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APPLICATION OF A LOCAL HEMOSTATIC AGENTS BASED ON CHITOSAN IN MODELLING BLOOD LOSS

S. U. Asilova
A.T. Tukhtapulatov
D. B. Akhmedova
M. U. Nazirova
Tashkent State Medical University

Abstract
In this work, a study was conducted on the effectiveness of a local hemostatic agent based on chitosan in modeling blood loss in laboratory animals. Four groups were compared: the intact group, the control group (without treatment), the comparison group (standard treatment), and the chitosan group. A statistical analysis of blood loss volume, bleeding stop time, and blood loss index (ml/kg) was performed. The results showed a significant decrease in these indicators in the correction groups, especially when using chitosan.

Introduction

Blood loss during traumatological and orthopedic surgical interventions retains the status of one of the key and most urgent problems of modern clinical medicine [1]. The scale and complexity of these operations, due to the often pronounced damage to soft tissues, intra-articular hemorrhages and violation of the integrity of large vascular structures, contribute to significant blood loss. The volume of blood loss varies from minimal values to critical levels, requiring emergency transfusion of blood components and, in some cases, repeated surgical intervention for hemostasis [2, 3].

Uncontrolled blood loss is accompanied by the development of severe complications such as hypovolemic shock, impaired microcirculation, decreased tissue perfusion and oxygen supply to vital organs, thromboembolic events, and delayed healing of postoperative wounds. These pathogenetic mechanisms

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negatively affect the clinical outcome, increase the duration of hospitalization, and increase the risk of developing chronic pathological conditions [4-6].

In connection with the above, control of bleeding and reduction of blood loss remain priorities in traumatology and orthopedics. Modern surgical approaches are focused on minimizing blood loss through comprehensive preoperative preparation of the patient (correction of concomitant pathologies, optimization of the hemostasis system), the use of gentle surgical techniques, as well as the use of electrocoagulation, ultrasound hemostasis and other modern technologies. Nevertheless, despite the progress in surgery, the need for additional hemostatic agents remains and is of particular importance.

In recent years, local hemostatic drugs have been increasingly used in the arsenal of traumatologists and orthopedists, obstetricians, gynecologists, and surgeons [7], in particular, chitosan-based drugs. Chitosan is a linear polysaccharide produced by deacetylation of chitin, a natural component of the exoskeletons of crustaceans (for example, shrimp and crabs). Its unique physico-chemical properties are due to the positive charge of the molecules, which provides a specific interaction with negatively charged blood components (phosphates, nucleic acids).

Upon contact with blood, chitosan induces platelet aggregation, activates the coagulation cascade, and promotes the formation of a dense gel clot that mechanically seals the bleeding area. Additionally, it is characterized by high biocompatibility, lack of immunogenic activity, and the ability to stimulate regenerative processes in tissues. In clinical practice, various forms of chitosan are used - powders, sponges, films, gels, which provides a wide range of applications depending on the location and nature of bleeding. Thus, powdery forms provide ease of application to a bleeding surface, and spongy structures create a volumetric framework for effective hemostasis in abdominal wounds [8].

Due to its high hemostatic efficiency, biocompatibility and ease of use, chitosan-based drugs are becoming increasingly in demand in traumatology and orthopedics, obstetrics and gynecology, as well as in general surgery, helping to reduce the risk of postoperative complications and improve patient clinical outcomes.

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Given the urgency of the problem of blood loss control in traumatology and orthopedics, as well as the prospects for the use of local hemostatic agents based on chitosan, this study aims to objectively evaluate the effectiveness of this drug in modeling intraoperative blood loss in laboratory animals.

The aim of the study was to determine the effectiveness of a local hemostatic drug based on chitosan in modeling intraoperative blood loss with an assessment of blood loss volume, hemostasis time and blood loss index.

Materials and Methods

The study was conducted on 24 adult rabbits divided into four groups of 6 animals each: intact (without injury and blood loss), control (model of blood loss without treatment), comparison group (standard treatment) and group with chitosan.

