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Evaluation of the anti-inflammatory effect of erythromycin on aseptic inflammation of temporomandibular joint in rabbit: a scintigraphic and histopathologic study

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Abstract – This article investigates the anti-inflammatory effects of erythromycin (EM) on aseptic inflammation of temporomandibular joint (TMJ) space using by Tc-99 m HIG scintigraphy and histopathology. In this experimental study, 33 adult male New Zealand White rabbits were divided into three groups. The animals in the first group were treated with EM 25 mg kg^{-1}; the animals in the second group were treated with methylprednisolone (MP) 2 mg kg^{-1} and the animals in the third group were given saline solution (control group). Each drug was given by intraperitoneal injection twice a day for 7 days. Two hours after the last injection, carrageenan was injected into right TMJ of rabbits for aseptic inflammation. After carrageenan injection, each rabbit was given an intravenous injection of 111 Mbq (3 mCi) Tc-99 m human HIG, and scanning was performed 4 h later. Later, all animals were killed and TMJ and periarticular tissues were resected. Histopathologically, the distance between synovial surface epithelium (SSE) and muscle layer (ML) in each section was measured by using SAMBA 200 Cell image processor with software. Scintigraphically, when lesion activity/adjacent region activity (L/N) was evaluated there was a significant difference between the control group and the other two groups ($P < 0.05$ for EM and MP). However, no significant difference was found between the EM and MP groups. Histopathologically, the mean distance between SSE and ML in the sections was found longer in the control group (78.6 ± 10.7 μm) than in the EM (44.38 ± 18.26 μm) and MP (44.05. ± 18.25 μm) groups. We think that administration of EM, which is a well-known macrolid antibiotic, might be very effective in the treatment of aseptic inflammation of TMJ space. Because corticosteroid administration has many side effects, EM may be a preferable drug in the treatment of inflammation of TMJ.

Ümit Ertaş[1], Sinan Tozoglu[1], Onder Sahin[2], Bedri Seven[3], Cemal Gundogdu[2], Bulent Aktan[4], Mustafa Yildirim[3]

[1]Department of Oral and Maxillofacial Surgery, School of Dentistry, Ataturk University, Erzurum, Turkey; Departments of [2]Pathology, [3]Nuclear Medicine and [4]Otolaryngology, Medical Faculty, Ataturk University, Erzurum, Turkey

Key words: erythromycin; Tc-99 m HIG scintigraphy; TMJ inflammation

Dr Ümit Ertaş, Diş Hekimliği Fakültesi, Ağız Diş Çene Hastalıkları ve Cerrahisi AD, Atatürk Üniversitesi, Erzurum, Turkey
Tel.: +90(442)2311799/231801
Fax: +90(442)2360945
e-mail: uertas1970@yahoo.com

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Inflammation is a complex response induced by a wide variety of stimuli commonly associated with infection or trauma. Commonly inflamed sites of temporomandibular joint (TMJ) tissues are the posterior attachment (retrodiscal pad, superior and inferior stratum), collateral ligaments and periarticular tissue (capsule, synovium, and TMJ ligaments) (1, 2). Pharmacologic management of TMJ inflammation is an adjunctive treatment used in combination with other modalities. Some temporomandibular disorders require only acute management. Drug therapy in these cases is a short-term solution and may constitute primary treatment. Other temporomandibular disorders are chronic and require long-term administration of medication (3).

There are two main categories of anti-inflammatory medication: non-steroidal anti-inflammatory drugs (NSAIDs) and corticosteroids being routinely used systemically for reduction of inflammation after oral and maxillofacial surgical procedures and inflammation of TMJ space. The primary clinical indication of corticosteroids is synovitis that is not infectious and poorly responsive to NSAIDs. Steroids have also been used to reduce myositis (3). Corticosteroids have a strong anti-inflammatory effect, but they have many side effects, such as gastric disturbance, hemorrhage tendency, renal impairment, teratogenic effect, suppression of the inflammatory, electrolyte imbalance, hypertension, osteoporosis, and hormonal suppression (3).

