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Effect of Bismuth Subgallate (Local Hemostatic Agent) on Wound Healing in Rats. Histological and Histometric Findings

Vinicius Augusto TRAMONTINA[1]
Maria Ângela Naval MACHADO[1]
Getúlio da Rocha NOGUEIRA FILHO[1]
Sung Hyun KIM[2]
Mário R. VIZZIOLI[3]
Sérgio de TOLEDO[1]

[1]Department of Periodontics, Faculty of Dentistry of Piracicaba, UNICAMP, Piracicaba, SP, Brazil
[2]Department of Periodontics, PUCPR and UTP, Curitiba, PR, Brazil
[3]Department of Oral Diagnosis (Pathology), Faculty of Dentistry of Piracicaba, UNICAMP, Piracicaba, SP, Brazil

The aim of this research was to evaluate the effect of bismuth subgallate on wound healing. In 40 Wistar rats, two standard wounds (3.5 mm x 2 mm) were made using a biopsy punch on the back of each animal. Test wounds were filled with bismuth subgallate and control wounds with 0.9% saline. At 1, 4, 7, 11 and 18 days, the qualitative evolution of the granulation tissue morphology was observed and digitalized histologic images were evaluated. There were no significant histological differences between test and control. Histometrically, there were statistically significant differences between test and control (ANOVA - days 1 and 4; Student t test, $p<0.05$ - days 7, 11 and 18) in terms of the following parameters: area of ulceration - day 1; distance between epithelial edges - day 4; area of granulation tissue - days 7, 11 and 18. It was concluded that bismuth subgallate is biocompatible to the healing tissue, and did not interfere with the normal development of wound healing.

Key Words: bismuth subgallate, wound healing.

INTRODUCTION

Hemostasis is essential in any surgical technique. However, bleeding may be difficult to control in some periodontic procedures, resulting in open wounds, with exposition of connective tissue after surgery and healing by second intention, such as gingivectomy and free gingival grafts. Saroff et al. (1) reported that most of the literature deals with indications, surgical procedures and healing of donor sites of free gingival grafts, but little attention has been paid to the care of donor areas, normally from the palatal mucosa. There are many means of achieving local hemostasis, such as sutures, collagen sponges (2), microcrystalline collagen (3-5), and calcium alginate (6,7).

Kim et al. (8) reported another alternative, a hemostatic compound called bismuth subgallate (BSG) used in medical otolaryngology (9-11). Bismuth subgallate is an insoluble compound that has a heavy metal (Bi) in its composition (12) (Figure 1) which has been used to treat open wounds, acute necrotizing gingivitis, syphilis and other diseases, as well as to control malodor in colostomized patients (13). Maniglia et al. (9)

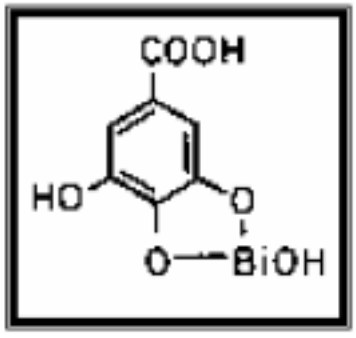

Figure 1. Molecular formula of bismuth subgallate.

Correspondence: Dr. Vinicius Augusto Tramontina, Rua Júlia da Costa 1993/51, 80730-070 Curitiba, PR, Brasil. e-mail: vtramontina@bol.com.br

reported the specific utilization of BSG as a hemostatic agent in otolaryngology in a 12-year retrospective study, using it during tonsillectomy and adenotonsillectomy, in which a low incidence of post-operative bleeding (0.28% of the cases) was achieved compared to 1.2% reported by Pratt and Gallagher (14), who utilized compression with gauze to obtain hemostasis. Other authors have reported the success of BSG to achieve hemostasis in cases of tonsillectomy and adenotonsillectomy (10,11,15), pointing out that this compound is very effective in stopping bleeding of small vessels and capillaries, reducing surgery time and avoiding post-operative bleeding. Thorisdothir et al. (13) reported that BSG acts on factor XII (Hageman) of coagulation, activating the coagulation cascade and leading to the early formation of the fibrin clot. In surgery, it could be of great help in cases that result in open wound and repair by second intention, i.e., donor site of free gingival grafts, gingivectomy, excisional biopsy areas, etc.

