Original Article

Anatomical considerations for applying the intra-articular injection technique in the rabbit temporomandibular joint

Marcia Molinet¹, Nilton Alves²,³,⁴

¹Escuela de Tecnología Médica, Universidad Austral de Chile, Puerto Montt, Chile; ²Master Program in Sciences, mention Morphology, Faculty of Medicine, Universidad de La Frontera, Temuco, Chile; ³Applied Morphology Research Centre (CIMA), Faculty of Dentistry, Universidad de La Frontera, 1145 Francisco Salazar Avenue, Temuco 4780000, Chile; ⁴Center of Excellence in Surgical and Morphological Research (CEMyQ), Faculty of Medicine, Las Encinas Avenue, Universidad de La Frontera, Temuco 4780000, Chile

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Abstract: This study aims to determine the parameters to be taken into account in order to carry out an intra-articular injection to induce osteoarthritis (OA) in the rabbit temporomandibular joint (TMJ). Eight TMJs from adult rabbits were used. One TMJ was dissected, enabling the fixing of reference points for entry into the joint. The most suitable needle for injection was selected, and the tissue was punctured 8 mm behind the posterior edge of the eye, at an angle of 45-55° above the horizontal. Black ink was injected through the needle to confirm that it was inserted into the joint cavity of the TMJ. The parameters used allowed the needle to be introduced into the joint cavity of the TMJ in all the test TMJs. Intra-articular injection for the induction of OA in the TMJ of rabbits is simple, quick and effective; however, the correct anatomical parameters must be taken into account to ensure the technique’s success.

Keywords: Intra-articular injection, rabbits, induced osteoarthritis

Introduction

Osteoarthritis (OA) is characterized by progressive degradation of the cartilage, remodeling of the subchondral bone, synovitis, and chronic pain [1]. OA commonly occurs in the joints which support the greatest loads (hip, knees, spinal column), but it can also affect the hands and the temporomandibular joint (TMJ). In the TMJ, it generally evolves with signs and symptoms such as tumefaction, pain on palpation, crepitation, and limited mandibular movement [2].

Pre-clinical studies allow the pathology to be assessed and described and the possible treatment of the disease to be determined [3, 4]. Studies in an animal model allow the effectiveness of new drugs and alternative treatments to be analyzed, and possible adverse events related to treatment to be assessed. The rabbit is used as an animal model to study TMJ OA because it presents structural and functional similarities to the human joint [4-7].

OA can be induced in the TMJ surgically [8] or by the application of chemical inducers [3, 6]. The advantage of inducing OA chemically is that the concentration of the substances can be controlled, modulating the progression and gravity of the lesions [6]. Furthermore, chemical induction causes less suffering in the animal, which is not subject to the risks involved in surgery. There are few studies which describe in detail the intra-articular injection technique used to induce this pathology and which assess its practice in different joints. The hypothesis of this study is: arthrocentesis is an easy and effective technique for the application of chemical inducers in the TMJ of rabbits used as animal model of OA. Therefore, the object of this study is to determine the parameters to be taken into account in order to carry out an intra-articular injection to induce TMJ OA in rabbits.

Materials and methods

Four adult rabbit heads (eight TMJs) were used for the study. The surgical material consisted of
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no. 15 and no. 22 scalpel blades, handles for the no. 15 and no. 22 scalpel blades, a tissue separator, a straight anatomical forceps, and a curved anatomical forceps. A model PI008 digital caliper was used to measure the distances between the reference points. For the intra-articular injections, we used 1 and 3 ml syringes, Chinese ink, and hypodermic needles of various sizes (21G x 1 1/2, 23G x 1, 25G x 1, 25G x 5/8 and 27G x 1/2).

Figure 1. A. Dissection of the left TMJ. The mandibular ramus (RM) and the components of the TMJ are observed, with the mandibular condyle (C) and the articular disc (D); B. Entry point of the needle 8 mm behind the posterior edge of the eye; C. Needle entry at an inclination between 45 and 55° above the horizontal.

The initial procedure consisted of dissecting one head selected at random, in order to demonstrate the anatomical characteristics of the structures composing the joint to obtain a better knowledge of the bone reference points and nearby structures (Figure 1A).

The depth of the joint below the skin was then analyzed to determine the best needle and syringe sizes to use for the injections, with the least possible damage to the tissues involved. Bone surface reference points were determined for the joint locations to ensure a clean delivery into the TMJ cavity. With this information, the selected needle was introduced into the joint, and Chinese ink was injected to prove the effective needle entry and delivery of the syringe contents into the joint cavity, without damaging the nearby tissues. The success of the injection was shown by the subsequent dissection of the TMJ.