Macrolides have been used for the treatment of infectious diseases in clinical medicine. EM is a well-known macrolide antibiotic and exerts an antibacterial activity by inhibiting bacterial protein synthesis via reversibly binding to the 50S ribosomal subunit of bacteria. It is active against *Staphylococcus aureus*, *Streptococcus pyogenes*, *Hemophilus influenza*, *Streptococcus viridans* and *Streptococcus pneumonia* (4).

Apart from their antibacterial activity, these agents exhibit a broad spectrum of pharmacologic effects including anti-inflammatory activity in humans and animals (5). Macrolides affect several pathways of an inflammatory process such as the migration of neutrophils, oxidative burst in phagocytes, and production of proinflammatory cytokines (5).

Polyclonal immunoglobulin G (HIG) has been introduced for imaging infection and inflammation, and several experimental and human studies have been published revealing the effectiveness of HIG in the diagnosis of inflammation (1–4, 6–9).

In this study, we aimed to investigate whether EM could be an effective and alternative drug to corticosteroids in the treatment of aseptic inflammation of TMJ in a rabbit model.

Material and methods

Animals

Thirty-three adult male New Zealand white rabbits (2.2–3.1 kg) were included in the study. Animals were housed in individual cages (50 × 40 × 40 cm) at room temperature. Animals were fed *ad libitum* consumption of pelleted feed mixture. The animals had free access to water and food at all times during the experimental period. After 2 weeks of adjustment, we initiated the study.

Treatment groups and management

All animals were separated into three groups: receiving EM, MP, or saline. The rabbits were injected with intraperitoneal medication twice daily for 7 days. For 7 days, EM was given at 25 mg $kg^{-1}day^{-1}$ and MP was given at 2 mg $kg^{-1}day^{-1}$.

Carrageenan-induced aseptic inflammation on TMJ

Two hours after ending treatment, rabbits were prepared for experimental acute inflammation model of TMJ. Experimental inflammation was induced by following the method proposed by Swift et al. (10). The hair around the right TMJ area was shaved to allow clear location of joint. A 0.2 mL volume of carrageenan solution (1%), which is an inflammatory agent like histamine, was injected into TMJ with a 30-gauge needle. The jaw was moved passively for 15 and 5 min after injection, respectively, in order to spread the chemical substances in the TMJ area (10, 11).

Tc-99 m HIG scintigraphy

After carrageenan injection, each rabbit was given an intravenous 111 Mbq (3 mCi) Tc-99 m human HIG, and scanning was performed 4 h later because the inflammation of carrageenan induction reaches the highest level at 4–5 h (10). The animals were killed with intramuscular injection of ketamine hydrochloride 50 mg kg^{-1} (Ketalar; Parke Davis, New York, NY, USA). Anesthetized animals were placed under a gamma camera. Right lateral head and TMJ space views were acquired using a gamma camera (Starcam 4000 XC/T; General Electric, St Albans, UK) equipped with low-energy, general-purpose, parallel-hole collimator. A specialist in nuclear medicine performed a quantitative analysis.

A static image in which the lesion showed the greatest activity was selected, and a region of interest (ROI) was traced around the greatest activity in the TMJ area. An ROI of similar size

and shape was drawn on the adjacent site of the TMJ in normal tissue (as the background) (Fig. 1a–c). For this image set, an uptake ratio was calculated as: lesion activity/adjacent region activity (L/N). The quantitative analysis was calculated dividing the mean counts in the TMJ region by the mean counts in the adjacent normal tissue to determine the Tc-99 m HIG uptake.

Histopathologic study

After the scintigraphic analyses were completed, all animals were killed by intracardiac pentothal administration. Immediately after killing, the right TMJ and periarticular tissues were removed *en block* by cutting the ramus mandible inferiorly and temporal bone superiorly, and orbita and zygomatic bone medially.