Harrison (16) reported that the main steps of wound healing are didactically divided into: a) clot formation and inflammation, b) epithelial healing, c) connective tissue healing, and d) wound maturation and remodeling. After attaining a stable clot, through conversion of fibrinogen into fibrin and polymerization of linked molecules forming a web that retains plasma fractions and cells, the inflammatory process takes place. It involves vascular, cellular and humoral reactions at the wound site, and it prepares the region for healing. Healing depends on the creation, by the inflammatory process, of a favorable environment for cellular metabolism via elimination of microorganisms, necrotic tissue and foreign particles. Epithelial healing occurs in which the keratinocytes form a monolayer of cells that migrate to the center of the wound until contact inhibition occurs, resulting in an epithelial seal of the wound area. This kind of healing prevents the ingrowth of irritants and the loss of fluids (nutrients for the connective cells), maintains the hydration of the wound and promotes an increase in wound strength. Connective tissue healing is the most complex and it depends on the stratification of the epithelial layers. The primary cell involved in this healing is the fibroblast that produces collagen and extracellular matrix, which are essential for regeneration and repair. After the initial inflammatory process, the granulomatous tissue, rich in macrophages, gradually becomes a granulation tissue (predominantly fibroblast), which shows that the connective tissue is healing properly. This process finishes when the collagen aggregation is completed and the extracellular matrix becomes hard. Then, maturation of tissue finally occurs.

Silverstein and Chvapil (17) reported that an ideal hemostatic product has the following characteristics: 1) high hemostatic action, 2) minimal tissue reaction, 3) no antigenic response, 4) bioabsorbable *in vivo*, 5) easy sterilization, 6) low cost, and 7) simulation of tissue structure.

There is much research concerning BSG and its efficiency to achieve hemostasis, but there is no research about its effect on tissues and on wound healing. Therefore, the aim of this investigation was to evaluate the effect of BSG on wound healing by using an animal model.

MATERIAL AND METHODS

Forty male adult albino Wistar rats (weight, 235-275 g), from the central biotery of UNICAMP (Campinas, SP, Brazil) were used. The animals were kept in cages for 20 days and received Labina® (Purina, São Paulo, SP, Brazil) and water *ad libitum*.

The animals were anesthetized with 3% sodic penthobarbital (*ip*, 40 mg/kg, Hypnol®, Fontoveter-Cristália, Itapira, SP, Brazil). A trichotomy of the back of the rats was then performed (approximately 5.0 cm x 5.0 cm), sufficient for 2 perforations (test and control, randomly made). These wounds were standardized with biopsy punches (Miltex®, Miltex Co., Lake Success, NY, USA) with a 3.5 mm diameter, on which a 2 mm mark from the edge of the punch was made, in order to standardize the depth of the incision. The incisions were always made by the same operator, as recommended by Bodner et al. (18) and Khanberg and Thilander (19), preserving approximately a 2 cm distance between both incisions. The pieces of tissue were subsequently excised at their base with the aid of a scissors and calipers, in order to maintain a uniform depth of 2 mm, as marked on the punch.

The wounds were filled with BSG (test wounds) which was prepared by adding 14 g of BSG to 15 ml of saline, so that a consistency similar to toothpaste was obtained, as described by Cozzi et al. (20). The control site was rinsed with a sterile swab and saline. After these procedures, the animals received no other treatment until the time of sacrifice.

