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Effects of tissucol™ and epsilon aminocaproic acid in the healing process following dental extraction in dehydrated rats

Efeitos do tissucol® e do ácido épsilon aminocapróico no processo de reparo alveolar em ratos desidratados

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ABSTRACT: A histological study was conducted of the alveolar bone healing process following tooth extraction of dehydrated rats after the implantation of fibrin adhesive (TISSUCOL™) associated to previous irrigation of the wound with a 5% epsilon aminocaproic acid solution (EACA). Seventy two rats were used, divided into three groups receiving different treatments after the surgical procedure. In group I, the gingival mucosa was sutured after extraction of the right upper incisor. In groups II and III, chronic dehydration was produced by water deprivation for 9 days (3 days in the preoperative period and 6 days in the postoperative period). In the animals of Group II, after tooth extraction, the gingival mucosa was sutured in the same way as performed in group I. In group III, after extraction, the dental socket was irrigated with 5% EACA, followed by implantation of the fibrin adhesive (TISSUCOL™). The mucosa was sutured in the same way as performed in the other groups. At 3, 7, 15 and 21 postoperative days, the animals were sacrificed in number of 6 for each group. Specimens containing the dental socket were removed and fixed in 10% formalin and decalcified in an equal part formic acid and sodium citrate solution. After routine processing, the specimens were embedded in paraffin for microtomy. We obtained 6 µm semi-serial slices that were stained with hematoxylin and eosin for histological evaluation. The results showed that the water deprivation in the pre- and postoperative periods caused a delay in the alveolar bone healing process. The use of the fibrin adhesive (TISSUCOL™) produced an improvement in the fibrinolytic picture caused by dehydration.

DESCRIPTORS: Tooth socket; Dehydration; Fibrin tissue adhesive.

RESUMO: Foi estudada histologicamente a reparação do alvéolo dental de ratos desidratados, após o implante de adesivo fibrínico (TISSUCOL®) associado à irrigação prévia da ferida com solução a 5% de ácido épsilon-aminocapróico. Foram empregados 72 ratos, divididos em três grupos, que receberam diferentes tratamentos após o procedimento cirúrgico. No Grupo I, após a extração do incisivo superior direito, a mucosa gengival foi suturada. Nos Grupos II e III foi provocada a desidratação crônica pela privação de ingestão de líquidos durante 09 dias (3 dias no pré-operatório e 6 dias no pós-operatório), sendo que, no Grupo II, após a extração dental, a mucosa gengival foi suturada de forma semelhante à do Grupo I; no Grupo III, logo após a exodontia, o alvéolo foi irrigado com a solução de ácido épsilon-aminocapróico a 5%, seguida de implante de adesivo fibrínico (TISSUCOL®) e foi feita sutura da mucosa de forma semelhante à dos demais grupos. Decorridos 3, 7, 15 e 21 dias após o ato operatório, os animais foram sacrificados em número de 6 para cada grupo. A seguir, as peças contendo o alvéolo dental foram removidas e fixadas em formalina a 10% e descalcificadas em solução de ácido fórmico e citrato de sódio em partes iguais. Após processamento laboratorial de rotina, foram incluídas em parafina para microtomia. Foram obtidos cortes semi-seriados com 6 micrômetros de espessura, seguidos de coloração em hematoxilina e eosina para estudo microscópico. Os resultados obtidos mostram que a hidroprivação no pré e pós-operatório ocasiona profundo retardo no processo de reparo em feridas de extração dental. O emprego de adesivo fibrínico (TISSUCOL®) melhora o quadro fibrinolítico provocado pela desidratação.

DESCRITORES: Alvéolo dental; Desidratação; Adesivo tecidual de fibrina.

INTRODUCTION

The alveolar bone healing process following tooth extraction is closely related to the mechanisms of haemostasis and fibrinolysis, since the formation and organization of the blood clot are

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basic conditions to guide the healing of the dental socket[1].