For histopathologic examination, the resected material was fixed with tamponed formalin (10%). After fixation, 5 mm thick specimens were cut horizontally including the TMJ region through the line from the superior margin of the meatus acusticus externus to the processus zygomaticus ossis temporalis (Fig. 2).The upper surfaces of sections were marked with India ink. TMJ and periarticular tissue were decalcified with EDTA (10%) for 2 days at 4°C. The marked surface was positioned superiorly and embedded within paraffin. After a 100 micron thickness of each paraffin block had been removed, embedded sections (4 μm) were cut serially (10 sections) with a leica RM 2115 microtome and stained with hematoxylin–eosin (HE). The distance between the synovial surface epithelium (SSE) and the muscle layer (ML) in each section was measured by using SAMBA 200 Cell image processor with software.

Statistical analysis

The results are given as mean ± standard deviation (SD). Statistical comparison for L/N ratios (scintigraphic evaluation) and for the distance between SSE and ML (histopathologic evaluation) of all groups was made using Kruskall–Wallis analysis. Mann–Whitney *U*-test was used to compare the differences between two groups. $P < 0.05$ was accepted as statistically significant. Statistically Package for the Social Sciences (version 10.0, SPSS Inc., Chicago, IL, USA) was used to analyze the data.

Results

Scintigraphic results

The mean values of the lesion to adjacent normal tissue ratio (L/N) for each image were calculated.

