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ABSTRACT

A total of 12 rabbits were divided into three groups and treated systemically with anticoagulants, either heparin (600 U/kg) or aspirin (0. 25 g/kg). The third group served as nontreated control. In each rabbit four skin, liver and kidney wounds were inflicted. Half of the bleeding wounds were treated topically either with gauze (controls) or collagen fleece (Novacol).

The anticoagulants significantly increased both the blood clotting time and the time of hemostasis of wounds treated with gauze. Topical administration of Novacol onto the bleeding wounds significantly shortened the bleeding time to values observed in control rabbit wounds treated with Novacol.

It is concluded that anticoagulant prophylaxis by heparin–like drugs or aspirin does not affect the hemostatic effectiveness of collagen which is achieved by different mechanisms than that of the above drugs.

INTRODUCTION

The high hemostatic effectiveness of pure collagen fibers textured in the form of woven fleece in otherwise intact animals has been documented[1–8]. Experimental evidence indicates that the hemostatic effectiveness of collagen is due to the adhesion of platelets to a fibrillar form of collagen, followed by a release–aggregation–clot formation cascade (the primary mechanism) or by changing osmolarity of the blood by the gelling effect of the collagen (secondary mechanism)[1, 4–6]. The main use of topical hemostatic agents is in the field of

cardiothoracic surgery, where many patients are on prophylactic anticoagulation therapy. Use of heparin-like drugs or small doses of aspirin are commonly used. Therefore, it is essential that the hemostatic agent is effective in this category of patients with modified hemocoagulation. From the theoretical point of view, heparinization should not interfere with induction of blood clotting by collagen fleece. Heparin acts as a cofactor of antithrombin III. Heparin binds to antithrombin III and facilitate the binding of antithrombin III with thrombin, and hence retard the fibrin formation.

The same applies to patients treated with aspirin, which reduces the clotting by inhibiting the synthesis of thromboxane A_2, which otherwise is a strong inducer of platelet aggregation.

The purpose of this study was to test the hemostatic effectiveness of hemostatic collagen fleece (Novacol) on bleeding skin, liver and kidney lacerations of heparinized and aspirin treated rabbits.

MATERIALS AND METHODS

1 Materials

Novacol, batch #3002 was supplied by Bioplex Corp., a subsidiary of Datascope Corp., NJ, U.S.A.

Sodium heparin, USP, 1000 U/m*l* was from Elkins and Sinns Corp., NJ

Aspirin (Bayer, Germany). Commercial tablets were dissolved in tap water, 0.5 g/5 m*l*.

2 Animal groupings and treatments

A group of 12 New Zealand white female rabbits, 2,5 to 3.8 kg body weight, were randomly divided into three groups:

Group	Number of rabbits	Treatment	Number of determinations		
			Skin	Liver	Kidney
1—Control	4	Gauze	8	8	8
		Novacol	8	8	8
2—Heparinized	4	Gauze	8	8	8
		Novacol	8	8	8
3—Aspirin treated*	3	Gauze	6	6	6
		Novacol	6	6	6

* 1 rabbit died due to anesthesia overdose.

Animals were anesthetized with nembutal sodium i.p. 40 mg/kg and mepromazine 0.2 m*l* with 10 mg/animal i.m. Animals receiving heparin were injected i.v. 600 units/kg 20 minutes prior to test. Animals treated with aspirin received 0.25 g/kg intragastrically per gavage two hours before the testing. All rabbits were shaved in the dorsal region.

3 Surgical wounds

The anticoagulation effectiveness of the treatment was determined by clotting time, measured from blood collected from lateral ear vein. In a drop of blood the first formation of fibrin fiber was determined by a stop watch (see **Table 1**).

a) Skin wounds

Four excisions of full thickness skin wounds, 2×2 cm square, were inflicted in each rabbit. The deep dorsal fascia with arterioles and venules was visualized and the branches cut with a scalpel. Time of hemostasis of an untreated wound (spontaneous hemostasis) as well as of wounds treated with 50–70 mg Novacol was determined. Novacol was compressed over the wound for 10–15 seconds.

b) Liver wounds

Liver edge excisions 1.5 cm wide and 0.3 cm deep were made on defined lobe of the liver with a scalpel. Four wounds were inflicted in each rabbit liver in a reproducible manner. The same amount of Novacol was evenly packed into the bleeding wound. Bleeding was considered under control when the seepage of blood into or through the hemostat stopped.

c) Kidney wounds

A small round punch biopsy needle, 4 mm diameter, was inserted under rotation approximately 2 mm deep into the upper lobe of the kidney cortex. Two similar wounds were inflicted in each kidney.