During blood coagulation, extrinsic and intrinsic mechanisms activate prothrombin into thrombin and, in the presence of Ca^{++}, produce the hydrolysis of fibrinogen into fibrin, bound with hydrogen bridges, and also activate coagulation factor XIII, a beta-globulin present in plasma in an active form. Factor XIII, a fibrin-stabilizing factor initially described by Laki, Lorand[10] (1948), is responsible for the cross reactions formed between fibrin monomers (producing the fibrin screen, that facilitates the growth of fibroblasts) and between collagen and fibrin[7]. Beck *et al.*[2] (1961) showed that there is no fibroblastic growth in the absence of factor XIII.

Cabrera *et al.*[4] (1988), studying the negative hydric balance effects on alveolar bone healing following tooth extraction, observed that the decrease in liquid ingestion produced a significant reduction in the vascularization of alveolar tissues, changes in the morphology of the healing process and a delay in the temporal evolution of the many healing stages.

The dehydration produces a decrease in the plasmatic and corpuscular volume, increasing the hematocrite and plasmatic protein levels[2,11], producing an increase in the blood viscosity. Cabrera-Peralta *et al.*[5] (1982) considered that these factors may determine the decrease or the blockade of the changes of substances through the capillary membrane, affecting cellular activity and changing the healing tissue formation process.

It seems, therefore, that the use of substances that maximize the physiological healing process could change the fibrinolytic conditions produced by dehydration. The adhesive system Tissucol™, basically composed of fibrinogen, aprotinine, factor XIII and thrombin has been used in surgeries as a tissue adhesive, and primarily has contributed in hard and soft tissues haemostasis in the oral cavity and in many clinical situations, when the fibrinolytic system is in prejudice[13].

Alves-Rezende, Okamoto[1] (1997) studied the alveolar bone healing in rats after Tissucol™ implantation and observed that the material was slowly phagocityzed during this process, which was not complete at the 21st postoperative day. They observed that connective and bone tissues were formed closely to the material, indicating its biocompatibility. Staindl[25] (1979) recommended the previous irrigation of the wound with Epsilon aminocaproic acid (EACA) before Tissucol™ implantation, with the aim to increase its biological properties.

Considering these data, the present study aimed to evaluate the alveolar bone healing process in dehydrated rats, after Tissucol™ implantation associated to previous irrigation of the extraction socket with 5% EACA.

MATERIAL AND METHODS

To perform this study, 72 male rats (*Rattus norvegicus*, Wistar) were used. The 220-250 g weight animals were anesthetized with Sodic Tionembutal (Abbott, Abbott Park, IL), with a 50 mg/kg of weight dosage. After syndesmotomy, the right upper incisor of each animal was extracted, using a specially adapted instrument[20].

The 72 rats were divided into 3 groups: Group I: Control; Group II: Dehydrated rats; Group III: Dehydrated rats that received the Tissucol® implant (Osterreichisches Institute fur Haemoderivate Ges M.B.H., Immuno Produtos, Rio de Janeiro, Brazil) associated to previous irrigation of the dental socket with 5% EACA (Química Farmacêutica Nikkho do Brasil, Rio de Janeiro, Brazil).

The chronic dehydration of the experimental rats was produced by water deprivation, that lasted 9 days, considering that 3 days were during the preoperative period and 6 days were during the postoperative period. The animals were separated in individual cages and maintained in a temperature of 30°C ± 2°C. They received food and water "ad libitum", except for 24 hours after surgery. The rats of Groups II and III were rehydrated in the sixth postoperative day with free access to water.

Immediately after extraction in groups I and II, the wounds were sutured with polyglactine 910 thread (Vycril, 4.0, Ehicon, São José dos Campos, Brazil). In group III, the extraction sockets received irrigation with a 5% EACA solution, followed by Tissucol™ implantation. After this, the wound was sutured in a similar way as in groups I and II.

Six animals from the groups were sacrificed at 3, 7, 15 and 21 postoperative days. The right upper jaw was removed and fixed in a 10% formalin solution for 24 hours and decalcified for a period of 30 days in a solution of sodium citrate and formic acid in equal parts[15]. After decalcification, the specimens were washed during 24 hours and dehydrated, diafanized and imbedded in paraffin. The longitudinal semi-serial slices were obtained with 6 μm in thickness and were stained with hematoxylin and eosin.

