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Effect of bismuth subgallate (a hemostatic agent) on bone repair; a histologic, radiographic and histomorphometric study in rats

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S. A. Puia, S. J. Renou, E. A. Rey, M. B. Guglielmotti, C. E. Bozzini: Effect of bismuth subgallate (a hemostatic agent) on bone repair; a histologic, radiographic and histomorphometric study in rats. Int. J. Oral Maxillofac. Surg. 2009; 38: 785–789.

Abstract. Bismuth subgallate (BS) is a hemostatic agent used for soft tissue surgery in otorhinolaryngology and dermatology. Its effect on bone repair has not been studied. The present study undertook a quantitative and qualitative evaluation of post-extraction bone healing in the presence of BS. Under intraperitoneal anesthesia, forty male Wistar rats, 80 ± 5 g body weight, underwent the extraction of both lower first molars. BS was placed in the right post-extraction socket (group E) and the contralateral socket served as control (group C). The animals were killed in groups immediately, 7, 14 and 30 days post-extraction. The mandibles were resected, radiographed and processed for embedding in paraffin. The mesial socket was sectioned along the bucco-lingual axis and stained with hematoxylin-eosin. Total tissue volume and trabecular bone volume of the apical third of the sockets were determined histomorphometrically. At 14 and 30 days post-extraction, group E exhibited bone tissue that resembled that of group C. Histomorphometric analyses showed no statistically significant differences between groups C and E. Bismuth subgallate did not interfere with post-extraction bone healing. Further studies will analyze the effect of this hemostatic agent on bone repair in aniticoagulated rats.

Keywords: bismuth subgallate; bone healing; hemostatic agent.

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Bismuth subgallate (BS) is an insoluble compound, which was used for treating disorders, such as Vincent's angina and syphilis, as well as for reducing colostomy odor and cleaning open wounds. BS has hemostatic properties, it acts on coagulation factor XII (Hageman factor), leading to the activation of the coagulation cascade and improving early formation of a fibrin clot[19]. In 1989, Maniglia et al[12] reported on the use of BS as a hemostatic agent in otorhinolaryngology; the authors observed that its use in tonsillectomy and adenotonsillectomy reduced the incidence of post-surgical bleeding. Other authors have confirmed this property of BS[2–4], demonstrating its effectiveness in stopping bleeding from small vessels and capillaries.

Hemostasis is essential in oral surgery, specifically in procedures resulting in open wounds that expose connective tissue. It is also important for correcting hemostatic failure during surgery on

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patients with clotting disorders. KIM et al[11] reported that BS was successful in improving hemostasis in periodontal surgery. REY et al[18] reported the use of BS in dental surgery on hemophilic patients.

The healing process following tooth extraction is a concern for the oral and maxillofacial surgeon. Several studies have proved the hemostatic efficacy of BS in soft tissues, but there are no data in the literature on the possible effects of BS on bone tissue repair. The aim of the present study was to perform a qualitative and quantitative evaluation of the post-tooth extraction bone healing process in the presence of BS in rats. This is a precursory study to look at the use of BS as a hemostatic agent in patients at risk of bleeding in oral surgery.

Materials and methods

The NIH guidelines for the care and use of laboratory animals were observed[13].

Forty male Wistar rats weighing 80 ± 5 g were used. Bone formation was assessed throughout the post-tooth extraction healing process. Following a previously described technique[8], the mandibular first molars were extracted under intraperitoneal anesthesia with 8 mg of ketamine hydrochloride (Ketalar, Parke-Davis, Morris Plains, NJ) and 1.28 mg of xylazine (Rompum, Bayer, Leverkusen, Germany) per 100 mg body weight. Bismuth subgallate was placed immediately in the right post- tooth extraction socket (E); the contralateral tooth socket remained untreated and served as a control (C).

The rats were fed regular chow and water *ad libitum*; no antibiotic therapy was administered. The animals were assigned to one of 4 groups ($n = 10$) that were killed by ether overdose at the following times: immediately post-tooth extraction, 7, 14, and 30 days post tooth-extraction. The mandibles were resected, fixed in 20% formalin solution and radiographed. They were decalcified in 5% formic acid and embedded in paraffin. Bucco-lingually oriented sections[8], 10 μm thick, were obtained at the level of the first molar mesial socket and stained with hematoxylin-eosin in order to perform histomorphometric studies based on stereologic methods[8,15,22]. The two histomorphometric parameters studied were total tissue volume (TV) and trabecular bone volume (BV). The parameters were determined on tracings obtained from overhead projections of the sections, using an image analyzing system (Kontron MOP AM 03, Carl Zeiss, Jena, Germany).

