Polish Journal of Veterinary Sciences Vol. 20, No. 2 (2017), 207–211

DOI 10.1515/pjvs-2017-0025

Original article

Injection in temporomandibular joint of rats. Description of technical protocol

R. Fuentes[1], C. Veuthey[1], A. Arias[1,2], D. Saravia[1,2,3], N.E. Ottone[1,4]

[1] Research Centre in Dental Sciences (CICO), Dental School, Universidad de La Frontera, Temuco, Chile
[2] Master Program in Dentistry, Dental School, Universidad de La Frontera, Temuco, Chile
[3] Universidad Adventista de Chile, Chillán, Chile
[4] Doctoral Program in Morphological Sciences, Universidad de La Frontera, Temuco, Chile

Abstract

The development of animal models for research has been very diffused. Osteoarthritis is a joint degenerative pathology that induces cartilage erosion, chondrocyte proliferation and osteophyte formation. The aim of this paper is to present a technical procedure to perform the injection of monosodium iodine acetate in the temporomandibular joints of rats to generate osteoarthritis and to contribute to future research analysis related to pathology progression and proper treatment performance. The use of rat models may be a complex process because of their size, but they can be compared to the human temporomandibular joint due to the similar characteristics and the possibility of performing diagnosis and treatment protocols in order to detect this pathology.

Key words: temporomandibular joint, injection, technical protocol, rat

Introduction

Osteoarthritis (OA) is a degenerative joint disease. About 80% to 90% of individuals of both sexes have evidence of OA by the time they reach the age of 65 years old (Hinton et al. 2002). It is characterized by erosion of cartilage, proliferation of chondrocytes and formation of osteophytes at the margins of the joint. The underlying bone is characterized by increased osteoclasts and the activity of osteoblasts, causing alteration of the bone contour and formation of subchondral cysts (Guingamp et al. 1997, Guzman et al. 2003, Wang et al. 2012). These changes occur over a long period of time, and are characterized both by degradation of the cartilage matrix and inhibition of matrix component synthesis. In the later stages of the disease, joint damage can lead to joint deterioration and pain. The temporomandibular joint (TMJ) presents the peculiarity of having articular surfaces covered with fibrocartilage, unlike the hyaline cartilage of other joints, so they can be affected by this degenerative process (Coronado et al. 2015).

The generation of animal models is relevant in research for its application to the clinic and surgery. To study the pathogenesis of this disease, many animal models have been developed to reproduce the

Correspondence to: R. Fuentes, e-mail: ramon.fuentes@ufrontera.cl, tel.: +56 45 27 34 153

physiopathology of OA in the TMJ (Montenegro and Rojas 2007) and to evaluate the properties of chondro-protective drugs. Regarding the clinical manifestation of OA in TMJ, it is characterized by an initial absence of symptoms, late onset and irreversible pathological changes, which may also be due to asymptomatic joint degenerative diseases in other territories. A suitable animal model can provide a useful way to understand the pathogenesis of OA in TMJ and to evaluate possible therapeutic interventions (Guingamp et al. 1997, Guzman et al. 2003, Wang et al. 2012, Orset et al. 2014), since early diagnosis enhances the effectiveness of the treatment and protects the joint by partially decreasing the progression of degenerative processes, reducing symptomatology and functional joint function.

The use of spontaneous or surgical methods for the development of OA was reduced due to the limited availability of special animals, the slow progression of the disease, and the high complexity in surgical intervention (Bendele 2001, Cledes et al. 2006). For these reasons, the method of intra-articular injection of monosodium iodine acetate (MIA) to induce lesions of osteoarthritis is widely used to induce OA of the knee (Guingamp et al. 1997, Guzman et al. 2003, Wang et al. 2012). MIA inhibits the dehydrogenase activity of glyceraldehyde-3-phosphate, thereby inhibiting glycolysis, which determines the death of chondrocytes in the articular cartilage of the specimen. Following intra-articular injection of MIA, cartilage lesions occur with loss of proteoglycans and functional impairment of the joint, similar to that which causes OA in humans (Guingamp et al. 1997, Guzman et al. 2003, Wang et al. 2012).

