Nurs Midwifery Stud. 2014 September; 3(3): e22063. DOI: http://dx.doi.org/10.17795/nmsjournal22063
Published online 2014 September 20. **Research Article**

Investigating the Effect of Zinc Chloride to Control External Bleeding in Rats

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Received: July 15, 2014; **Revised:** August 12, 2014; **Accepted:** August 13, 2014

Background: Despite all progresses in surgical science, bleeding caused by traffic accidents is still a challenge for surgeons to save patients' lives. Therefore, introducing an effective method to control external bleeding is an important research priority.
Objectives: This study aimed to compare haemostatic effect of zinc chloride and simple suturing to control external bleeding.
Materials and Methods: In this animal model study, 60 male Wistar rats were used. An incision (two cm in length and half a cm in depth) was made on shaved back of rats. The hemostasis time was measured once using zinc chloride with different concentrations (5%, 10%, 15%, 25%, and 50%) and then using simple suturing. Skin tissue was assessed for pathological changes. Due to abnormal distribution of variables in Kolmogorov-Smirnov test, the data was analyzed using Kruskal-Wallis test Mann-Whitney U tests.
Results: In all the groups, complete hemostasis occurred. Hemostasis times of different concentrations of zinc chloride were significantly less than that of the control group ($P < 0.001$).
Conclusions: Zinc chloride was effective to control external bleeding in rats.

Keywords: Hemostasis; Zinc Chloride; Rat

1. Background

Bleeding, particularly caused by traffic accidents remains one of the main causes of traffic accident deaths. Saving life through stopping or minimizing blood loss until advanced medical aid is the main target of healthcare team (1). Using a tourniquet, applying external pressure, suturing and in some cases elevating the bleeding site are the best options currently available to control external bleeding. But using a tourniquet in some parts of the body such as trunk, neck and the axilla (armpit) is not feasible or associated with several complications including nerve and muscle damage (2, 3). Simple suture is a standard method to control external bleeding; however, it is possible only in medical centers and by trained staff. Using topical agents, which act by stimulating hemostasis at skin surface, is another way to control bleeding (4-6). Zinc chloride is a chemical agent with acidic property and formula of $ZnCl_2$. Zinc chloride is used frequently as a hemostatic agent to control local bleeding in dental surgeries (5). Moreover, it is widely used in water purify as a protein coagulant (7). Zinc chloride is potentially a very strong haemostatic agent regarding significant amount of proteins in blood. In fact, zinc chloride exerts its haemostatic effect through a chemical reaction with blood proteins, and this makes zinc chloride a very efficient haemostatic agent. Therefore, it could be an effective haemostatic agent to control bleeding even in patients with abnormal body haemostatic system. Although haemostatic effect of zinc chloride has been already found, its effect in controlling external bleeding has not been assessed and compared with suturing as a standard method.

2. Objectives

This study aimed to compare the haemostatic effect of zinc chloride and simple suturing to control superficial venous bleeding.

3. Materials and Methods

3.1. Animals

This study was performed at Isfahan University of Medical Sciences from December 2013 to March 2014. In this study, 60 male Wistar rats weighting 180-230 grams were randomly divided into six groups, each containing 10 rats. One week before the study, animals were kept at 21 ± 1°C with 12 hours light/dark cycle (lights on from 8:00 AM to 8:00 PM). They had access to standard rat chaw and water ad libitum.

3.2. Surgery

Rats were anesthetized by intraperitoneal (IP) injec-

tion of a Ketamine/Xylazine mixture (ketamine 100 mg/kg and xylazine 10 mg/kg). The 10% Ketamine and 2% xylazine (Alfasan, Netherland) were purchased from local supplier. An incision of two cm in length and half a cm in depth was made on shaved back of rats by scalpels No. 10 (Figure 1).

3.3. Zinc Chloride Administration and Measuring Hemostatic Time

Zinc chloride was purchased from Merck Company (Darmstadt, Germany). Aqueous solutions of zinc chloride were prepared in five concentrations of 50%, 25%, 15%, 10% and 5% (w/v) in distilled water; each concentration was used in one group of rats. Half a milliliter of solutions was applied to the incision site by an insulin syringe. The time of hemostasis was measured using a chronometer. The hemostasis time was considered as the time required for complete drying of bleeding and no blood discharge from the incision site (Figure 2). The mean of measured times in ten rats of each group was considered as the haemostatic time for each zinc chloride concentration. Simple suturing using nylon 3-0 was used as the standard method of hemostasis in the control group. All simple sutures were performed by one surgeon. After controlling external bleeding, each rat was treated intraperitoneally with 50 mg Cefalotin (1g Cefalotin/10 mL, Aspen Pharmacare Australia Pty Ltd, Australia) to prevent infection.