Following previous reports, TV was defined as bone tissue plus marrow spaces and was measured in the area located above a line drawn tangential to the upper border of the mandibular canal and perpendicular to the external aspect of the buccal plate; BV was defined as BV / TV (%), measured in a rectangle outlined on the apical third of the socket[8–10]. The results were statistically analyzed using unpaired Student's t test, and are presented as mean ± stan-standard deviation. Statistical significance was set at $p < 0.05$.

Results

Radiographic finding

Immediately after surgery, the periodontal cortical in both C and E was visualized as a radiopaque line limiting the sockets; it was uniformly radiolucent in group C (Fig. 1A) and highly radiopaque in group E (Fig. 1B). At 7 days, the periodontal cortical was less distinct and a similar radiopaque image was observed in the fundus of both C and E tooth sockets. At 14 and 30 days, both C and E sockets exhibited radiopaque bone tissue of similar density filling the socket.

Histologic findings

Histologic studies performed immediately post-tooth extraction showed C sockets to be filled with blood coagulum and debris from the periodontal membrane attached to the periodontal cortical. The E sockets (Fig. 2A) exhibited many particles of BS immersed in the blood coagulum and remnants of the periodontal membrane (Fig. 2B). At 7 days, woven bone exhibiting extensive bone surfaces showing active osteogenesis was found in the apical portion of C and E sockets, and granulation tissue was observed in the remaining portions of the sockets. Isolated particles of BS surrounded by macrophages were observed in the granulation tissue in E sockets (Fig. 3). Fourteen days post-surgery, both C (Fig. 4A) and E (Fig. 4B) sections exhibited woven bone lined with active osteoblasts filling the entire socket, which was completely covered with epithelium. The BS particles were no longer evident at 30 days, both C (Fig. 5A) and E (Fig. 5B) sections exhibited lamellar bone tissue filling the sockets, covered with well-differentiated keratinized stratified epithelium.

Histomorphometric measurements

Data collected immediately post-tooth extraction served as baseline reference

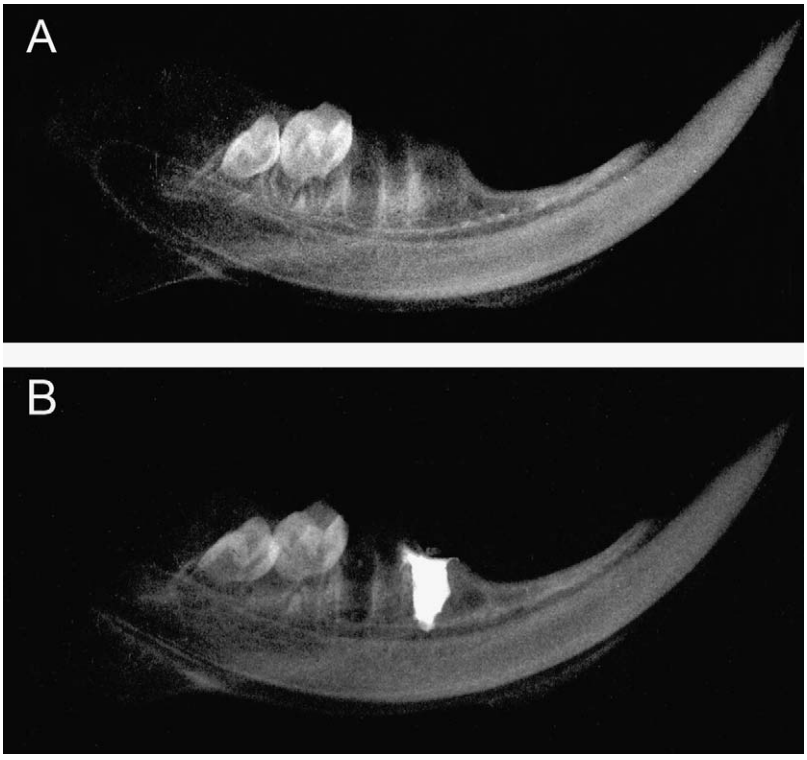

Fig. 1. Radiograph taken immediately post surgery. (A) Control. (B) Experimental. Note the socket filled with BS (radiopaque).