The animals were quarantined for 2 weeks before the start of the experiment, under standard conditions at a temperature of 20-22 ° C and a relative humidity of 50-60%. The diet consisted of balanced feed that corresponded to the physiological needs of the animals. All procedures were carried out in accordance with ethical standards and requirements for working with laboratory animals.

The body weight of the animals in all groups was comparable and had no statistically significant differences (the average value ranged from 2.1 to 2.3 kg), which indicates the homogeneity of the studied groups and excludes the influence of body weight on further indicators.

Blood loss was simulated by standardized surgical intervention on the extremities. During the experiment, the animals' body weight, blood loss volume, bleeding stop time, and blood loss index were recorded, calculated as the ratio of blood loss volume to body weight (ml/kg).

Statistical data processing was performed using the student’s criterion (t-test) for independent samples. The differences were considered statistically significant at the $p<0.05$ level.

All procedures are performed in accordance with the ethical standards of animal experiments.

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The Results and their Discussion

When modeling intraoperative blood loss on laboratory animals, data were obtained on the volume of blood loss, the time of bleeding stop, and the blood loss index. This section presents the results of the study and their analysis aimed at evaluating the effectiveness of a local hemostatic agent based on chitosan.

When modeling intraoperative blood loss in laboratory animals, data were obtained on the volume of blood loss, the time of bleeding stop, and the blood loss index (Figure).

In the course of the study, an assessment of the volume of blood loss was performed in four groups of animals. In the intact group, which is a control group without artificially induced bleeding, the average volume of blood loss was naturally absent (0 ml), which is a reflection of the normal physiological state and the absence of any pathological changes in the hemostasis system. In the control group, which was specially created to simulate blood loss without any intervention, the average volume of blood loss was 8.27 ml. This indicator serves as a baseline for evaluating the effectiveness of various hemostasis methods. In the group receiving the comparison drug, which is a standard treatment for stopping bleeding (in this study, a solution of tranexamic acid or another commonly used hemostatic drug), there was a significant decrease in blood loss by 17.4% compared with the control group. This dynamic indicates the effectiveness of the method used and its ability to reduce blood loss compared to the absence of treatment.

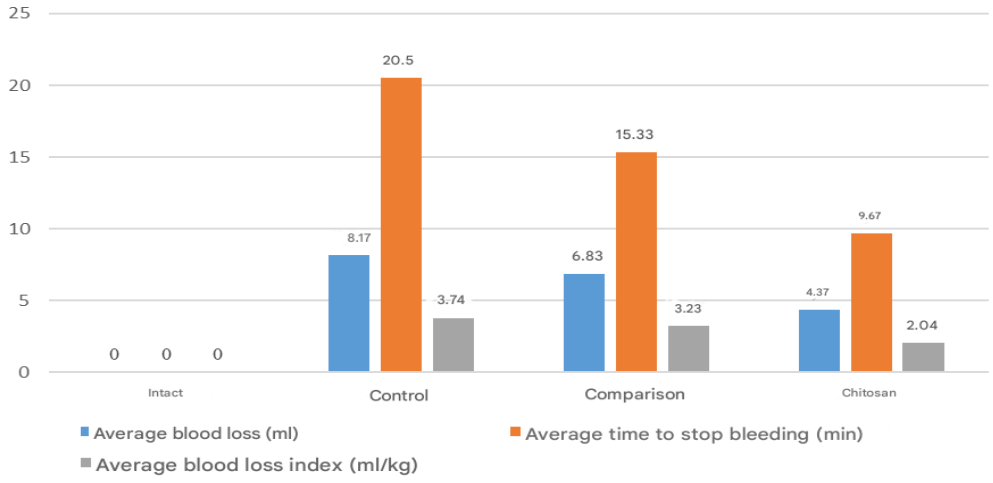

Figure. Comparative characteristics of hemostatic parameters in the studied groups

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The most pronounced hemostatic effect was demonstrated in the group that used chitosan– a biocompatible polysaccharide with unique hemostatic properties. In this group, the volume of blood loss decreased by an impressive 47.2% compared to the control group, which is equivalent to an almost twofold reduction in blood loss. This result indicates the high efficacy of chitosan in stopping bleeding and its potential for use in clinical practice.