The TMJ is divided by the articular disc, which forms a compartment above and a smaller one below. Injection into the lower compartment is difficult, both in humans and animals, because of its limited space (Wang et al. 2012), while injection into the upper compartment, even in rats, is technically and manually preferable (Wang et al. 2012).

Thus, in this technical protocol injection of MIA into the upper compartment of the TMJ rat is carried out to seek to induce OA-like lesions throughout the joint.

The aim was to develop a working protocol for the application of monosodium iodine acetate (MIA) in temporomandibular joints(TMJ) of rats, to generate osteoarthritis.

Materials and Methods

For the implementation of this protocol of MIA injection Sprague-Dawley rats, obtained from ethical source were used. They were females, from 200 to 230 g in weight. The anatomy of the TMJ of these rats will initially be described. Later the technical procedure of injection of MIA into the TMJ will be described.

The rats used to perform the anatomic study of the TMJ and subsequent injection procedure were anesthetized with intraperitoneal ketamine-xylazine (80 mg/kg and 10 mg/kg, respectively). Euthanasia was performed with an overdose of the same anesthetic. These procedures were approved by the Research Ethics Committee at Universidad de La Frontera, with Resolution No 149-13.

Results

Description of the anatomy of the temporomandibular joint in rats

The temporomandibular joint (TMJ) is a complex structure of bones, ligaments and joint capsule. Many diseases occur in this diarthrodial-like joint such as fractures, ankylosis, degenerative disease and neoplasia. Animal models allow the study of TMJ and are used for therapeutic trials. Rat models are often considered in medical research because they are economical and easy to handle. The TMJ of the rat is surrounded by a thin capsule, consisting of a fibrous tissue and a synovial lining. The capsule extends from the edge of the mandibular fossa to the neck of the condyle. The joint space is separated into two spaces: one inferior, disc-mandibular and one superior, disc-temporal. From superficial to deep, on the outer surface of the joint, there are the following elements: skin, subcutaneous cellular tissue, lymph nodes, parotid gland, superficial temporal artery, facial and transverse facial arteries and the masseter and temporal muscles. Once the soft tissues are removed, the zygomatic arch, the condyle, the mandibular angle and the temporal bone can be observed. The mandibular angle has a prominent shape. The position of the condyles is divergent along the axial axis. The glenoid fossa is flat, without eminence.

Condyle: This is an area that comprises a thick layer of cartilage, mainly hyaline type, with superposed layers of chondrocytes that mature to the transition zone with bone tissue.This is seen in the articular surface of the mandibular condyle. The axis of the rat condyle is sagittal for propulsive movement, whereas it is transverse in humans for three-dimensional movements (opening/deduction/propulsion) (Orset et al. 2014).

Temporal bone: As with the articular surface of the condyle, there is a fibrous layer that increases in