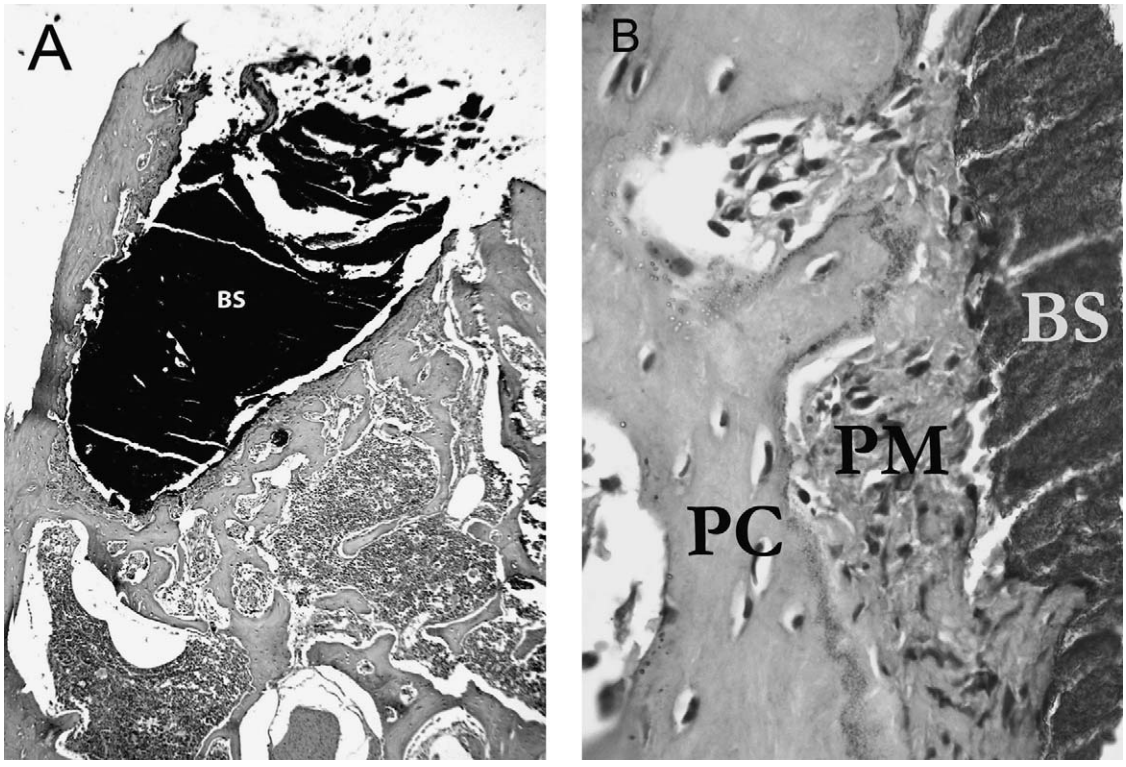

Fig. 2. Microscopic section of the mesial aspect of the first molar socket immediately post surgery in an experimental animal. (A) Note the presence of BS completely filling the socket. (B) Note the presence of BS immersed in the blood coagulum and the debris from the periodontal membrane (PM) attached to the periodontal cortical bone (PC). (H-E, Orig. Mag. ×40 (A) and ×100 (B)).

values to establish comparison with results obtained at the different experimental time points (Table 1). TV and BV were found to increase steadily throughout the 30-day experimental period.

Discussion

The results demonstrate that placing BS immediately in the post-tooth extraction socket does not interfere with the subsequent bone healing process. The present histologic and histomorphometric study was conducted using a well-characterized model of active osteogenesis described by the authors' laboratory[5–10,21].

Tooth socket wound healing provides a suitable model for the study of bone formation and can be considered a sensitive indicator of bone damage under different experimental conditions[9–10,21]. It has been reported that complete extraction of rat molars is difficult[16], but the surgical technique used in this model allows removal of the entire molar and the post-tooth extraction, wound-healing process to be studied without the interference of root or bone rests.

Tramontina et al[20] performed a histologic and histomorphometric study of the healing process of soft tissues in the presence of BS and concluded that the drug did not interfere with the normal course of the healing process. They found many macrophages surrounding free BS particles, but none of the samples showed giant cells or any other sign of foreign body reaction.

Other local hemostatic drugs that have been used extensively, such as biological tissue adhesives, do not alter the normal healing process in bone or connective tissues[1,14,17]. These drugs are costly, which is a serious disadvantage for their standard use. Antifibrinolytic drugs, such as Σ-aminocaproic acid[14,19], do not impair the healing process.