Two wounds in each organ were treated with Novacol, the other two wounds were covered with gauze and served to determine spontaneous hemostasis. In order to minimize the variability of results, the testing was performed by the same research team. Time was determined when the bleeding stopped. This was recognized by no further enlargement of the blood stained area on the Novacol or on the gauze.

Table 1 Hemostatic effectiveness of collagen fleece—Novacol

Group treatment	Blood clotting time (sec)	Hemostasis (in seconds)					
		Skin	*t*–value	Liver	*t*–value	Kidney	*t*–value
Control Spontaneous clotting +Novacol	135	75±15 48±11	4.13*	125±40 45±25	5.12*	105±35 35±15	5.12*
Heparin Spontaneous clotting +Novacol	600	320±120 65±40	5.82*	210±65 55±25	6.82*	205±60 50±35	6.81*
Aspirin Spontaneous clotting +Novacol	270	85±25 45±20	3.24*	160±45 70±35	3.87*	145±45 45±25	4.60*

Variability is given by $X \pm SD$.

* Statistical significance calculated by Student *t*–test shown by *t*–values. These correspond at degree of freedom ranging from 14 to 26 to $p<0.01$ or $p<0.001$.

Table 2 Effect of Novacol collagen fibers on the bleeding of wounds in heparin or aspirin treated rabbits

Group	Untreated		Treated with Novacol	
	No. of determinations	Bleeding time	No. of determinations	Bleeding time
Control	25	104.4±15.0	46	44.4±7.89**
Heparin	28	286.2±54.0	48	56.2±5.00***
Aspirin	18	126.2±20.4	37	61.8±6.00***

Bleeding time presented in seconds. The variability is shown by $X \pm SEM$.
Asterisks refer to ** $p<0.01$ or *** $p<0.001$.