3.4. Pathological Study

Three days after the treatment, all rats were anesthetized using IP injections of a mixture of Ketamine and Xylazine (Ketamine 100 mg/kg and Xylazine 10 mg/kg). Then rats were placed in a prone posture on the operating table and previous sites of treatment were resected. The resected tissues were immediately fixed in formalin and sent for pathology study. Based on defined pathological grading (8, 9), pathology results were classified into six groups including zero: no change, 1: minor inflammatory infiltration without edema, 2: mild to moderate inflammatory infiltration with mild edema, 3: mild to moderate inflammatory infiltration and moderate edema, 4: moderate inflammation with neutrophils scattered and diffuse edema, 5: severe inflammation of the tissue and edematous changes, fibrosis and hemorrhage.

3.5. Ethical Considerations

Animal handling and all experiments were performed in accordance with the international guidelines set out in the Guide for the Care and Use of Laboratory Animals (Institute of Laboratory Animal Resources, 1996). The study protocol was approved by local research council and the animal research ethics committee at Isfahan University of Medical Sciences, Isfahan, Iran (grant number: 9378, ethical code: 1912).

3.6. Statistical Analysis

Data analysis was performed using SPSS software version 13 (SPSS, Inc., Chicago, IL, USA). Kolmogorov-Smirnov test was used to assess normal distribution of data. Kruskal-Wallis test was used to compare the mean hemostasis times in zinc chloride groups and Mann-Whitney U test was used to compare the mean hemostasis times between zinc chloride groups and that of control group (suturing technique).

4. Results

4.1. Hemostatic Results

Hemostasis time of the six groups were shown in Table 1. Complete hemostasis was occurred in all groups. However,

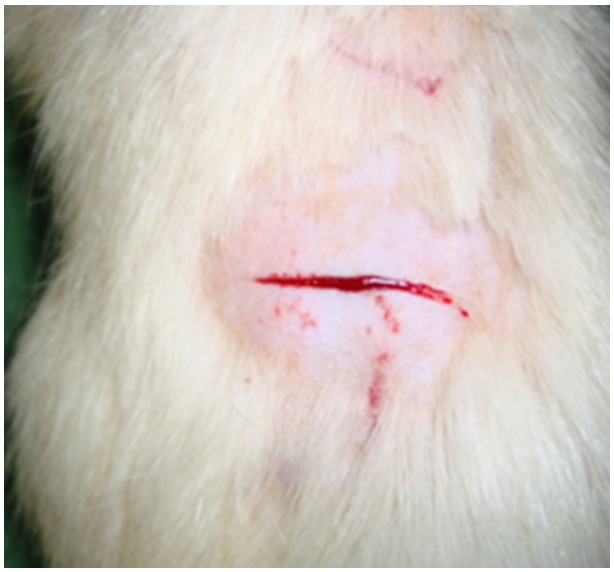

Figure 1. External Bleeding in Wistar Rat

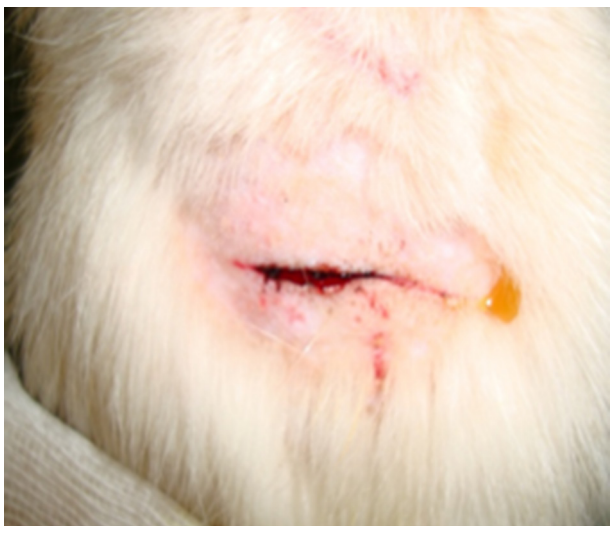

Figure 2. Control of External Bleeding by Zinc Chloride (50%)

Table 1. Hemostasis Time Using Different Concentrations of Zinc Chloride and Suturing Technique to Control External Bleeding

Groups	Zinc Chloride 5%	Zinc Chloride 10%	Zinc Chloride 15%	Zinc Chloride 25%	Zinc Chloride 50%	Suture	P Value
Haemostatic times							< 0.001
Mean ± SD (second)	42.00 ± 4.19	30.80 ± 1.68	21.20 ± 1.31	14.10 ± 1.37	8.20 ± 0.919	84.00 ± 4.05	
Median	40.00	32.40	19.80	12.30	7.00	80.00	
Interquartile range test	3.50	2.50	2.40	2.30	1.90	3.90	