The ideal hemostatic drug should exert high hemostatic action, cause minimal tissue reaction and no antigenic response, should be bioresorbable, easily sterilized and affordable, and should mimic tissue structure.

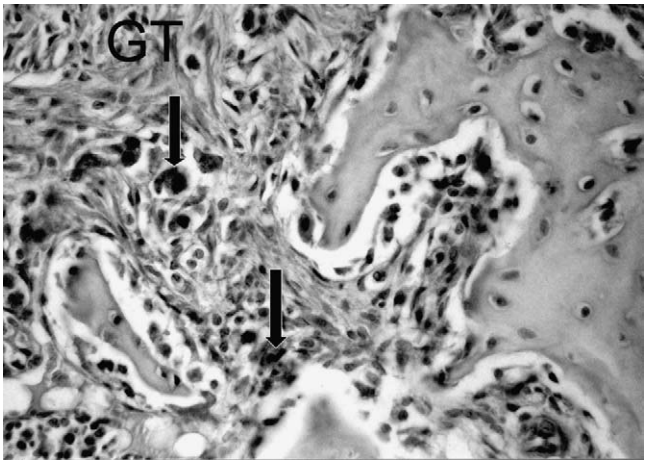

Fig. 3. Microscopic section of the mesial aspect of the first molar socket 7 days after surgery; evidencing active osteogenesis, granulation tissue (GT), and the presence of macrophages containing bismuth subgallate (arrowed). (H-E, Orig. Mag. ×400).

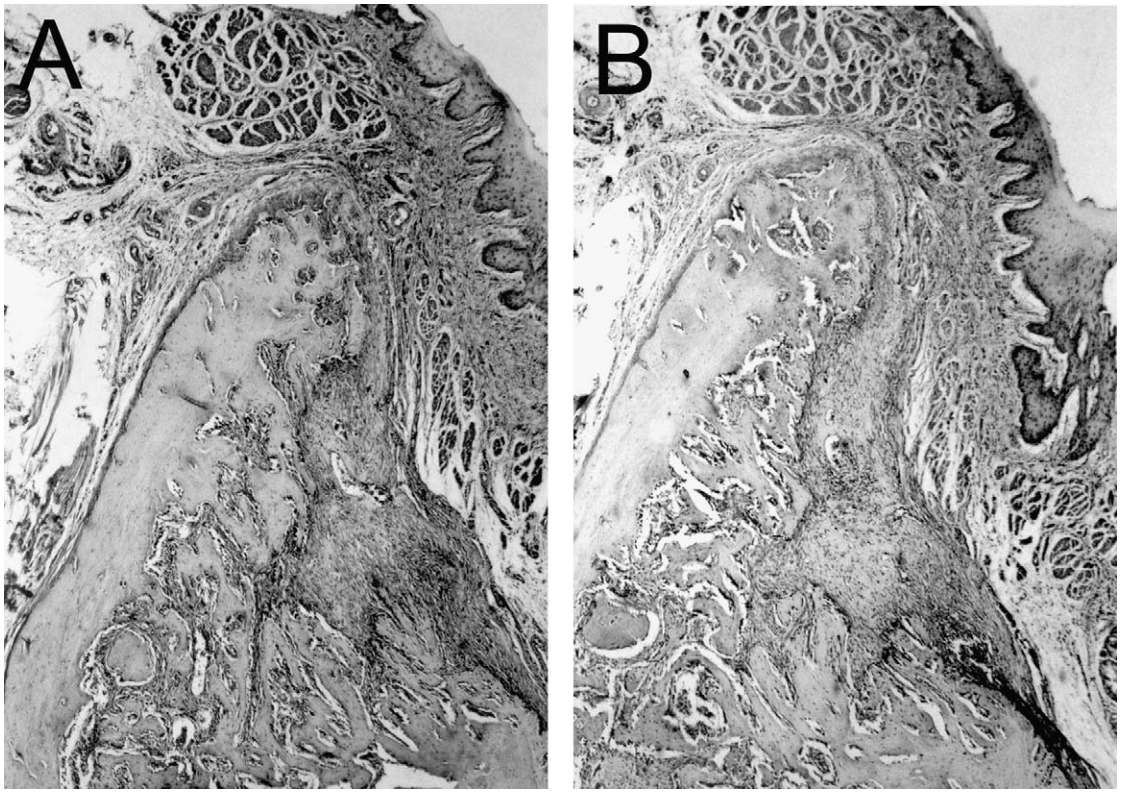

Fig. 4. A and B: Microscopic section of the mesial aspect of the first molar socket 14 days after surgery. Note the presence of woven bone almost completely filling both the control and experimental sockets (H-E, Orig. Mag. ×40).