Results

A 25G 5/8 hypodermic needle with a 1 ml syringe was selected for the intra-articular injection into the TMJ. This decision was based principally on the ease of manipulation and the depth of tissue to be penetrated by the needle to reach the joint cavity. Approximately 2/3 of the total needle length was introduced.

An entry point for the selected needle was established 8 mm behind the posterior edge of the eye (Figure 1B). Entry into the joint cavity was guided by the rabbit’s anatomy, by locating the zygomatic arch. The reference point for the needle entry was established at this site, sliding deeply down the zygomatic process of the temple. In order to identify the points more easily, the zone was shaved first.

The needle perforated the skin, ideally in a postero-superior direction and at an inclination ideally between 45 and 55° above the horizontal (Figure 1C). If the angle mentioned above is used, direct entry into the joint cavity is assured.

In the dissection, it was observed that the injected Chinese ink was found in the joint cavity of the TMJ.

The technique described here enabled the needle to be introduced into the joint cavity of the TMJ in 100% of the test joints.
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Discussion

In experimental studies with animals, those with the greatest similarity to human anatomy are usually chosen; however, the animal model will always present differences from humans. The rabbit is an animal model very widely used to study TMJ OA, since it shares many anatomical similarities with human TMJ, as well as lateral physiological movements not present in other mammals [9]; however it also differs in some characteristics which must be pointed out. The rabbit TMJ is reinforced laterally by a bony plate which projects from the zygomatic arch; it has no real mandibular fossa because the cranium does not completely overlie the mandibular condyle [5-7]. It must be remembered that preclinical studies form part of the basic evidence for decisions made in clinical practice [10]. They are important for understanding the mechanisms of a disease and for safely testing the effectiveness of new interventions [11]. In preclinical studies intended to test the effectiveness of a new therapy, it is important to ensure that the inducer effectively generates changes compatible with OA in the joint before testing a new treatment. Alves et al. [12] state that morphological changes in TMJs can be observed using cone-bean computerized tomography (CBCT) 25 days after the chemical induction of OA. The abnormalities become more evident at 45 days, as corroborated by Güler et al. [6]. These authors say that CBCT is an important tool which can be used to monitor the development of chemically induced TMJ OA in rabbits [12].

TMJ OA is a condition which has a negative impact on patients’ quality of life [13]; anti-rheumatic drugs [6] or alternative therapies have to be tried to assist with the treatment and improve their quality of life. New TMJ OA treatments can only be tested in humans after preclinical trials to verify their effectiveness and identify any adverse effects. This is done through the use of an animal model in which TMJ OA is induced. The surgical model is a technique used to induce OA in rabbit TMJs by exposing the joint in order to inject the inducer substance directly [3] and to cause joint degeneration by the displacement of the articular disc or the mandibular condyle [7, 14]. This is a successful technique, producing cartilage degeneration and anterior displacement of the articular disc, but it can injure the adjacent structures [8] which may affect the assessment of the results. OA can also be induced chemically by arthrocentesis, which is quick and effective and does not expose the animal to risks of post-operative infections, possibly leading to loss during the study. This method has the further advantage of avoiding confusion, since with the surgical model inflammatory processes may be triggered by the surgery and not just by the action of the chemical OA inducer.

Some studies have induced OA using intra-articular injections, without surgery or without opening the joint capsule [6, 15]. However, these studies have not described in detail the anatomical parameters used to locate the joint and guarantee a successful application, making the technique difficult to reproduce. Kapila et al. [4] describe the point of needle penetration as 5 to 10 mm behind the posterior edge of the eye in order to find the mandibular condyle. They add that this structure can be moved by opening and closing the mouth to ensure that the procedure has been carried out correctly. Artuzi et al. [16] determined an intra-articular injection site 5 mm from the posterior margin of the zygomatic process to induce TMJ OA in rabbits. Our study is very similar to these in terms of the distance, direction, and reference points described; however, we detail the exact point of penetration and the inclination of the needle, as well as the insertion length to reach the articular cavity of the TMJ without damaging the adjacent structures. We also point out that if the angle of inclination is much greater, there is a risk of damaging the nearby muscles. However, if the angle is reduced considerably, the ocular globe and/or the content of the socket may be damaged. Furthermore, if the needle does not reach the necessary depth, the superficial vascular and nervous elements will probably be harmed.

Conclusion

The present study describes in detail the technique known as arthrocentesis used in the chemical induction of TMJ OA in adult rabbits. It is a quick and safe technique which can be reproduced easily in preclinical studies.

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Disclosure of conflict of interest

None.

Address correspondence to: Dr. Nilton Alves, Faculty of Dentistry, Universidad de La Frontera, 1145 Francisco Salazar Avenue, PO-BOX 54-D, Temuco, Chile. Tel: 56-45-2325000; E-mail: nilton.alves@ufrontera.cl

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