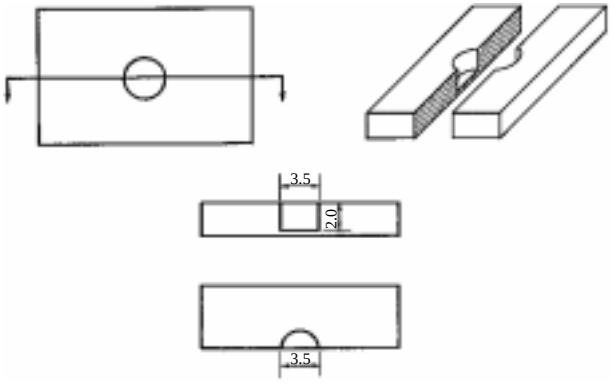

Figure 2. Schematic drawing of skin samples and how they were cut for histological processing.

The animals were sacrificed 1, 4, 7, 11 and 18 days by an overdose of ether, and the skin area containing the wounds was immediately excised and trimmed. The specimens were then stored in 10% formalin for 24 h.

The skin samples (test and control sides) were cut transversally within the diameter of the wound (Figure 2). After routine embedding procedures, 7-µm thick sections were obtained, beginning in the middle of the wound, and stained with hematoxylin and eosin (H&E). The first slide was used to perform the histometric measurements, and the other slides were used to evaluate histological findings. Histometric measurements were made using a light microscope (Diastar, New York, NY) connected to a microcamera (Sony, CCD-IRIS color video camera, Tokyo, Japan). The image was digitalized by a specific image digitalizing and measuring program (Mocha 1.2 Image Analysis software, version 1992-1994, Jandel, San Raphael, CA). The unit used to measure the sections was the "pixel". The slides were analyzed histometrically at a magnification of 2.5X, using the following parameters: 1st day: measurement of ulcerated area (wound area); 4th day: measurement of the linear distance between the edges of the wound (epithelium to epithelium); 7th, 11th and 18th days: measurement of the area of granulation tissue.

Statistical Analysis

Histometric measurements from test and control wounds were submitted to analysis of variance (ANOVA) and, considering that interaction between the causes of variation (period and/or treatment) was found, data were again submitted to the Student t test (at level of 5%).

RESULTS AND DISCUSSION

On the first day, control wounds had discontinuity of epithelium and exposed connective tissue, with some areas of superficial necrosis, revealing the presence of moderate inflammatory infiltration (PMNL and some macrophages). Test wounds were very similar to the control wounds, but with a visible amount of BSG. There was a larger quantity of macrophages in the test wounds.

On the fourth day (Figure 3), control wounds had decreased and it was possible to observe the migration of epithelial cells from the edges of the wound, presenting a very thin layer or showing connective tissue still exposed. Moderate infiltration of PMNL was still present, and below it, it was possible to observe the proliferation of fibroblasts and the presence of neoforming vessels (angiogenesis) and a few thin collagen fibers. On the fourth day, test wounds were similar to control wounds (amount of fibroblasts and neoforming vessels). The basic difference was the presence of BSG and a greater quantity of macrophages in and around it.

On the seventh day, control wounds (Figure 4) were covered by epithelium and there was a drastic reduction in wound size. The presence of cellular infiltration was poor at this phase. Granulation tissue was more fibrous than cellular, different from the 4-day specimens. On the seventh day (Figure 5), the presence of BSG was less evident than on the fourth day in the test wounds, which indicates that it was partially absorbed or digested by macrophages that can be seen surrounding the BSG. The wounds were covered by an epithelium layer and granulation tissue was developing normally, very similar to control. The collagen fibers were still immature, as in the control group (thin and poorly stained when compared to the normal fibers). BSG was located in a deep portion of connective tissue.

