Local Hemostatic Effect of Aqueous Ozone in Cutting Wound Surface

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(Accepted for publication, February 19, 2014)

Abstract: The medical use of ozone has been based on its antibacterial and oxidative characteristics. Currently ozone is being discussed in dentistry as a possible alternative oral antiseptic agent. In this study, we examined the hemostatic effect of water and gel contains aqueous ozone in animal testing. The mean of bleeding time using ozonated water and ozonated gel were observed for significant difference compared with no treatment. 0.5 ppm diluted ozonated water shortened bleeding time the same as 4.0 ppm ozonated water. These results suggest that ozonated water and gel which contains aqueous ozone show hemostatic ability which is almost equal to the Bosmin solution and Liquid Thrombin.

Key words: Hemostatic effect, Ozone, Ozonated water, Ozonated gel

Introduction

Ozone, an allotrope of oxygen, is a reactive gas and is present in large quantities in the upper layer of the Earth’s atmosphere1-4). The medical use of ozone has been based on its antibacterial and oxidative characteristics. Therefore, ozone has been widely used as prevention of hospital-acquired infection in the medical field. Currently, ozone is being discussed in dentistry as a possible alternative oral antiseptic agent, antibacterial effect of artificial denture and cleaning solution for root canal treatment. Its high antimicrobial power, including against oral pathogens, without resistance development, has been reported not only for gaseous ozone5,6), but also for aqueous ozone7-9). In the concentrations currently used in dentistry, ozone gas has been found to decrease the viability of oral cells significantly9). In comparison, aqueous ozone has been used for sterilizing and washing in dental practice because of a high level of biocompatibility to fibroblasts, cementoblasts, and epithelial cells where it comes into contact with resident oral cells, periodontal disease and apical periodontitis11-13). We would like to report our experiments that ozonated water and gel which contain aqueous ozone show significantly local hemostatic ability.

Materials and Methods

Animals

This study was approved by the animal facility of Inagawa Research Center, Osaka Dental University Institute of Dental Research. All animals received adequate care in accordance with the animal experiment regulations of Osaka Dental University. Five-week-old, male, specific pathogen free, and ICR mice weighing 24.5 to 28.9 g were used as both donors and recipients.

Hemostatic experiments

Before these experiments, 20 mg/kg body weight of pentobarbital sodium (Somnopentyl® injection for animals, Kyoritu Seiyaku, Tokyo, Japan) were injected into the mice. The details of test solutions were shown in Table 1. Epinephrine 0.1 % (Bosmin® solution, Daiichi Pharmaceutical Co., Ltd., Japan) (BS), Thrombine, JP 1000 unit/ml (liquid Thrombin®, Sankyo Co., Ltd., Japan) (LT), Aluminium chloride 25%, Cetylpyridinium chloride 0.5 %, Lidocaine, JP 5.25 % (dental TDZ solution, Bee Brand Medico Dental Co., Ltd., Osaka, Japan) (TDZ), glycerin (Nacalai, Kyoto, Japan) (Gl), ozonated gel (VMC Co., Ltd., Osaka, Japan) (OG), normal saline solution (NS) were used for comparison. No treatment was used as the control condition. Ozonated water (OW) was treated with ozone which was generated by an ozone generator (Ozone Oral Irrigator®, BE SONIC, Tokyo, Japan) in purified water. This condition would result in an ozone concentration of 4 ppm was defined as the 100 % ozonation state. The method of measurement of bleeding time was based on the literature4). 40 mg/kg body weight of pentobarbital sodium was administered intraperitoneally into the mice. The tails were cut at
Table 1. Descriptions of samples used in this study

<table>
<thead>
<tr>
<th>Code</th>
<th>Samples</th>
<th>Composition</th>
<th>Manufacturers</th>
</tr>
</thead>
<tbody>
<tr>
<td>OG</td>
<td>Ozonated gel</td>
<td>Glycerin, 1000 ppm of ozone</td>
<td>VMC Co., Ltd., Japan</td>
</tr>
<tr>
<td>OW</td>
<td>Ozonated water</td>
<td>Purified water, 4ppm of ozone</td>
<td>Authors’ laboratory-made</td>
</tr>
<tr>
<td>BS</td>
<td>Bosmin® Solution</td>
<td>Epinephrine 0.1%</td>
<td>Daiichi Pharmaceutical Co., Ltd., Japan</td>
</tr>
<tr>
<td>LT</td>
<td>Liquid Thrombine</td>
<td>Thrombine, JP 1000 unit/ml</td>
<td>Sankyo Co., Ltd., Japan</td>
</tr>
<tr>
<td>TD</td>
<td>TDZ dental solution</td>
<td>Aluminium chloride 25%</td>
<td>Bee Brand Medico Dental Co., Ltd., Japan</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Cetyldiperoxide 0.5%</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Lidocaine, JP 5.25%</td>
<td></td>
</tr>
<tr>
<td>NS</td>
<td>Normal saline solution (JP)</td>
<td>Sodium chloride 0.9%</td>
<td>Authors’ laboratory-made</td>
</tr>
<tr>
<td>Gl</td>
<td>glycerin</td>
<td>glycerin</td>
<td>Nacalai, Kyoto, Japan</td>
</tr>
</tbody>
</table>

the length of 10 mm from the head of the tail by razor when the mice were posed recumbent posture under general anesthesia. The cutting wounds of mice were dipped into 2mL of test solution after cutting of mice tails. Non-dipping cutting wound of mice were applied to no treatment condition. The bleeding blood was absorbed by the chromatography paper (20×400 mm S1A, Advantec) every 30 seconds. The observation of no adherent blood was defined the bleeding time.