BS presents high hemostatic activity, can be easily sterilized using conventional methods, and is relatively inexpensive. The present investigation demonstrates that the BS does not impair the bone healing process, and that the drug particles are no longer present at the end of the bone repair process. This precursory study demonstrates that BS does not interfere with post-tooth extraction bone repair. Future studies will evaluate the effect of BS on bone repair in anticoagulated rats.

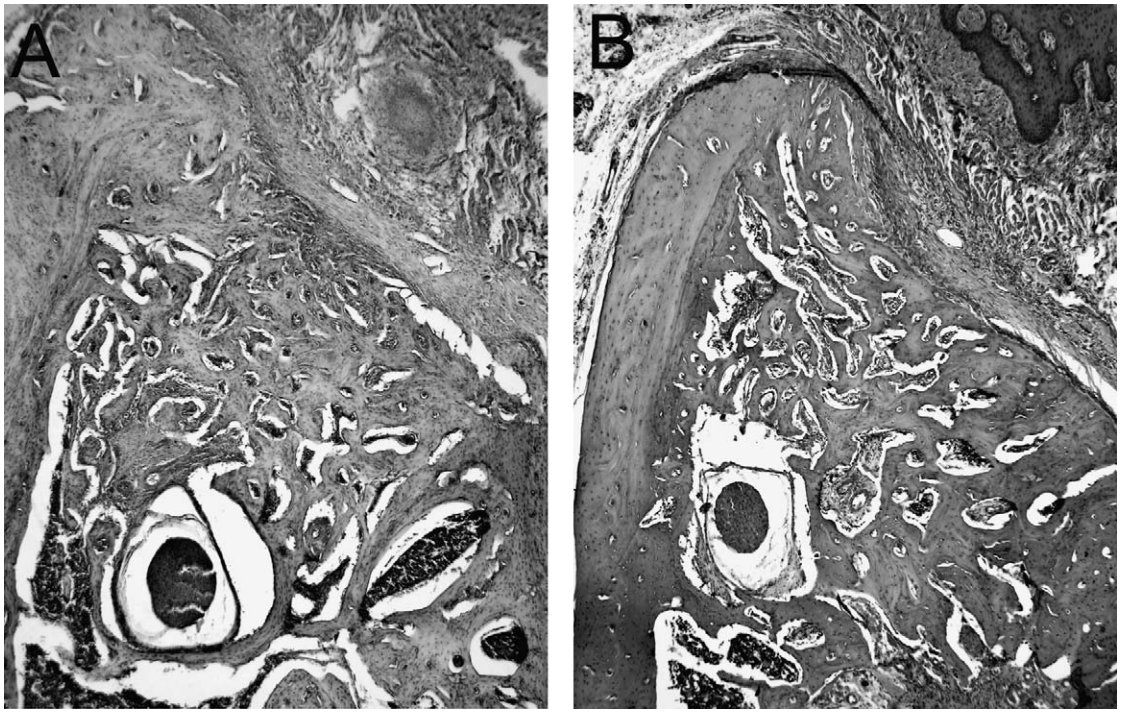

Fig. 5. A and B: Microscopic section of the mesial aspect of the first molar socket 30 days after surgery. Note the presence of lamellar bone almost completely filling both the control and experimental sockets (H-E, Orig. Mag. ×40).

Table 1. Histomorphometric study of bone healing 0, 7, 14 and 30 days post-tooth extraction.

	TV cm^2				BV %			
	0 hs	7d	14d	30d	0 hs	7d	14d	30d
Control	57 ± 12	$10\ 2 \pm 13$	146 ± 36	152 ± 36	0	33 ± 5	42 ± 6	54 ± 4
$p < 0.05$	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
BS	56 ± 11	106 ± 19	135 ± 33	152 ± 30	0	34 ± 7	41 ± 7	51 ± 5

BS: Bismuth Subgallate; TV: Total Tissue volume expressed in $cm^2$of projection; BV: Trabecular Bone volume in the apical third expressed as a %; n.s.: no statistically significant differences.
Values are given as mean ± standard deviation.

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Competing interests

None declared.

Ethical approval

Was given by the Ethics Committee of the School of Dentistry - University of Buenos Aires - Argentina.

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