The extraction socket was divided into three thirds: cervical, middle and apical, considered from the bone crest towards the "*fundus*" of the socket. A qualitative analysis was performed of the neoformed connective tissue and bone trabeculae in the different postoperative stages.

RESULTS

Group I (3 days) – The extraction socket was filled with blood clot and a great number of macrophages could be observed in its interior. In the lingual wall, it was possible to observe well vascularized periodontal ligament remainder and rich in fibroblasts. In the middle and apical thirds, adjacent to the remnants of periodontal ligament, small portions of blood clot and the presence of a moderate number of fibroblasts, capillaries and amorphous fundamental substance were observed.

Group II (3 days) – The extraction socket was partially filled with a less organized blood clot, with the presence of a discrete number of macrophages. Observing the three thirds of the extraction socket, no evidence of connective neoformation was noted. The periodontal ligament remainder was poor in blood vessels and some fibroblasts showed degeneration signs.

Group III (3 days) – The extraction socket was filled with blood clot and a great number of macrophages. The periodontal ligament remainder showed a moderate number of fibroblasts and blood vessels. Next to the middle third, adjacent to the periodontal ligament, we could observe moderate fibroblastic proliferation and formation of a small amount of amorphous fundamental substance; some neoformed capillaries were also observed.

Group I (7 days) – Practically the entire dental socket was filled with neoformed connective tissue, showing different characteristics. At the level of the cervical third, small isolated bone spikes were observed next to the lingual wall. Also, the presence of remainders of the blood clot was observed. At the middle and apical thirds, the bone neoformation was more intense. Small and immature bone trabeculae with many osteoblasts were observed on their borders (Figure 1).

Group II (7 days) – The extraction socket showed the presence of a disorganized blood clot in many points, with a discrete number of macrophages (Figure 2). Next to the bone wall, the connective tissue was less vascularized with a discrete number of fibroblasts. Small bone spikes could be observed at the level of the middle third, with a discrete number of osteoblasts on their borders.

Group III (7 days) – The extraction socket was filled with neoformed connective tissue, showing different characteristics. At the cervical third, the connective tissue with no bone differentiation and rich in fibroblasts and blood vessels was observed. Small areas with blood clot were seen. Next to the middle and apical thirds, bone trabeculae showing osteoblasts on the border were observed. The ossification was more intense near the alveolar bone wall (Figure 3).

Group I (15 days) – The extraction socket showed bone trabeculae with different characteristics. At the cervical third, thin bone trabeculae with many osteoblasts on their border were ob-

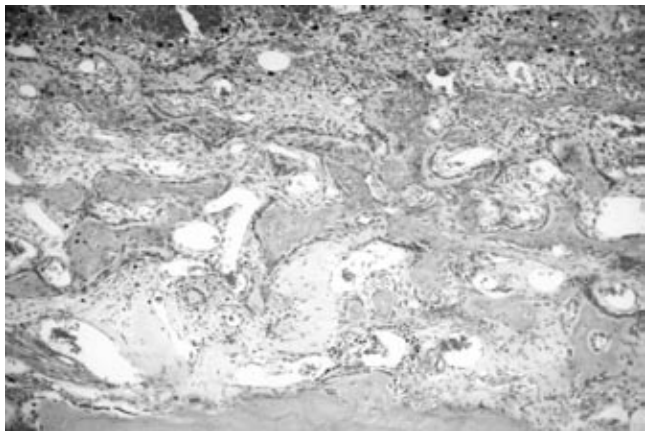

FIGURE 1 - G. I, 7 days: control. Middle third of the extraction socket showing small neoformed bone trabeculae, with numerous osteoblasts on their borders. (HE, 63 X).