On the eleventh day, control sites showed an epithelial tissue whose characteristics were very similar to a normal one, including an initial formation of keratin and specialized adnexae. Granulation tissue had a predominance of fibers that were thicker than those seen earlier, but they were still immature when compared to adjacent normal ones. The pattern of granulation tissue develop-

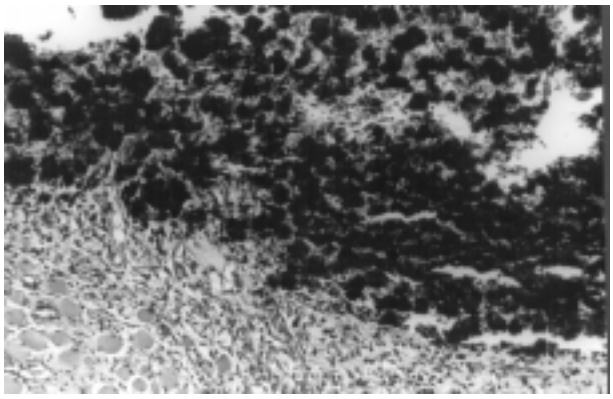

Figure 3. Photomicrography of a test wound showing the presence of BSG (at the top) within the connective tissue (4 days - original magnification 10X).

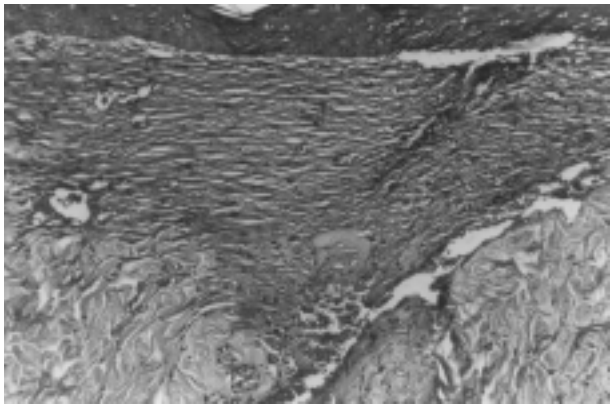

Figure 4. Photomicrography of a control wound showing the quality of the granulation tissue (7 days - original magnification 10X).

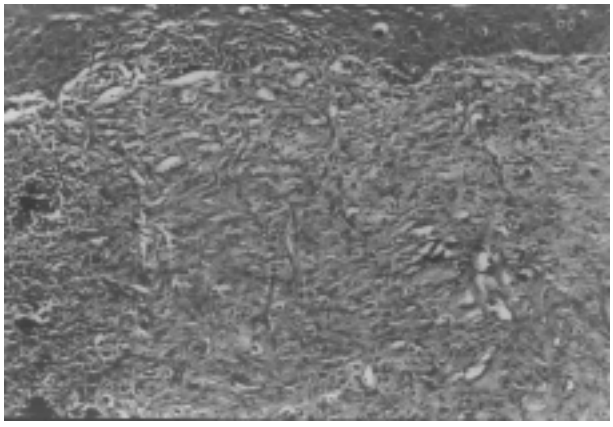

Figure 5. Photomicrography of a test wound showing the quality of the granulation tissue. Note the presence of BSG in the left side and the well formed epithelium (at the top) (7 days - original magnification 10X).

ment in the test wounds was very similar to control wounds, including early formation of keratin and specialized adnexae at the epithelium. A small portion of BSG was still present in the deep connective tissue.

On the eighteenth day, control sites showed a small area of granulation tissue, characterized by a considerable predominance of collagen fibers quite similar to the normal area. Test sites continued to develop similarly to control ones, but they still showed small quantities of BSG surrounded by a few macrophages. This did not prevent the development of granulation tissue.

Histometric evaluation of 5 slides of each group on the first day showed an area of ulceration of 230,231 pixels for the test group compared to 119,838 pixels for the control group. The difference between these means was statistically significant (Student t test, $p<0.05$). On the fourth day, the distance between epithelial edges (five measures for each group) was 648.45 for the test group and 316.02 for the control group. The difference between these means was also statistically significant (Student t test, $p<0.05$). The results concerning the areas of granulation tissue on the seventh, eleventh and eighteenth days are shown in Figure 6.