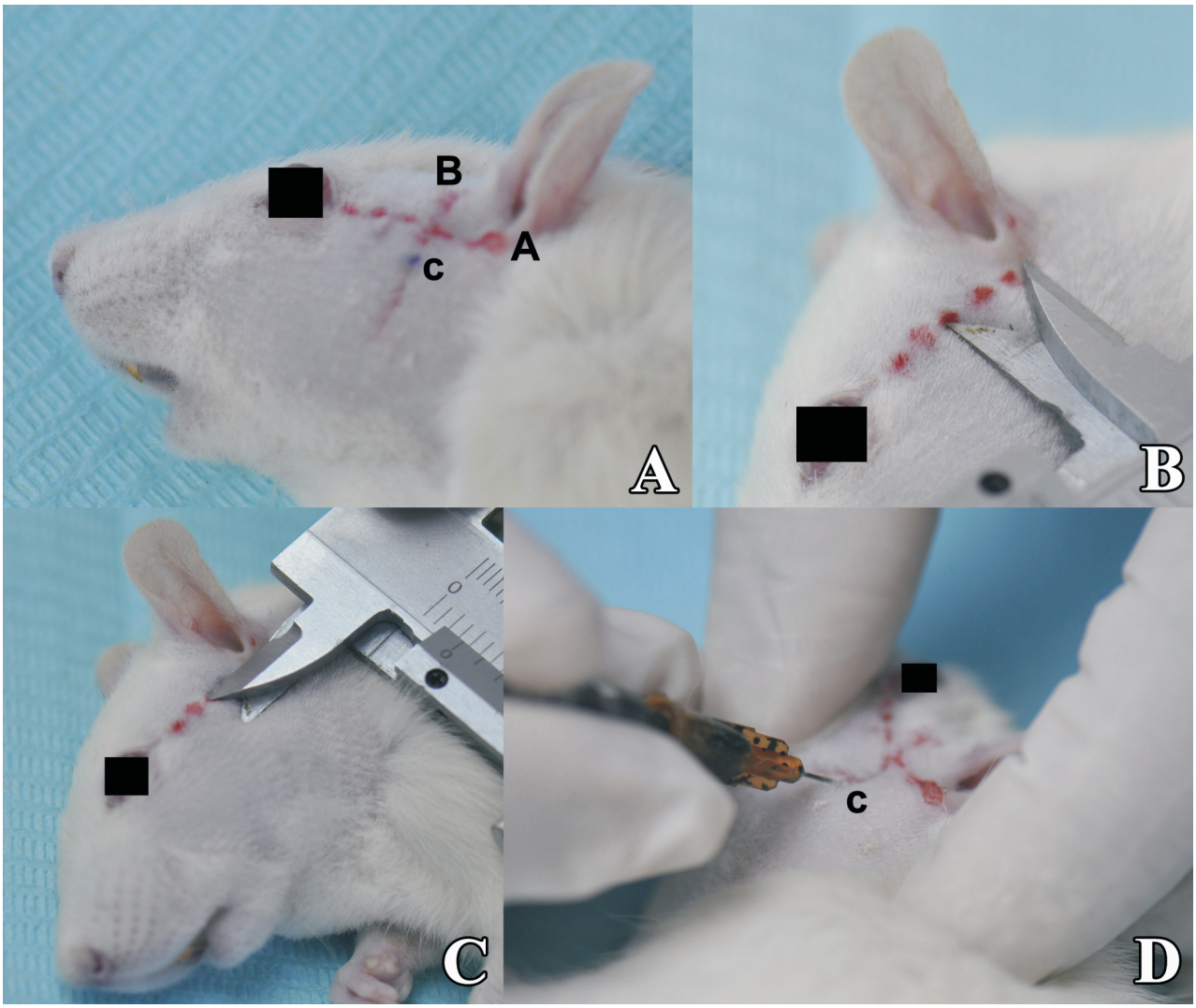

Fig. 1. Recognition of anatomical landmarks for establishing location of anatomical structures. **A.** Line **A** from external ear to eyeball, parallel to and above the zygomatic arch. Line **B** located 5 mm anterior to the external ear, and perpendicular to line A, corresponding to the location of the condilar process. **c**, corresponds to the point of insertion of the needle (see Fig. 1. C.). **B.** Measurement of the location of the condilar process, 5 mm anterior to the external ear. **C.** The measurement for the exact location of the needle is made from the anterior point, 3 mm above line A. **D.** Oblique introduction of the needle, with an inclination angle of 45º at point of insertion (c).

thickness from an anterior to a posterior position on the joint surface of the temporal bone. There are also layers of chondrocytes superimposed in smaller numbers than in the condyle.

Joint disc: The articular disc is biconcave, less thick in the central area; And is composed of fibrous connective tissue. It divides the articular joint into two compartments: upper and lower.