Table 2. Frequency of Pathological Grades (Grades 0 to 5 Based on the Severity of Pathological Inflammation) Three Days After Exposure to Different Concentrations of Zinc Chloride and Suturing Technique [a]

Pathological grade	Zinc Chloride 5%	Zinc Chloride 10%	Zinc Chloride 15%	Zinc Chloride 25%	Zinc Chloride 50%	Suture
Grade 1	10 (100)	10 (100)	10 (100)	3 (30)	10 (10)	10 (100)
Grade 2	0 (0)	0 (0)	0 (0)	7 (70)	9 (90)	0 (0)
Total	10 (100)	10 (100)	10 (100)	10 (100)	10 (100)	10 (100)

[a] Data are presented as No. (%).

significant difference was observed between hemostasis times in the six groups ($P < 0.001$). Moreover, significant differences were observed between hemostasis time in groups with different concentrations of zinc chloride and the control group. Hemostasis time in all experimental groups were significantly less than that of the control group ($P < 0.001$).

4.2. Pathological Results

All wounds were in grade 1 on the third day after the experiment except the wounds in the groups in which 25% and 50% concentrations of zinc chloride were applied (Table 2). No wound was in grades 0, 3, 4 and 5.

5. Discussion

The present study aimed to compare the haemostatic effect of zinc chloride and simple suturing in controlling external bleeding. We showed that hemostasis time was significantly shorter in groups of zinc chloride compared to simple suturing. A number of studies conducted on local haemostatic agents indicated the efficacy of these materials in reducing haemostatic time and patients' need for blood or blood products, leading to improved prognosis of patients after surgery (10-14). Most of local agents currently used to stop external bleeding stimulate hemostasis on the cut surface and require normal haemostatic systems to exert their functions. Zinc chloride unlike well-known haemostatic agents, exerts its haemostatic effect through a chemical reaction with blood. This makes it a very efficient agent. In the current study, we also studied inflammatory and pathological effects of zinc chloride on exposed tissues. Pathology study showed that zinc chloride even at very high concentrations, did not cause inflammation greater than grade two, and immune system reaction to this haemostatic agent did not differ significantly from simple suturing. In two studies, Nouri et al. sought the haemostatic effect of ferric sulfate and Ferric Chloride on external and hepatic bleeding. They reported that ferric sulfate is an effective haemostatic agent (8, 9). Pathological changes following administration of ferric sulfate was consistent with changes occurred in the present study. Nouri et al. noted that skin tissue of Wistar rat had a slight inflammatory reaction to ferric sulfate as a foreign body (8). According to Kim haemostatic agent which does not need normal body haemostatic system to exert its effect. Moreover, acidic property of zinc chloride should be considered. This chemical agent coagulates the blood proteins and makes a barrier to prevent outflow of blood from vessels. Such a barrier also blocks zinc chloride to enter vessels and preventing its potential systemic side effects (8).

According to Rethnam viewpoint, a good haemostatic agent should not interfere with tissue healing, stop bleeding in a shortest possible time, be easily portable and compatible with life and impose minimum complication to patient with a reasonable price (15). Considering the definition of a haemostatic agent provided by these researchers, unique features of zinc chloride such as not requiring normal haemostatic system for function, make this chemical substance an extremely effective topical haemostatic agent to control external bleeding along with other methods.

Because no study has investigated the hemostatic effect of zinc chloride in controlling external bleeding, we expected that lower concentrations of zinc chloride could not control external bleeding. Therefore, different concentrations of zinc chloride were used in this study.

However, complete hemostasis was occurred in all the groups. Due to the financial limitation, we did not use concentrations lower 5%. Therefore, it is suggested to perform further investigations using lower concentrations of zinc chloride in controlling external bleeding in an animal model to determine the lowest concentration of zinc chloride able to control external bleeding.

Acknowledgements

The authors would like to appreciate kind assistance and financial support provided by the Vice-Chancellor for Research at Isfahan University of Medical Sciences.

Authors' Contributions

Saeed Nouri: Acquisition of data, analysis and interpretation of data, study supervision, study concept and design and statistical analysis. Mohammad Reza Sharif: Drafting of the manuscript, critical revision of the manuscript for important intellectual content, administrative, technical, and material support. Fatemeh Tabatabaei: Drafting of the manuscript and critical revision of the manuscript for important intellectual content. Shima Farokhi: Drafting of the manuscript, critical revision of the manuscript for important intellectual content administrative, technical, and material support

Financial Disclosure

No benefits in any form were received or would be received from a commercial party related directly or indirectly to the subject of this manuscript.

Funding/Support

This study was supported financially by the Vice-Chancellor for Research of Isfahan University of Medical Sciences.

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