Statistical Analyses

Results were expressed as the mean ± the standard deviation, and the data were analyzed using the paired Student’s t-test. p values less than 0.05 * was considered significant from the control condition.

Observation of mice blood

A portion of blood was added to the 2mL of test solution to observe the macroscopically change of the blood in comparison the difference among the test solution to mice blood.

Results

Effect of local hemostatics on bleeding time was shown in Fig. 1. The mean of bleeding time of OG (120±5 sec.) and 4ppm of OW (120±15 sec.) were observed significant difference from control (300±10 sec.), NS (305±25 sec.) and Gl (365±55 sec.). The mean of bleeding time of BS (115±25 sec.), LT (225±15 sec.) and TDZ (130±60 sec.) were also significantly shortened in comparison with control condition. Concentration effect of OW on bleeding time was shown in Fig. 2. Hemostatic ability was observed even at 0.5 ppm of diluted OW as same as 4.0 ppm. The observation of mice blood blended with local hemostats were shown in Figure 3. Hemolysis was macroscopically found in the case of OW, BS and LT. Blood clot appeared in TDZ. Difference of the color was observed between Gl and OG.

Discussion

Interest in ozone use in dentistry is due to the infectious diseases associated with the oral cavity. Ozone presents great advantages when used as a support for conventional treatments, for example, to dental caries, periodontal procedures, and endodontic treatment. However, to our knowledge, there have been a few reports on hemostatic effect of ozone in the past few decades. Hemostasis is a process which causes bleeding to stop, meaning to keep blood within a damaged blood vessel. It is the first stage of wound healing. Most of the time this includes blood changing from a liquid to a solid state. Hemostasis has three major steps: vasoconstriction, temporary blockage of a break by a platelet plug, and blood coagulation, or formation of a clot that seals the
hole until tissues are repaired. Vascular spasm is the first response of the blood vessels to injury. The vasoconstriction reduces the amount of blood flow through the area and limits the amount of blood loss. This response is triggered by factors such as a direct injury to vascular smooth muscle, chemicals released by endothelial cells and platelets, and reflexes initiated by local pain receptors. Vascular spasm is much more effective in smaller blood vessels. Platelets play one of the biggest factors in the hemostatic process. Being the second step in the sequence they stick together to form a plug that temporarily seals the break in the vessel wall. As platelets adhere to the collagen fibers of a wound they become spiked and much stickier. We investigated that the local hemostatic effect of ozone by using OW and OG. OW was treated with ozone generated by an ozone generator (Ozone Oral Irrigator®, BE SONIC, Tokyo, Japan) in purified water. OG (VMC Co., Ltd., Osaka, Japan) was a glycerine solution dissolved ozone molecules. BS which has local vasoconstrictive effect affects the injected mucosa of the oral cavity, suppression of bloating, bleeding effect and increase the effect of adjunctively-used local anesthetic agent. Therefore, 5-10 times diluted BS is used as the efficient method of local application, eye-drop, nose-drop, spray and tampon. BS in this study also exhibited significant hemostasis effect. The bleeding time of OW and OG were also equal to BS. The significant hemostasis effect of LT is followed by thrombin which promotes a blood-clotting system. Thrombin solution is effective hemostatic ability of blood capillary and parenchyma organ. Thrombin is often used by the local spray at external injury and diseased bleeding in the bleeding of surgery. In our experiments, thrombin was significantly shortened in comparison with no treatment condition. Interestingly, the bleeding time of OW and OG were superior to LT. TDZ was also significantly shortened in comparison with control condition. Arrest of bleeding of TDZ was occurred to the astringent effect of aluminum chloride due to the denaturalization of protein and clotting of blood. Therefore, the color of bleeding blood was turned to the black when TDZ was applied to the cutting wound of mice tail. On the other hand, no clotting blood was macroscopically observed in OW, BS and NS. These results indicate that no significant cytotoxic activity was occurred due to the same isotonicity as extracellular fluid. The color of OG contained blood was turned to the yellow. This color change was probably due to the oxidation of hemoglobin and producing the bilirubin. No direct correlation was occurred between oxidation of hemoglobin and hemostasis because hemostasis ability of OG was same as OW. The ozonated water and gel significantly shortened bleeding time compared with non-treatment. The 0.5 ppm of diluted ozonated water also shortened bleeding time as same as 4.0 ppm of ozonated water. These results suggest that ozonated water and gel which contain aqueous ozone show hemostatic ability which is almost equal to the Bosmin solution and Liquid Thrombin. Our experiments have revealed that hemostatic activity of ozonated water and gel in animal experiments, we currently focus on the application of ozonated water for the clinical treatment of periodontal desease, implant, and dental extraction.

References
8. Nagayoshi M, Fukuzumi T, Kitamura C, Yano J, Terashita M and Nishihara T. Efficacy of ozone on survival and...


