IMMUNOHISTOCHEMICAL EXPRESSION OF PCNA, STRO-1 AND CD44 IN EXPERIMENTALLY PRODUCED PERIAPICAL PERIODONTITIS IN VIVO

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ABSTRACT

Objective: *To determine the immunohistochemical expression of PCNA, STRO-1 and CD44 and their role in the experimentally produced periapical periodontitis.*

Materials and Methods: *In this experimental study, Sprague-Dawley (SD) male rats (n=50), each weighing 200 were collected and pulp of each rat was extirpated from the mesial root of maxillary 1st molar for the development of peri-apical infection and the root canal was exposed to oral flora for 4 weeks. After 4 weeks, maxillary 1st molars mesial roots together with the surrounding alveolar bone and muscle tissue were excised and fixed in 10% neutral buffered formalin solution for 5 days and then decalcified in 10% EDTA solution for 4 weeks. All the tissue samples were then processed, and stained with hematoxylin and eosin, and were evaluated immunohistochemically using an anti-PCNA, anti- STRO-1, and anti- CD44 polyclonal antibodies.*

Results: *In this study, we assessed the periapical infection of the mesial root of the maxillary Ist molar, which was schematically developed in Sprague-Dawley (SD) male rats (n=50). Histological study of H&E stained sections reveals periapical periodontitis composed of chronic inflammatory cells mostly lymphocytes, along with the alveolar bone resorption around the apex of the mesial root of maxillary Ist molar. Immunohistochemistry showed positive staining for PCNA, STRO-1, and CD44. Strongly positively stained cells were found around the apex of the mesial root of the maxillary Ist molar.*

Conclusion: *Periapical inflammatory tissues expressed proliferative cell marker PCNA and Mesenchymal Stem Cell markers such as STRO-1, and CD44, suggesting the presence of MSCs. The PCNA, STRO-1, and CD44 positive cells were proliferated dental stem cells that were involved in the healing of the periapical periodontitis.*

Keywords: *PCNA, STRO-1, CD44 , MSCs. periapical periodontitis, proliferating dental stem cells*

INTRODUCTION

Stem cells are operationally defined as unspecialized cells that have the ability of prolonged self-renewal and can differentiate into one or many types of mature cells. Stem cells can be found in the embryo as well as in adult cells. Totipotent embryonic stem cells have the ability to divide into stem cells and other daughter cells. On another hand, adult stem cells (ASCs) have the lesser ability of self-renewal as they can differentiate into only one type of lineage cells.[1,2]

Mesenchymal stem cells are one of protype ASCs which were first extracted from bone marrow and can differentiate into mesodermal and non-mesodermal tissues like osteoblasts, adipocytes, and chondrocytes.[3] Stro-1 and CD44 are markers of

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mesenchymal stem cells and have been used in many studies for the identification and isolation of MSCs.[4,5]

Microbial infection is among the major causative factors that result in various pulpal and peri-apical pathologies.[6] Bacteria and their byproducts are considered to be the primary etiologic agents of pulpal necrosis and periapical lesions. Therefore, the elimination of bacteria and their byproducts is one of the most important steps in endodontic treatment.[7] Representative inflammatory periradicular pathologies are universal and involve mainly the apical periodontium, with no predominance of race, sex or age.[8] Inflammation of tooth-supporting structures accompanied by insufficient repair process usually leads to severe damages of the tissues such as bone loss and subsequent tooth loss.[9] The dental tissues are considered a rich source of mesenchymal stem cells. These dental stem cells have multifactorial potential such as high proliferation rates, potential to differentiate into several cell types and have a very important therapeutic role in oral tissue regeneration.[10, 11]

In this study, we aimed to identify the role of mesenchymal stem cells in healing of periapical inflammatory lesions induced in the maxillary molars of experimental rats using immunohistochemistry.

MATERIALS AND METHODS

This experimental study was conducted at Tokyo Dental College (223,206) in which Sprague-Dawley (SD) male rats (n=50), each weighing 200g, were used for in vivo study and were anesthetized with an intraperitoneal injection containing sodium thiopental (Ravonal; Tanabe, Osaka, Japan), 0.3 ml/100g bodyweights of the rat.