Zygomatic arch: The zygomatic arch is prominent, convex to lateral, and extending from the temporal bone, above the TMJ to which it covers in its anterior half, until reaching the facial mass at the level of the nasal bones. From its origin descends to cover the anterior half of the TMJ, reaching the anterior margin of the neck of the condylar process. It extends anteriorly, until exceeds the anterior margin of the mandibular branch, from where it ascends to finally enter into contact with the nasal bones. Due to this arrangement, it partially and superficially covers the temporomandibular joint by the lateral side (in contrast to what occurs in the human, which is above).

Based on the results of this study, it is possible to say that regarding the histopathological characteristics, there is a great similarity between rat and human TMJ. As in the rat, in the human condyle there is a thick layer of fibrocartilage, but with more layers of chondrocytes superimposed than on the articular surface of the condyle of the rat. The temporal articular surface of the human is also similar to that of the rat, except for the smaller number of chondrocyte layers

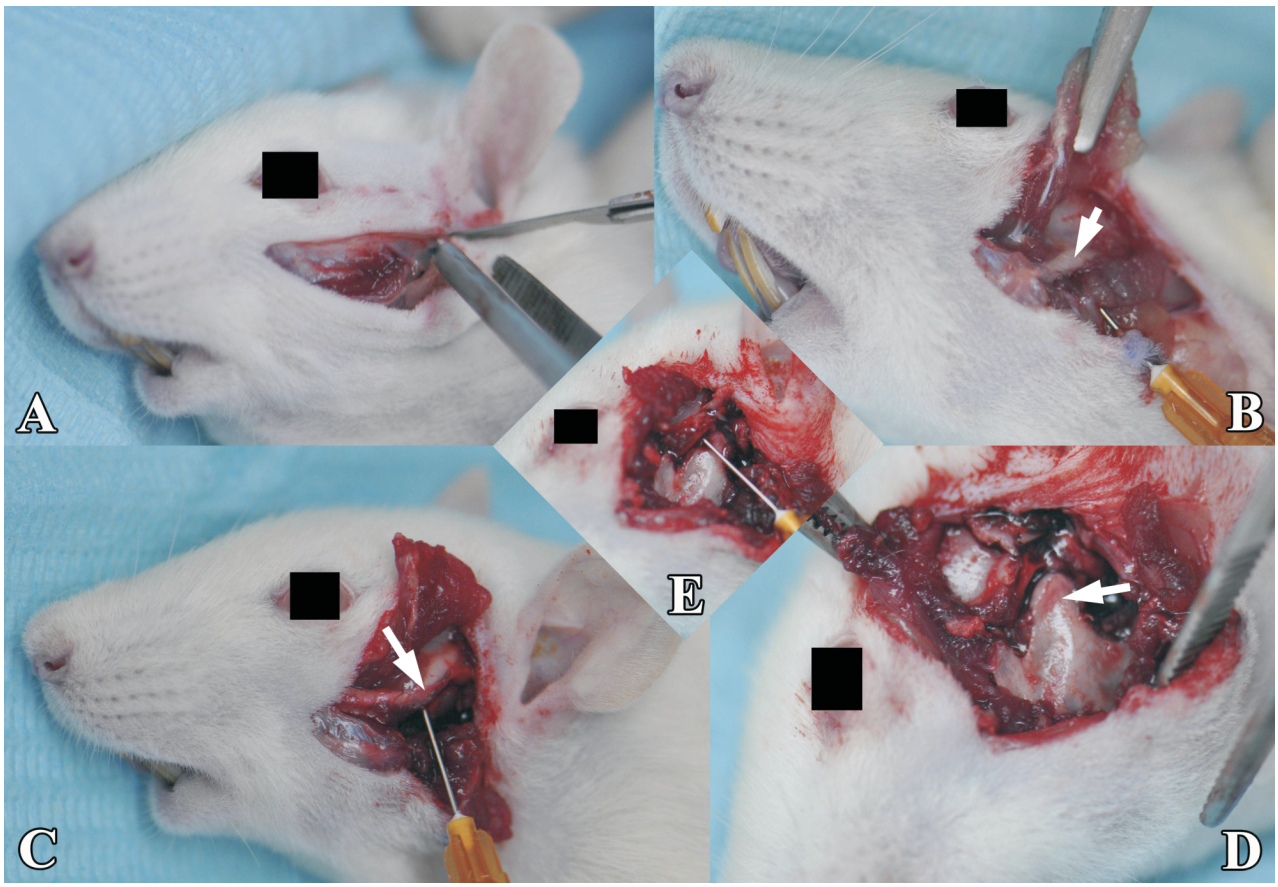

Fig. 2. Dissection of the TMJ of the rat, with needle injection position, for identification of anatomical landmarks necessary for proper insertion of the needle into the upper compartment of the TMJ. **A.** Plane surface dissection (skin and subcutaneous tissue). **B.** Recognition of the zygomatic arch (white arrow). **C.** Viewing of the zygomatic arch (white arrow) and its relationship to the condylar process. The needle is located in the injection position. **D.** Removal of the zygomatic arch and visualization of the condylar process (white arrow). **E.** Relationship of the needle to the condylar process and its entry into the upper compartment of the TMJ.

and the greater thickness of the fibrous layer. The shape of the disc is also similar, since the articular disc of the rat is biconcave and consists of fibrous connective tissue, as in the human. The TMJ of the rat also contains a human-like synovial membrane.

Anatomical reference points for needle insertion and SIA injection

Initially a line (A) was drawn from the external ear to the eyeball, parallel and over the zygomatic arch of the rat (Fig. 1A.). Subsequently, the condyle was located superficially, about 5 mm in front of the external ear, covered by the zygomatic arch. At the 5 mm level a line (B) was drawn, perpendicular to line A (Fig. 1A,B). There, in the cross-linking of both lines (A and B), the needle was inserted obliquely, at an