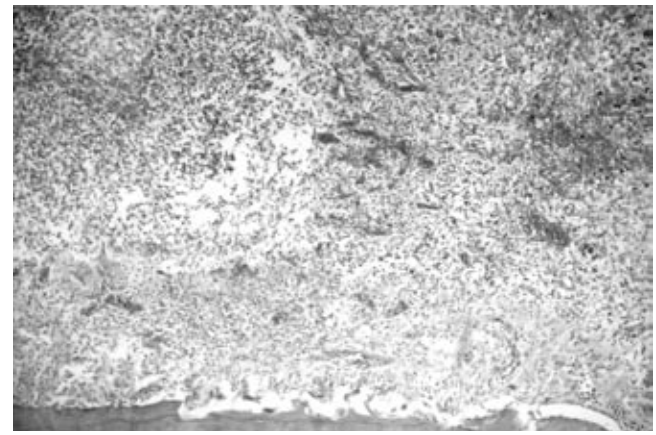

FIGURE 2 - G. II, 7 days: Middle third of the extraction socket showing small neoformed bone spikes (HE, 63 X).

served. Next to the middle and apical thirds, more developed bone trabeculae were noted, usually next to the bone alveolar wall. Numerous osteoblasts could be observed on their border.

Group II (15 days) – On all of the extension of the extraction socket, small areas occupied with blood clot without organization were observed, showing a discrete number of macrophages and lymphocytes. Connective tissue showing a discrete number of fibroblasts was noted. In many points, this tissue showed little vascularization and, at the middle and apical thirds, small bone spikes were observed, with a discrete number of osteoblasts on their border (Figure 4).

Group III (15 days) – The extraction socket, except for some points occupied with blood clot, was filled with neoformed connective tissue, showing different characteristics. Next to the cervical third, the ossification was discrete and isolated bone trabeculae were noted, with numerous osteoblasts on their borders. At the middle and apical thirds, the bone trabeculae were more developed; otherwise, in some points they were thin, with large intertrabecular spaces (Figure 5).

Groups I, II and III (21 days) – At the three thirds of the dental socket, the presence of neoformed bone trabeculae was observed. Compared to the control group (Figure 6), the ossification was less intense in Group II (Figure 7) than in Group III (Figure 8). Therefore, the bone trabeculae were thinner, showing greater amounts of connective tissue without bone differentiation.

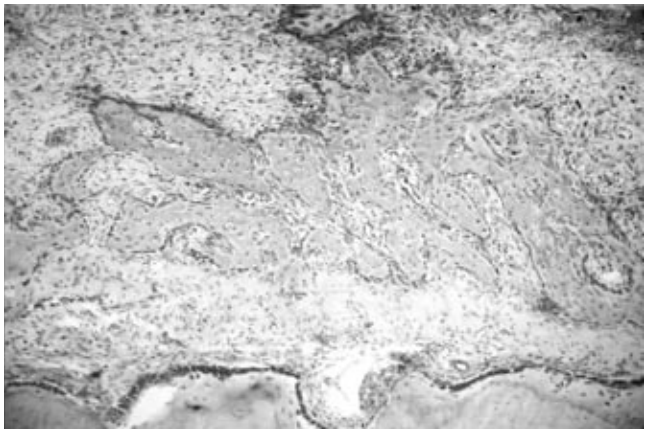

FIGURE 3 - G. III, 7 days: Middle third of the extraction socket showing the "lingual" side with thin bone trabeculae (HE, 63 X).

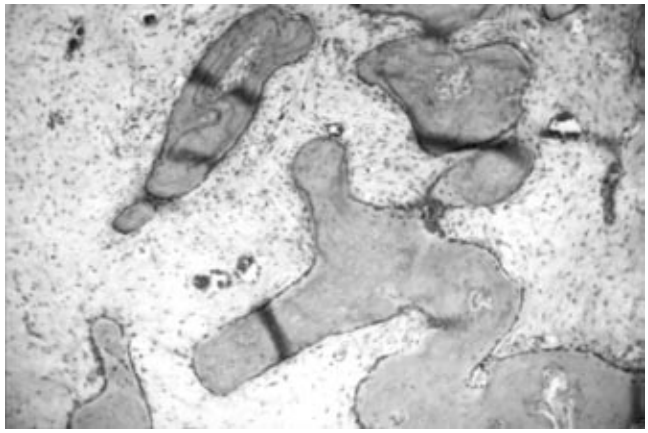

FIGURE 4 - G. II, 15 days: Middle third of the extraction socket, with the "lingual" side showing isolated small spikes (HE, 63 X).