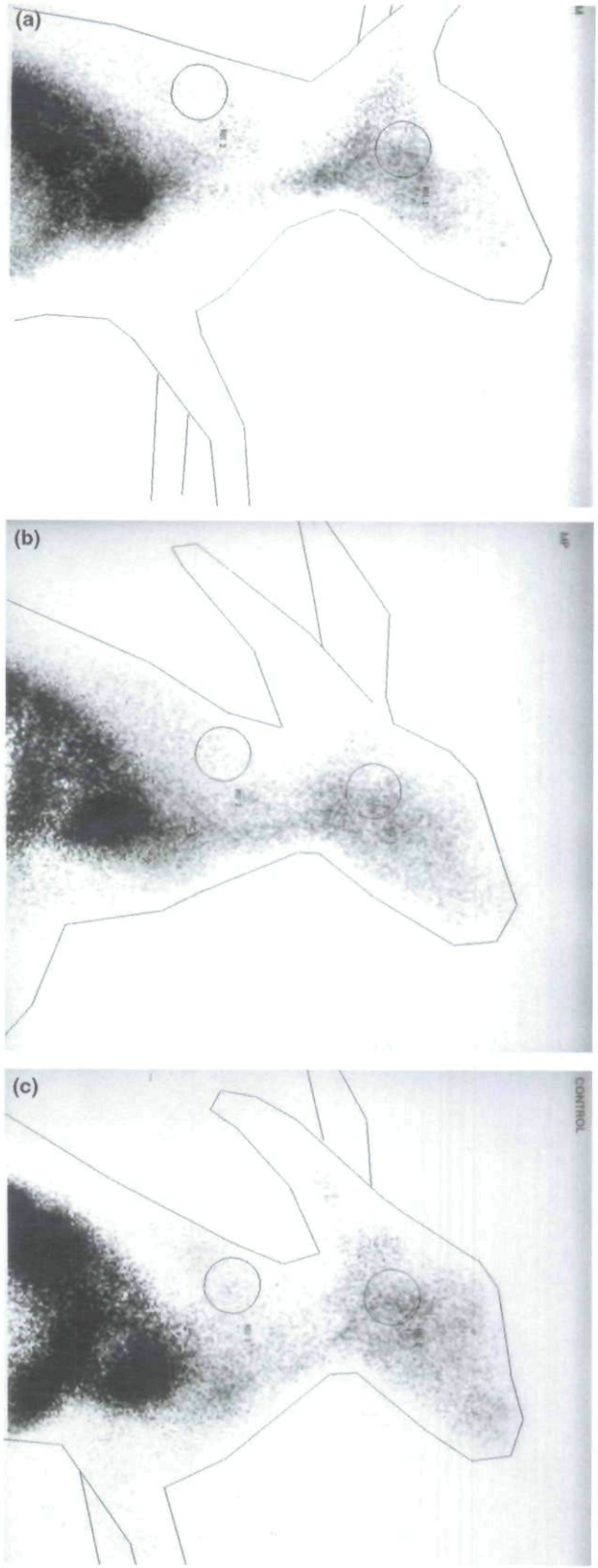

Fig. 1. Right lateral static images with Tc-99 m HIG in rabbits. (a) EM. (b) MP. (c) Control group.

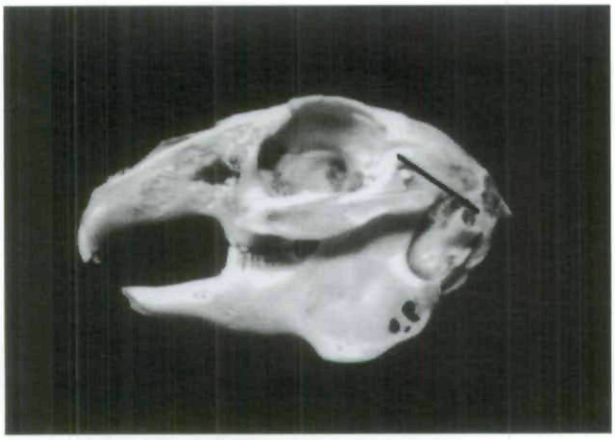

Fig. 2. The line through the superior margin of the meatus acusticus externus with processus zygomaticus ossis temporalis.

The mean and SD for EM, MP and control groups were 2.50 ± 0.12, 2.46 ± 0.12 and 4.12 ± 0.09 respectively.

There was a significant difference between the control group with MP and EM groups in terms of L/N ratios ($P < 0.05$). Nevertheless, there was no statistically significant difference between the EM and the MP groups ($P > 0.05$).

Histopathologic findings

Microscopically, we observed that the distances between SSE and ML in sections of the control group (78.6 ± 10.7 μm) was longer than those of MP (44.05 ± 18.25 μm) and EM (44.38 ± 18.26 μm) groups (Figs 3 and 4) ($P < 0.05$). The values MP and EM groups were similar ($P > 0.05$).

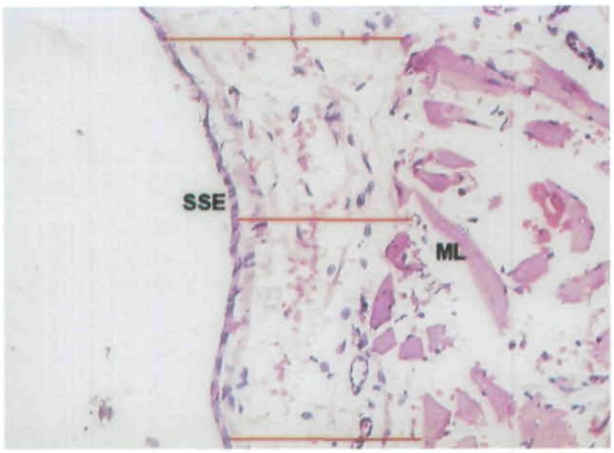

Fig. 3. The distance between the synovial surface epithelium (SSE) and the muscle layer (ML) in the section of the erythromycin (EM) group.

