and wound healing. J Theor Biol, 21: 219-234, 1986.
16. Weigel PH, Frost SJ, McGary CT, LeBoeuf RD: The role of hyaluronic acid in inflammation and wound healing. Int J Tissue React, 10: 355-365, 1988.
17. West DC, Kumar S: Hyaluronan and Angiogenesis. In: Evered D, Whelan J (ed.). The biology of Hyaluronan. Ciba Foundation Symposium. UK, Chichester, 187-207, 1989.
18. Pandolfi M, Hedner U: The effect of sodium hyaluronate and sodium chondroitin sulfate on the coagulation system in vitro. Ophthalmology, 91: 864-866, 1984.

19. Larsen NE, Leshchiner EA, Parent EG, Hendrikson Aho-J, Balazs EA, Hilal SK: Hyalan gel composition for percutaneous embolization. (Vascular site, the gel induces formation of a solid blood/gel coagulum). J Biomed Mater Res, 25: 699-710, 1991.

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