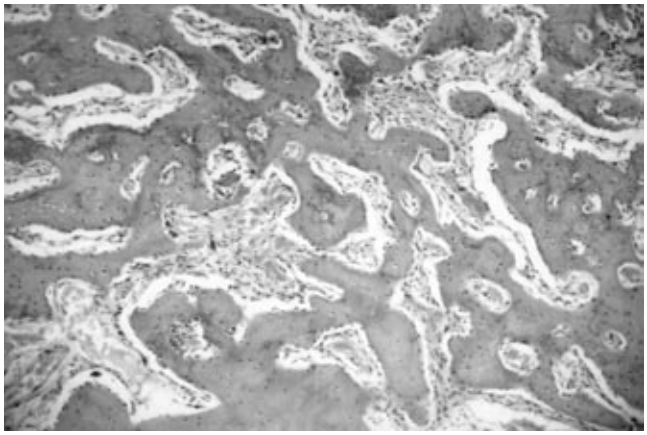

FIGURE 5 - G. III, 15 days: Middle third of the extraction socket, with the "lingual" side showing thin bone trabeculae with numerous osteoblasts on their borders (HE, 63 X).

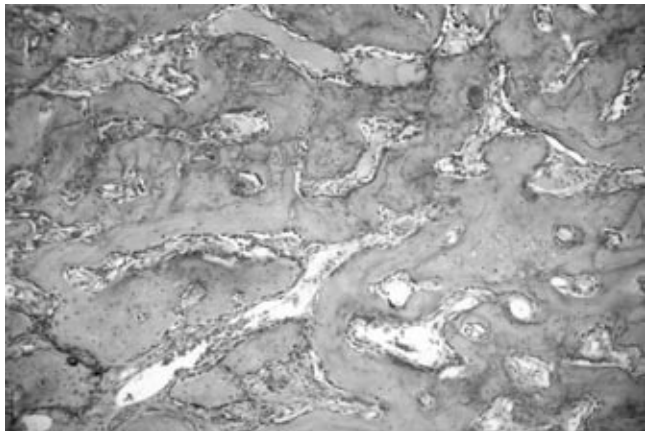

FIGURE 6 - G. I, 21 days: Middle third of the extraction socket showing bone trabeculae (HE, 63 X).

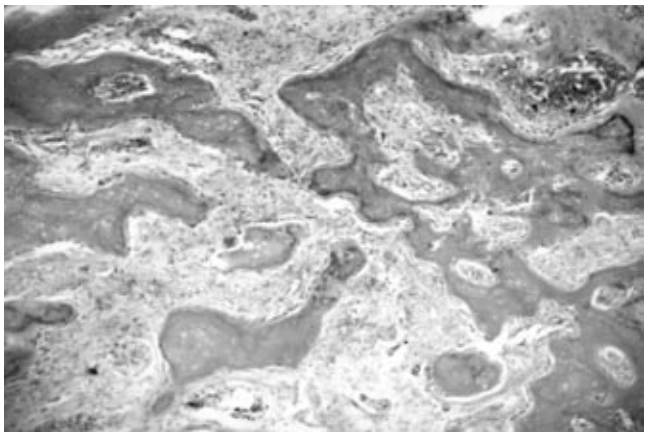

FIGURE 7 - G. II, 21 days: Middle third of the extraction socket showing fewer bone trabeculae with osteoblasts on their border (HE, 63 X).

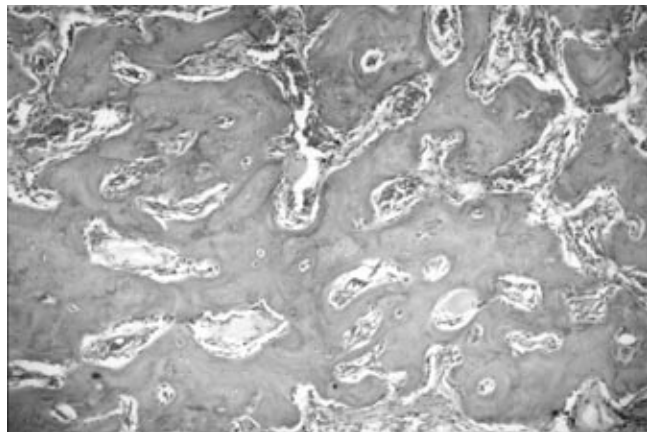

FIGURE 8 - G. III, 21 days: Middle third of the extraction socket, showing well developed bone trabeculae (HE, 63 X).

