Vasocontractile effect of SeraSeal® in rat thoracic aorta

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Hemostasis is a primary patient management issue in the oral, and maxillofacial surgery.[1] Most conventional hemostatic techniques are being used to minimize blood loss, such as manual pressure, ligature, and the application of a tourniquet.[2] The other hemostatic techniques are sealing of bleeding vessels with thermal methods, and topical hemostatic agents.[3] Hemorrhage can largely be controlled through local methods such as, direct pressure, electrocoagulation, or hemostatic agent.[1] SeraSeal® is one of the primary agents, which is recently available in clinical practice. The vascular action of SeraSeal® is not yet investigated. The objective of this study is to examine whether SeraSeal® has a vasoconstrictor hemostatic effect on rat thoracic aorta.

This study was performed from January to March 2010 in Gulhane School of Medicine, Ankara, Turkey. Six locally bred Wister male adult rats (weighing 250-300 g) were used. The Local Animal Ethics Committee of our institute approved the study protocol. In this study, we used the same experimental protocol by Seyrek et al.[4] Rats were anesthetized with a mixture of xylazine (Rompun, 2% solution; Bayer, Leverkusen, Germany), and ketamine (Ketalar, 30 mg/kg [Parke-Davis, Morris Plains, NJ, USA]). Then, thoracic aorta were dissected out and cleaned of adhering fat, and connective tissue. Aortic ring segments were cut (3 mm in length) and mounted in isolated tissue baths containing oxygenated 10 ml Krebs-Henseleit solution (composition in mmol/l: 118 sodium chloride, 4.7 potassium chloride (KCl), 2.5 calcium chloride, 7 magnesium sulfate, 1.2 water, 1.2 dipotassium phosphate, 25 sodium hydrogen carbonate, and 10 D-glucose) at 37°C for isometric tension measurements by a force-displacement transducer (model FT03 [Grass Instruments, Astro-Med Inc, West Warwick, RI, USA]), and amplified with a strain gauge amplifier (model P122 [Grass Instruments, Astro-Med Inc, West Warwick, RI, USA]), and analyzed by using data-acquisition, and analysis software (Polyview version 2.0 [Grass Instruments, Astro-Med Inc, West Warwick, RI, USA]). The segments were equilibrated under final resting force of one g for 1.5 hours. After the equilibration period, arterial rings were challenged with 68 mM KCl in order to test the viability. Since a higher contraction indicated functional integrity of vascular smooth muscle, the rings that were contracted more than one g were included in the experimental protocol (number of rings=14). The tissues were washed every 10 minute (min) during an additional 30-min waiting period. Tissues were tested for endothelium integrity with acetylcholine (ACh [10^{-6} M]) after contracting them with phenylephrine (PE [10^{-6} M]). In endothelium-intact tissues, control contractile responses to different concentrations of SeraSeal® were recorded. Contraction to the test material was tested at same concentrations in the presence of nifedipine (10^{-6} M) in different rings. The rings were incubated for 30 min with nifedipine before the experimental procedure was applied.

The endothelium was denuded from 4 rings to test if mediators released from it are involved in vasoactive effect. Contraction to the test material was tested at the same concentrations in endothelium-denuded rings. The (n) represents the number of animals from which the aortic rings were obtained. The KCl was purchased from Merck Co, (Darmstadt, Germany), PE, acetylcholine, and nifedipine were purchased from Sigma Chemical Co. (St. Louis, MO, USA). SeraSeal® was purchased from Wortham Laboratories, Inc (Chattanooga, TN, USA). SeraSeal®-evoked contraction response is expressed as the arithmetic mean ± SEM of the KCl response in each corresponding tissue. Groups were compared by using Mann-Whitney U test to determine significant differences among the means of the data groups. A p-value of $p<0.05$ was accepted as significant difference. The baseline values for the level of precontraction (1.06 ± 0.06 g), and the final active tonus after addition of 68 mM KCl (1.38 ± 0.18 g) were similar in all experiments. SeraSeal® elicited concentration-dependent contractions (n=6). SeraSeal® induced contractions were statically significant at 3 doses (one ml/10 ml, 1.3 ml/10 ml, 2 ml/10 ml). Mechanical rubbing of the endothelium did not significantly alter contractile responses induced by SeraSeal® (**Table 1**, n=4). In addition, relaxation induced by ACh (10^{-6} M) was significantly reduced in endothelium-denuded aorta (endothelium intact: 35.46 ± 4.56, endothelium denuded (%): 1.79 ± 1.03% of PE contraction). Pre-incubation with nifedipine also did not alter contractile responses induced by SeraSeal® (**Table 1**, n=4). Our results show that SeraSeal® induces dose-dependent contraction in the rat aorta. The contractions induced by SeraSeal® are not dependent on either calcium influx from membranial calcium channels, or endothelium. The vasoconstrictor property of SeraSeal® at high doses might allow proper

Table 1- Contraction to SeraSeal® at 2 ml/10 ml dose in thoracic aorta rings under basal tonus. Data are expressed as % contraction of 68 mM KCl. Each value is expressed as mean ± SEM.

SeraSeal® (2 ml/10 ml)	Mean ± SEM
Control	30.86 ± 3.89
Endothelium-denuded rings	39.84 ± 7.86
Nifedipine (10^{-6} M)-incubated rings	40.15 ± 8.45

KCl - potassium chloride, SEM -standard error of the mean

control of hemorrhage. SeraSeal® is a primary hemostatic agent, which includes active bovine protein such as factor II, VII, IX, and X. Thus, SeraSeal® provides coagulation factors which improve hemostasis. In addition to this hemostatic affect, the vasoconstrictor property of SeraSeal® at high doses may improve its efficiency as a hemostatic agent.

Endothelium plays a crucial role in the regulation of cardiovascular homeostasis, and produces many biologically active substances that participate in the regulation of vascular tone, cell growth, inflammation, and hemostasis. Endothelium-derived relaxing factors are nitric oxide (NO), prostacyclin, and endothelium-derived hyperpolarizing factor. Endothelin, which is released from the endothelium is one of the contractile factors.[5] In the present study, contractile responses induced by SeraSeal® were not altered by mechanical rubbing of the endothelium. This result demonstrates that SeraSeal® do not induce contractile responses by inhibiting, or activating vasoactive agent release from the endothelium of the aorta. Rat aorta is a well-characterized tissue model for assessing the effects of SeraSeal® on vascular reactivity, and for making the responses more readily quantifiable, and reproducible. Smooth muscle relaxation in dental pulp vessels, and rat aortas are similar. Therefore, the response to various known vasoactive agents of pulpal vessels is not different from rat aorta.[4] There are several limitations of our study. First, we used 2 ml/10ml of SeraSeal® in our study. This concentration may be below the clinically applied concentrations. Higher concentrations of SeraSeal® may cause a change in pH of Krebs-Henseleit solution. This may have harmful effect on the viability of our preparations. Second, tissue concentrations of SeraSeal® may decrease due to distribution via circulation in clinical conditions. In our experimental setup, we used a fixed concentration of SeraSeal®, and we could not mimic actual clinical situation. Taken as a whole, SeraSeal® is a hemostatic agent including Factor II, VII, IX, and X. It has a vasoconstrictor effect on rat thoracic aorta. We demonstrate that the vasoconstrictor effect of SeraSeal® is not related to calcium, and endothelium. Further studies are needed to explain the exact mechanism of the vasoconstriction.

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