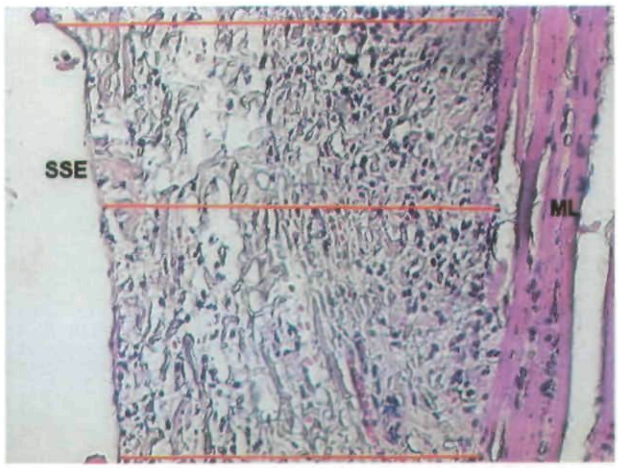

Fig. 4. The distance between the synovial surface epithelium (SSE) and the muscle layer (ML) in the section of the control group.

Discussion

In this study, we aimed to investigate whether EM would have an anti-inflammatory activity in experimental aseptic inflammation of TMJ.

According to our quantitative scintigraphic findings and semi-quantitative histopathologic findings, administration of EM significantly reduced aseptic inflammation when compared with the control group. These results show that EM has an anti-inflammatory effect in the TMJ space. EM inhibits both chemotaxis and random migration of neutrophils *in vitro* (12). The chemotactic dysfunction of neutrophils induced by EM may be secondary to intrinsic cellular abnormalities or to alterations of mediators influencing neutrophil function. Thus, EM may alter normal cellular calcium ion mobilization and disrupt cytoskeletal functions such as chemotaxis. EM may decrease the synthesis of chemoattractants and thus reduce neutrophil accumulation at the sites of inflammation (12).

Recently, the anti-inflammatory effect of EM has been studied in many diseases such as diffuse panbronchiolitis, chronic bronchitis, bronchiectasis, chronic sinusitis, pleurisy, and otitis media (5, 13–15). However, mechanism of these clinical improvements is still unclear (11, 12). This effect may be attributed to the inhibitory effect of EM on the production of cytokines and proinflammatory mediators (5).

We found a significant difference between the MP group and the control group with respect to L/N ratios and the distance between SSE and ML. This result confirms a well-known anti-inflammatory effect of MP. We found no significant difference between the EM and MP groups scintigraphically and histopathologically. This result also reveals that

EM has a similar anti-inflammatory effect as MP. Although low-dose long-term EM was used as a treatment of airway inflammation, Ianora et al. (5) reported that short-term macrolide antibiotics were only administered once 1 h before carrageenan injection into the pleural cavity. The anti-inflammatory activity of macrolide antibiotics and their ability to reduce the production of proinflammatory mediators and cytokines were investigated both *in vitro* and *in vivo*. This study shows that EM has anti-inflammatory activity.

Various radiopharmaceuticals have been used to measure joint inflammation. Indium-111-labeled HIG accumulates at various sites of infection and inflammation. Tc-99 m HIG is an alternative tracer that has shown similar results in a preliminary series (16, 17). Breedveld et al. (18) showed in an animal model that Tc-99 m HIG collects at sites of inflammation, and that quantitative studies could be performed to assess the severity of the inflammation. Liberatore et al.(19) compared Tc-99 m HIG, Tc-99 m albumin nanocolloid, and Tc-99 m leukocyte scintigraphy for evaluating joint inflammatory activity. They found that Tc-99 m HIG scan was more sensitive and accurate than the other scan types in terms of inflammatory activity. De Bois et al. (20) compared Tc-99 m HIG with Tc-99 m hydroxymethylene diphosphonate (HDP) conventional bone scintigraphy for assessing joint inflammation. They showed that Tc-99 m HIG scan was more specific and sensitive than Tc-99 m HDP scans for detecting and measuring inflammatory activity. Pons et al. (21) indicated that Tc-99 m HIG scintigraphy allows detection and measurement of joint inflammation and quantitative analysis can measure more objectively the degree of activity. In this study, we were able to identify the inflammation of TMJ in rabbits with a higher accumulation of Tc-99 m HIG both visually and quantitatively.

In conclusion, EM might be a preferable drug in the treatment of TMJ inflammation because of its anti-inflammatory and anti-bacterial effects and its potentially lesser side effects than corticosteroids.

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