DISCUSSION

Bergel[3] described the use of a fibrin sealant as a hemostat in 1909. In 1915, Grey[9] described the use of fibrin for haemostatic purposes during cerebral surgery. In 1940, Young, Medawar[26] described the use of fibrinogen as an adhesive to achieve peripheral nerve attachment. Cronkite *et al.*[6] provided the first description of using both fibrinogen and thrombin as a biologic glue for the purposes of skin grafting in 1944. The use of a fibrin sealant containing concentrated fibrinogen was described for neural anastomoses in 1972 by Matras *et al.*[14] (1972). In 1983, Gestring, Lerner[8] described clinically useful chemical and cryoprecipitation methods for producing concentrated fibrinogen for use in fibrin sealants.

Fibrin sealant has been used for enhancement of local haemostasis after dental extractions for over two decades. Fibrin sealing mimics the last phase of blood clotting, i.e., the conversion of fibrinogen into fibrin[12]. During wound healing the clot material undergoes gradual lyses and is completely absorbed within two weeks. Aside from its adhesive and haemostatic properties, the sealant has been found to enhance wound healing. Studies comparing the effectiveness of fibrin sealant in patients undergoing dental extractions who are therapeutically anticoagulated with warfarin have shown equally successful outcomes with respect to haemostasis[23].

Yücel *et al.*[27] (2003) encouraged the use of fibrin glue on wound healing in the oral cavity. They reported the excellent results with the product in septic open wounds in the oral cavity. It seems that one of the most significant pathogenic factors for the development of bleeding after oral surgery is the activation of fibrinolysis in the oral cavity. The literature suggests an advantage of using fibrin sealant at the time of dental extractions in combination with antifibrinolytic agents. It appears to reduce bleeding at the time of dental extractions in patients with coagulopathies from hemophilia or therapeutic anticoagulation with warfarin[21,22]. In 1989 a study by Sindet-Pedersen *et al.*[24] demonstrated that the maintenance of oral anticoagulant therapy in conjunction with oral surgery does not result in severe bleeding complications in patients receiving a tranexamic acid mouthwash postoperatively.

An animal study developed by Alves-Rezende, Okamoto[1] (1997) suggested that the biological properties of Tissucol™, increased in association with EACA, might have contributed to the dental extraction wound healing in rats under stress. These authors believed that Tissucol™ in association with EACA would be useful for haemostatic procedures.

The biological compatibility of the applied material is increased when the surgical wounds are previously irrigated with EACA solution[1,17-19]. The obtained results are in accordance with data of the literature that describes the reversal of a fibrinolytic state in the presence of Tissucol™[1,16-18].

Our results observed at postoperative days confirm the tendency to healing changes in Group II, in great part reversed in Group III. The use of a fibrin adhesive in Group III practically produced the reversal of a fibrinolytic situation caused by dehydration. While the disorganized blood clot was

evident in some specimens of Group II, until late periods of observation (21 days) in Group III, the presence of Tissucol™ provided the fibrin screen necessary for fibroblastic aggregation and for the healing process to evolve.

In face of the complexity of the events involved in the healing of dental sockets, other researches are necessary to assert the clinical use of fibrin adhesive in managing healing alterations in dehydrated patients.

CONCLUSIONS

According to the experimental conditions followed in the present study, it is possible to conclude that:

1. Water deprivation at the preoperative and postoperative periods caused a delay in the connective tissue neoformation in the dental socket healing process;
2. The use of the fibrin adhesive Tissucol™ produced an improvement of the fibrinolytic situation caused by dehydration.

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