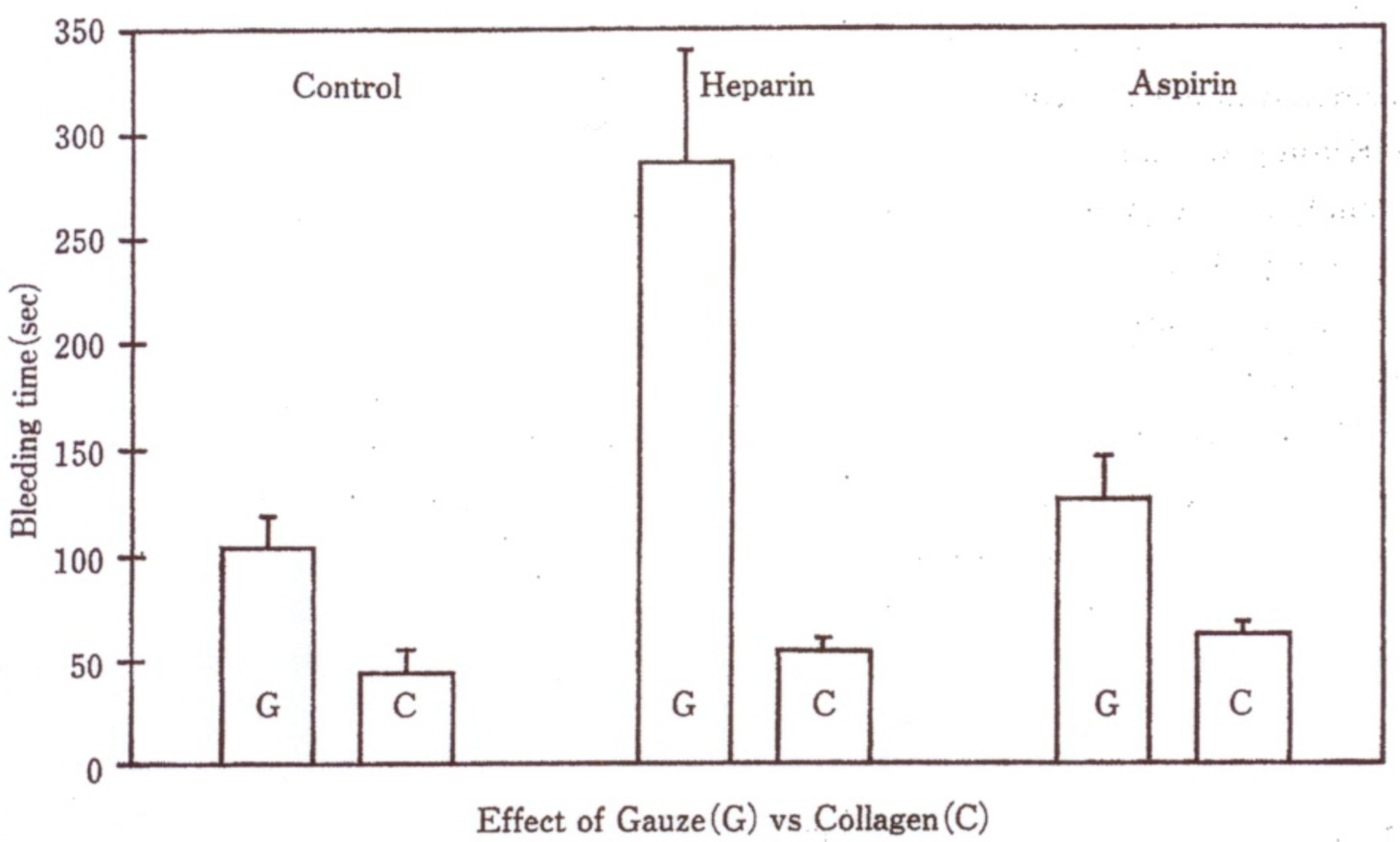

Fig. 1 Effect of hemostatic collagen fleece on bleeding of wounds in heparin or aspirin treated rabbits

RESULTS

The values of hemostasis for the three organs tested presented as time in seconds, when the bleeding of gauze or Novacol treated wounds completely stopped, are presented in **Table 1.** The data show that either treatment, heparin or aspirin, significantly increased the spontaneous bleeding time. Only in the skin wounds, the increased bleeding induced by aspirin was not statistically significant. In all other situations, the heparin treatment tripled or doubled the bleeding time, the effect of aspirin was less, achieving prolongation of the bleeding by 50% when compared to untreated control group data.

Bleeding wounds inflicted in skin, liver or kidney of rabbits treated either with heparin or aspirin clotted significantly faster when treated topically with Novacol. In fact, hemostasis

of these animals was not statistically different from Novacol treated wounds of control rabbits. Due to the same trend of the results, reflecting either the anticoagulation treatment modality or the effect of Novacol, we summarized the appropriate data for further statistical treatment as shown in **Table 2** (and **Fig. 1**).

The results shown in **Table 2** strengthen the previous conclusion. Both systemically administered anticoagulants significantly increased the bleeding time of any wound. Topical treatment of bleeding wounds with Novacol significantly reduced the bleeding of rabbits treated with either anticoagulant to the time found in control rabbits. Therefore, **Table 2** gives the mean values and standard error of the mean summarized data for all tested wounds, nontreated and treated with Novacol, regardless of the bleeding site.

DISCUSSION

The results of this study are in agreement with the established mechanism of clotting as induced by collagen on one side[4–6] and with the knowledge on the effect of heparin or aspirin on blood coagulation. Also, because collagen fibers induce blood coagulation by a mechanism other than that involved in heparin or aspirin effect, our finding of effective hemostasis by collagen fleece in all rabbit wounds, regardless of treatment, is a logical finding. In each animal in all three treatment groups, the average time to achieve hemostasis for lesions treated with Novacol is less than the corresponding one from the untreated lesions. Statistical analysis showed that the differences among the treatment groups under Novacol do not differ at the 0.05 level. In other words, although both heparin and aspirin increase bleeding times (as is well documented), application of collagen hemostatic fleece reduces the times to achieve hemostasis to essentially the same level that it achieves in the control animals.

The selected dose of heparin (600 U/kg body weight) was effectively prolonging the bleeding time approximately three times when compared to control–nontreated rabbits. The effectiveness of aspirin at the dose of 0.25 g/kg body weight on blood coagulation was evident, but much less than heparin. On average, the coagulation was delayed in aspirin treated rabbits by 50% of values for control rabbits.

In our models of wound bleeding the blood flow was strongest in the liver avulsion, followed by kidney puncture. Skin bleeding was marginal.

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