Stainless steel tungsten carbide bur 06 was used to make the access cavity in the upper 1st molar mesial root. Then pulp was extirpated from the mesial root of maxillary 1st molar using 06 to 25 mm conventional reamers and files, as the scheme for the development of peri-apical infection (showed in Fig. 1 a, b, c and d) and the root canal was exposed to oral flora for 4 weeks in order to develop peri-apical periodontitis.

After 4 weeks all the animals were sacrificed by cervical dislocation and the maxillary 1st molars mesial roots together with the surrounding alveolar bone and muscle tissue were excised and fixed in 10% neutral buffered formalin solution for 5 days and then decalcified in 10% EDTA solution for 4 weeks. All the tissue samples were then processed, and stained with hematoxylin and eosin (H&E).

For immunostaining, the tissue sections were treated with 30% hydrogen peroxide and methanol solution for 15 min at room temperature to inactivate endogenous peroxidase activity. Tissue samples were blocked with 1% BSA in PBS for 30 min at room temperature. Samples were incubated with the PCNA, Stro-1, primary antibodies (dilution of each antibody, 1:200) in PBS containing 1% BSA for 1 hour at room temperature. Specimens were then washed and incubated with a biotinylated secondary antibody for 45 min at room temperature. After washing with PBS 3 times for 3 minutes each time, samples were stained with NICHIREI-Histofine simple-stain DAB (NICHIREI) and counter-stained with hematoxylin. All these paraffin-sections were observed by using UPM Axiophoto (Carl Zeiss) microscope.

RESULTS

The histological examination of H&E stained tissue sections of periapical infection of the mesial root of the maxillary Ist molar, which was schematically developed in Sprague-Dawley (SD) male rats (n=16) by extirpating root canal, which were exposed to oral flora for 4 weeks, reveals periapical periodontitis composed of chronic inflammatory cells infiltrate, mainly composed of lymphocytes, along with the alveolar bone resorption around the apex of the root. (Fig. 2)

Histological study of PCNA stained sections shows numbers of PCNA strongly positively stained cells around the apex of the mesial root of the maxillary Ist molar. (Fig. 3)

Similarly, the histological study of sections showed numbers of positively stained cells for STRO-1 and CD 44 around the apex of the mesial root of the maxillary Ist molar. (Fig. 4 and 5 respectively).

DISCUSSION

Peri-apical lesions usually occur when diseased pulp is not treated on time thus the microbes and products of inflammation leach out into the peri-apical area through apical foramen. This change results in the loss of bone tissues, which can be diagnosed during routine radiographic examination. Histolog-

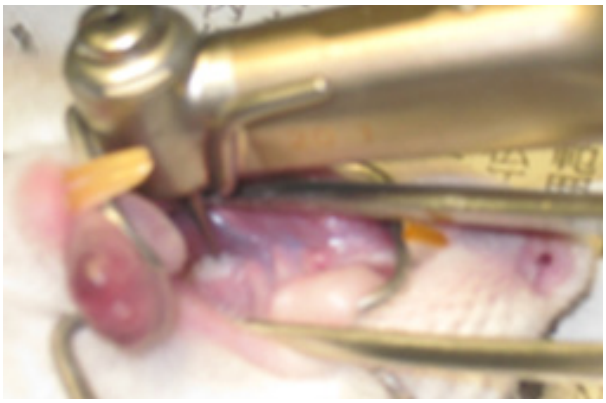


Figure 1a:

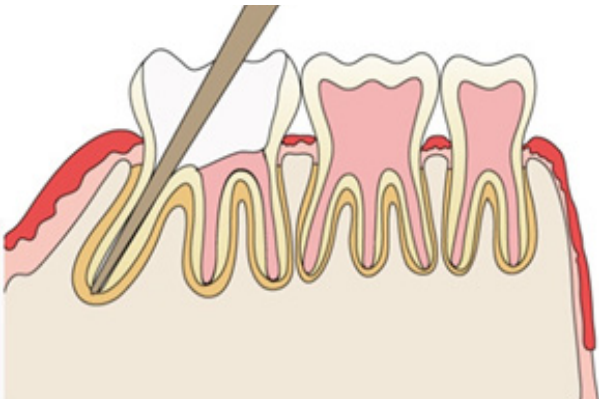


Figure 1b:

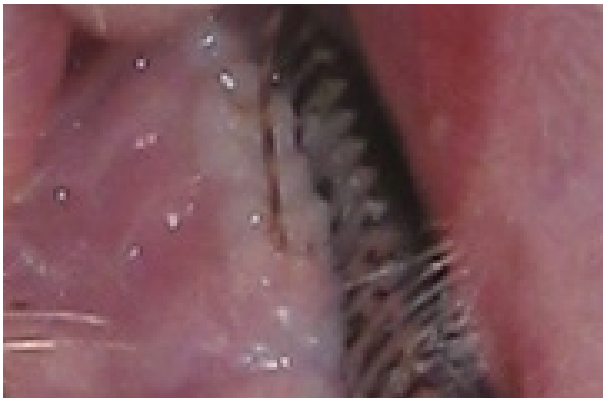
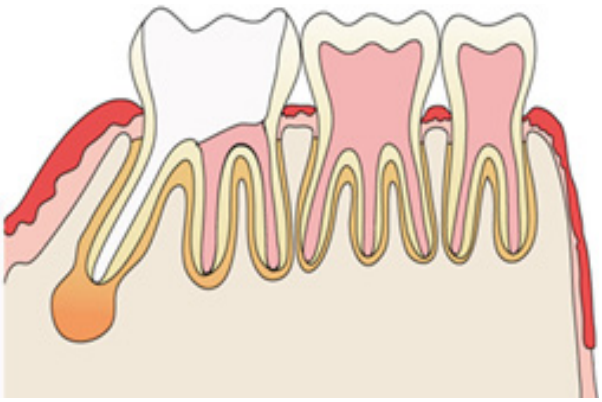


Figure 1c:



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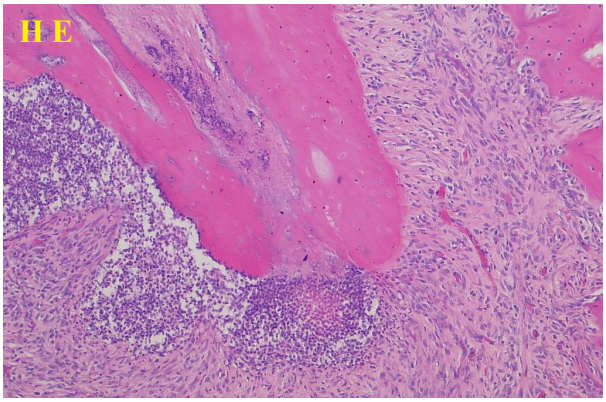

Figure 2:

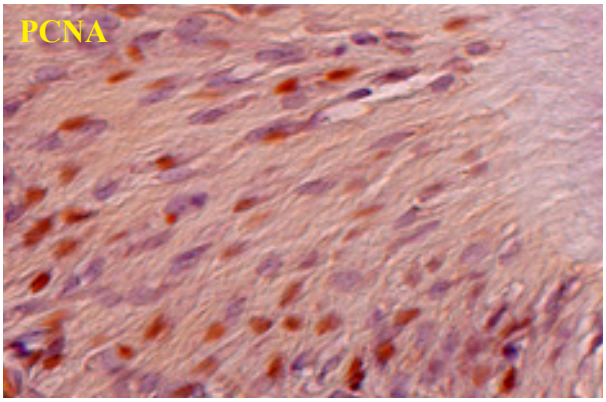

Figure 3:

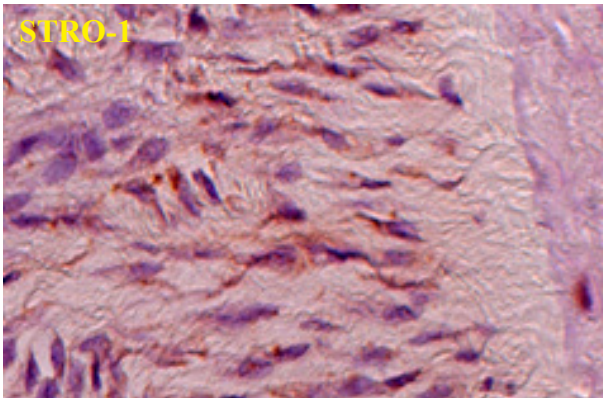

Figure 4:

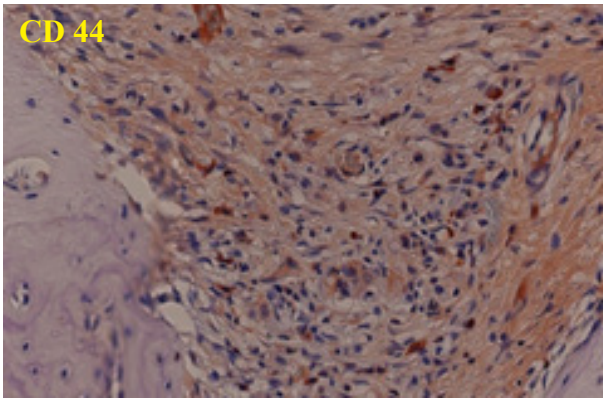

Figure 5:

ically this tissue contains various chronic inflammatory cells with collagen and neovascularization. When source of infection is removed by extraction or endodontic treatment, this area undergo healing and various studies have suggested that these resolving periapical lesions have stem cells that have potential to differentiate and regenerate bone.[12-14]

In this experimental study, we evaluate the proliferation of mesenchymal stem cells in peri-apical inflamed tissue. We developed the periapical lesions in SD rats that became radiographically apparent after 14 days, achieving the maximum size after 28 days of pulpal exposure to the oral flora. Our results agreed with those found by Yamasaki et al[15] Yu SM, and Stashenko P.[16]

Anti-PCNA antibody has been used to identify proliferating cells in paraffin-embedded tissue sections. As the use of the antibody recognizing PCNA could be useful for evaluating cell proliferation during wound healing, we compared PCNA expression with the expression of other markers.[17]

PCNA has a longer half-life, and it can therefore probably continue to be recognized for a while after completion of cell division.[18]

Sargolzaei et al19 have used Proliferant cell nuclear antigen (PCNA) along with other markers for the evaluation of the proliferative ability of 16 periapical granulomas and 8 radicular cysts, in which PCNA positive cells were present in 22 of the 24 cases examined. This supported our study, the PCNA strongly positive result.

Takeuchi et al[20] investigate the effects of a self-assembling peptide (SAP) nanofibre hydrogel on healing of surgical periodontal defects in rats by checking the expression level of PCNA-positive cells along with other markers in vitro interactions between rat periodontal ligament (PDL) cells and SAP hydrogel and expression levels of PCNA-positive cells were significantly greater than those in other groups. This supported our study.

Yang et al[21] evaluated the expression of the mesenchymal stem cell markers such as STRO-1 and CD146 in the pulp revascularization treatment for the immature permanent tooth with periapical periodontitis in an animal model. This supported our study, the positive STRO-1 cells.

Estrela et al[22] observed CD 44 immunostaining in mesenchymal cells located in the outer portion of the abscess and periapical cyst specimens. This supported our study, the positive CD 44 cells.

James Liao and his colleague found that the cells they isolated from peri-apical inflammatory lesion were strongly positive for mesenchymal stem cell markers like STRO-1, CD90 and CD 146 that agrees to our results.[14]

CONCLUSION

Periapical inflammatory tissues expressed proliferative cell marker PCNA and Mesenchymal Stem Cell markers such as STRO-1, and CD44, suggesting the presence of MSCs. The PCNA, STRO-1, and CD44 positive cells were proliferated dental stem cells that were involved in the healing of the periapical periodontitis.

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