angle of 45 degrees, from lower to upper and lateral to medial. At the time of insertion of the needle, it must be considered that it is made about 5 mm below and behind the level of the zygomatic arch, which, as described above, superficially covers the anterior half of the TMJ, and the needle must be inserted underneath it. The lower margin of the zygomatic arch serves as a guide for the exact location of the needle (Figs. 1C,D).

The needle was inserted 7 mm in depth until it reached the condyle of the TMJ, which serves as a reference above which, at about 3 mm, it would be possible to access the TMJ compartment located above the articular disc. At this level, black ink was injected and later TMJ was dissected. The black color of the ink within the joint capsule was detected and, in this way, the injection site was correctly identified due to the arrival of the ink in the TMJ (Figs. 1D, 2A-E).

Anatomical points essential for performing this procedure are located 5 mm behind the zygomatic arch and 5 mm anterior to the external ear, where the condylar process of the articulation is located in depth, above which it is posible to access the compartment located above the articular disc (Figs. 2C-E).

Discussion

There is a great diversity of pathologies that can affect the temporomandibular joint, leading to the development of temporomandibular disorders, causing pain and dysfunction of the stomatognathic system. Among them is osteoarthritis, a disease which can affect any joint, such as the TMJ. It is a degenerative disease of the joint, characterized by structural changes in the articular cartilage and bone, together with a secondary inflammatory process, with periods of exacerbation and remission (Guingamp et al. 1997, Hinton et al. 2002, Guzman et al. 2003, Wang et al. 2012, Coronado et al. 2015).

In reference to the animal models for the generation of the disease, the TMJ of the rat presents certain similarities with the TMJ of the human, which allows the results obtained in the experimental model to reproduce in patients (Porto et al., 2010). In both cases, TMJ is a diarthrosis (synovial) joint with an articular disc (Herring 2003, Cledes et al. 2006, Vasconcellos et al. 2007, Fuentes et al. 2016).

In this sense, it is very important to take into account the anatomical landmarks for the identification of the injection site, such as the external ear and the zygomatic arch. A point located between both structures, 5 mm from each, is recommended as the site of introduction of the needle, obliquely, with an angle of 45 degrees, in the inferior-superior and latero-medial (Wang et al. 2012).