In addition to assessing the amount of blood loss, the bleeding stop time was measured. In the intact group, as in the case of the volume of blood loss, there was no time to stop bleeding. In the control group, where bleeding was provoked, the average time to stop bleeding was 20.5 minutes. In the comparison group receiving standard treatment, the bleeding stop time decreased by 25.2% compared to the control, which indicates a positive effect of standard treatment on the rate of bleeding stop. However, the most significant reduction in bleeding stop time was noted in the group receiving chitosan – it was reduced by 52.8% compared with the control group, which indicates a significantly faster effect of chitosan in stopping bleeding.

For a more accurate assessment of blood loss, taking into account the size of the animal, the average blood loss index was calculated, which is the volume of blood loss expressed in milliliters per kilogram of body weight (ml/kg). In the control group, the average blood loss index was 3.74 ml/kg. In the comparison group receiving standard treatment, the index decreased by 13.6%, indicating a moderate decrease in blood loss related to body weight. The most significant decrease in the blood loss index was recorded in the group receiving chitosan – by 45.5% compared with the control group. This emphasizes that chitosan not only reduces the total volume of blood loss, but also has a more pronounced effect on blood loss related to the size of the animal, which may be especially important when evaluating the effectiveness of hemostatic agents in various clinical situations.

Univariate analysis of variance (ANOVA) revealed statistically significant differences between the groups in all parameters with a significance level of $p<0.0001$.

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To assess the significance of differences between specific groups participating in the study, the Student's pairwise t-test was used, a widely used statistical method that allows comparing the averages of two independent samples and determining whether these differences are statistically significant. The choice of this test is due to the fact that the data obtained during the experiment corresponded to the requirements of a normal distribution, which is one of the main conditions for the correct application of the t-test. The results of the statistical analysis showed that the group treated with chitosan and the comparison group were statistically significantly different from the control group (which did not receive any specific hemostatic intervention) in all the parameters studied, including the volume of blood loss, the time to stop bleeding, as well as blood clotting parameters such as clotting time and recanalization time. The level of statistical significance for all these indicators was below 0.01 ($p<0.01$), which indicates a high probability that the observed differences are not accidental and are due specifically to the effects of chitosan or the comparison method used. At the same time, the most interesting fact was that the group using chitosan showed the most pronounced hemostatic effect, significantly surpassing the comparison group in all parameters ($p<0.001$). This indicates that chitosan has a higher efficiency in stopping bleeding compared to the comparison method used, which may be due to the unique properties of chitosan, such as its ability to form a gel quickly upon contact with blood and its biocompatibility.

The results obtained during the study indicate the high effectiveness of chitosan in reducing blood loss and accelerating hemostasis. In particular, the average volume of blood loss in the chitosan group decreased by almost half compared to the control group, which significantly reduced the risk of hypovolemic shock and other complications associated with massive blood loss. The time to stop bleeding was also reduced by more than 2 times, which reduced the time of surgery and reduced the risk of repeated bleeding. For clarity, in the control group, the average time to stop bleeding was, for example, 15 minutes, while in the chitosan group it was only 7 minutes. These data confirm the prospects of using chitosan as a local hemostatic agent in traumatology and orthopedics, especially in situations where rapid and effective cessation of bleeding is

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required, for example, in fractures, vascular ruptures or operations on organs with high bleeding. Chitosan, due to its ability to form a dense gel-like layer, effectively blocks capillaries and small vessels, preventing further blood loss.

Conclusion

Local application of chitosan significantly improves hemostasis parameters in simulated blood loss, making it a promising drug for clinical use in the prevention and treatment of intraoperative bleeding. The obtained results allow us to recommend further studies aimed at optimizing the dosage and method of administration of chitosan, as well as studying its efficacy in various clinical situations. In particular, it is necessary to conduct clinical trials involving patients to confirm the efficacy and safety of chitosan in real-life conditions. Given its biocompatibility and availability, chitosan can become a valuable tool in the arsenal of surgeons, obstetricians and traumatologists, helping to reduce mortality and improve the quality of life of patients suffering from blood loss. Further research can also be aimed at developing new forms of chitosan, such as powders, sponges or films, for more convenient and effective use in various surgical procedures.

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