Thus the objective of this paper was to evaluate BSG in order to obtain information about tissue reactions. BSG was applied to an open standardized skin wound, and healing occurred by second intention. This model was used to simulate the real situation of an open

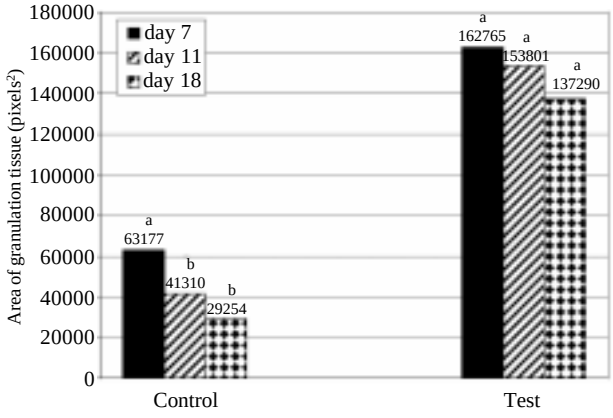

Figure 6. Mean values of the area of granulation tissue at days 7, 11 and 18. Columns with different letters indicate a statistically significant difference for the same day of evaluation. At day 7, there was no statistically significant difference between test and control groups; however at days 11 and 18, the area of granulation tissue was significantly greater at test (BSG) sites.

wound at the palate of patients who would receive a free gingival graft, and the palate would be the donor site.

The results showed that BSG in early periods (first and fourth days) caused an inflammatory response characterized by PMNL and macrophages differing only in number from the control. This suggests that the number of PMNL macrophages is greater in BSG groups because of the physical presence of the product within the tissue. However, none of the samples showed giant cells or any characteristic of foreign body reaction, which is an important finding.

In general, test and control wounds evolved quite similarly, and it was possible to see that, after 7 days, both test and control showed complete covering of the ulcer by a layer of healthy epithelium, which clinically means that wounds filled with BSG could be covered by epithelium in the same way as an untreated wound. Observed macroscopically, test and control wounds had similar lateral contraction.

Histometric measures showed a larger ulceration area on BSG wounds in the first day, that could be explained by the presence of BSG filling the wound to the top and its acting as a barrier to initial contraction. This filling could be a factor that contributes to wound protection against trauma and bacteria. Although at 7, 11, and 18 days, there were larger areas of granulation tissue, there was a slight delay in healing of test wounds. An interesting finding is that, as the test wounds were healing, BSG was located deeply in the tissues and in a small quantity (it had been absorbed mostly by macrophages). This did not significantly hinder the normal healing of this tissue (connective tissue and epithelium). Large areas of granulation tissue at test sites could be explained by the physical presence of BSG in contact with connective tissue.

We conclude that BSG can be used as a hemostatic agent in periodontal surgery, leading to healing by second intention, without deleterious effects to the connective tissue.

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RESUMO

Tramontina VA, Machado MAN, Nogueira Filho GR, Kim SH, Vizzioli MR, Toledo S. Efeito do subgalato de bismuto (agente hemostático local) no processo de reparação de feridas em ratos. Achados histológicos e histométricos. Braz Dent J 2002;13(1):11-16.

O objetivo do presente trabalho foi avaliar o efeito do subgalato de bismuto (SGB) no processo de reparação de feridas. Em 40 ratos Wistar, duas feridas padronizadas foram feitas no dorso do animais utilizando-se um bisturi circular para biópsia (“punch”) de 3,5mm X 2,0mm. As feridas teste foram preenchidas com SGB e as controle, com salina 0,9% e avaliadas com 1, 4, 7, 11 e 18 dias. A evolução qualitativa do tecido de granulação foi avaliada histologicamente e imagens digitalizadas foram medidas histometricamente. A avaliação histológica não demonstrou diferenças significativas entre teste e controle e histometricamente, houve diferenças significativas (ANOVA - dias 1 e 4; teste *student*, $p<0,05$ - dias 7, 11 e 18) em relação aos parâmetros analisados, ou seja, no dia 1: área de ulceração; dia 2: distância entre bordas epiteliais; dia 7, 11 e18: área de tecido de granulação. Pode-se concluir que o SGB apresenta-se biocompatível aos tecidos em reparação e não interferiu significativamente com o desenvolvimento do processo de reparação.

Unitermos: subgalato de bismuto, processo de reparação.

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