To ensure correct introduction of the needle and posterior MIA injection, it is recommended that fhould previously be perfomed an ink injection with the same technique, followe by a subsequent dissection of the TMJ to identify the ink inside the joint capsule. This ensures a correct injection mechanism performance (Wang et al. 2012).

Although in basic biology, the species of animal used in relation to the response of mesenchymal cells is similar in all cases, the use of rats as an experimental model for research and development of pathologies of the ATM is important, not only for achieving an experimental reproducibility as close to the human ATM, but also for the use of a reduced number of animals, obtaining the maximum benefit and allowing the anatomy of the TMJ to be faithfully reproduced (Herring 2003, Cledes et al. 2006).

In conclusion, a technical procedure is presented for the correct introduction of the needle into the upper compartment of the TMJ of rats, in order to achieve subsequent injection of MIA to produce osteoarthritis. This is a fundamental process for the generation of an animal model that allows this pathology to be studied in relation to its appearance and chronological evolution and then create another animal model to study the treatment of this disease.

References

Bendele AM (**2001**) Animal models of osteoarthritis. J Musculoskelet Neuronal Interact 1: 363-376.

Cledes G, Felizardo R, Foucart JM, Carpentier P (**2006**) Validation of a chemical osteoarthritis model in rabbit temporomandibular joint: a compliment to biomechanical models. Int J Oral Maxillofac Surg 35: 1026-1033.

Coronado L, Iturriaga V, Bornhardt T, Fuentes R (**2015**) Evaluación de los protocolos de aplicación de ácido hialurónico en procesos degenerativos óseos de la articulación temporomandibular. Una revisión de la literatura. Av Odontoestomatol 31: 77-84.

Fuentes R, Ottone NE, Bucchi C, Cantin M (**2016**) Análisis de los términos utilizados en la literatura cientifica para referirse a la cápsula articular y ligamentos articulares de la articulación temporomandibular. Int J Morphol 34: 342-350.

Guingamp C, Gegout-Pottie P, Philippe L, Terlain B, Netter P, Gillet P (**1997**) Mono-iodoacetate-induced experimental osteoarthritis: a dose-response study of loss of mobility, morphology, and biochemistry. Arthritis Rheum 40: 1670-1679.

Guzman RE, Evans MG, Bove S, Morenko B, Kilgore K (**2003**) Mono-iodoacetate-induced histologic changes in subchondral bone and articular cartilage of rat femorotibial joints: an animal model of osteoarthritis. Toxicol Pathol 31: 619-624.

Herring SW (**2003**) TMJ anatomy and animal models. J Musculoskelet Neuronal Interact 3: 391.

Hinton R, Moody RL, Davis AW, Thomas SF (**2002**) Osteoarthritis: diagnosis and therapeutic considerations. Amer Fam Physician 65: 841-848.

Montenegro RMA, Rojas RMA (**2007**) Factors that regulate the morphogenesis and human mandibular growth. Int J Odontostomat 1: 7-15.

Orset E, Chaffanjon P, Bettega G (**2014**) Temporomandibular joint model: anatomic and radiologic comparison between rat and human. Surg Radiol Anat 36: 163-166.

Porto GG, Vasconcelos BC, Andrade ES, Silva-Junior VA (**2010**) Comparison between human and rat TMJ: anatomic and histopathologic features. Acta Cir Bras 25: 290-293.

Vasconcellos HA, Sousa EM, Cavalcante ML (**2007**) Temporomandibular joint classification: Functional and anatomic aspects. Int J Odontostomat 1: 25-28.

Wang XD, Kou XX, He DQ, Zeng MM, Meng Z, Bi RY, Liu Y, Zhang JN, Gan YH, Zhou YH (**2012**) Progression of cartilage degradation, bone resorption and pain in rat temporomandibular joint osteoarthritis induced by injection of iodoacetate. PLoS One 7: e45036.