Induction of haemarthrosis in the TMJ of rats: Validation by MR imaging (MRI) and histology

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SUMMARY. Objective: To develop an animal experimental model for the induction and assessment of joint damage caused by haemarthrosis in the temporomandibular joint (TMJ) in rats. Methods: Both TMJs of 10 young male Wistar rats were injected with autologous blood with additional heparin and Gd-DTPA. Two rats served as controls and were injected with physiological saline solution with additional heparin and Gd-DTPA. All rats were subsequently scanned with magnetic resonance imaging (MRI) to verify the accuracy of the intra-articular injection. The experimental rats were sacrificed after 6 and 24 h, and 2, 3 and 4 days, respectively. The rats of the control group were sacrificed after 6 and 24 h. Histopathological analysis was performed. Results: MRI showed that all TMJs, except one were correctly injected. One injection site was considered doubtful but after renewed injection proved to be correct. Histopathological examination showed a correct injection of blood in all joints, but showed no signs of inflammation or intra-articular damage. Conclusion: We have established a reproducible animal model for the induction of haemarthrosis in the TMJ of rats. Histopathological analysis showed neither signs of intra-articular damage nor inflammation. © 2008 European Association for Cranio-Maxillofacial Surgery

Keywords: temporomandibular joint, trauma, haemarthrosis, ankylosis, animal model, cartilage

INTRODUCTION

Trauma to the temporomandibular joint (TMJ) may cause several conditions such as an accelerated deforming arthrosis and in the long term the development of a fibrous or osseous ankylosis (Kaban et al., 1990; Chidzonga, 1999; Manganello-Souza and Mariani, 2003; Ferretti et al., 2005; Yun and Kim, 2005; Erol et al., 2006). Both deforming arthrosis and ankylosis can demonstrate similar clinical symptoms: restriction of movement of the mandible, limitation of mouth opening, joint pain and joint sounds. These functional impairments usually have psychosocial consequences and give way to problems with chewing, eating, swallowing, speaking, yawning and kissing. The treatment is intensive, often invasive and expensive.

The underlying pathophysiological mechanism after trauma leading to these chronic invalidating problems has not yet been elucidated. Several authors (Miyamoto et al., 2000; Yücel et al., 2002; Oztan et al., 2004; Yun and Kim, 2005; Erol et al., 2006) refer to the hypothesis of an intra-articular haematoma or haemarthrosis as the causal event although this is not supported by fundamental scientific research.

Intra-articular fractures of the condylar process of the mandible and trauma of the TMJ in general create an intra-articular haemorrhage. Blood-induced arthropathy is the most common clinical phenomenon in patients with severe haemophilia, and can cause severe handicaps (Houglund, 1967; Arnold and Hilgartner, 1977; Zeman et al., 1991; Roosendaal et al., 1999). In experimentally induced haemarthrosis in the knees of Beagle dogs, one of the earliest effects observed is proliferation and inflammation of the synovial tissue (Roosendaal et al., 1999).

Studies show that canine cartilage exposed to whole blood in vivo for a relatively short time (4 days) exhibits lasting biochemical and histobiochemical changes of the cartilage matrix, changes in chondrocyte metabolic activity, and changes in the synovium (Roosendaal et al., 1999). These results suggest that a single episode or a limited number of intra-articular bleeding episodes, although not directly related to degenerative joint
destruction, can cause lasting changes in articular cartilage that will inevitably lead to joint destruction. In further studies (Hooiveld et al., 2003, 2004) it appeared that the animals’ capacity to restore matrix turnover was inhibited for at least 2 weeks. These effects were prolonged when the experimental joint bleedings were combined with loading of the affected joint (Hooiveld et al., 2003, 2004).

The purpose of this study is to develop an experimental model in rats for the induction and assessment of joint damage after haemarthrosis in the TMJ.

**ANIMALS, MATERIALS AND METHODS**

**Animals**

Fourteen young mature male Wistar rats with a mean weight of 298.6 g and a standard deviation of 15.5 g were obtained from the animal facilities of the Radboud University Nijmegen. They were housed in groups of 2. The animals were fed a standard commercial diet with water *ad libitum*. Approval for this study was obtained from the Radboud University Nijmegen Animal Experiments Ethical Committee (DEC) on December 12, 2005, registered under number RU-DEC 2005-124.

The animals were then assigned to five experimental groups and one control group of two rats each. The remaining two rats were kept as spares in case of drop outs in the experimental groups. An haemarthrosis was created in both TMJs of the animals of the first five groups. Rats of group I were sacrificed 6 h after injection, group II after 24 h, group III after 2 days, group IV after 3 days and group V after 4 days. From the control group VI, one rat was sacrificed after 6 h and the other after 24 h.

Since the animals reacted and behaved as before the operation, there were no indications to administer analgesic medication.

**Experimental haemorrhages**

Initial pilot studies were performed to assess the topographic anatomy of the TMJ region and to evaluate methods to induce an intra-articular haematoma and contrast on magnetic resonance imaging (MRI), as determined in the pilot study. Then the rats were shaved on both sides at the TMJ region. With a small felt-tip pen, the site of injection was marked. As determined in the pilot study the marking point was located 2 mm pre-auricular; on a line connecting the upper side of the auditory duct with the lateral canthus.

On the marking point 0.2 ml of the autologous blood-mixture was injected into the joint with a direction of the injection-needle from posterior—lateral to anterior—medial. This was performed under distraction of the TMJ by closing the mouth of the rat and manipulating the lower jaw in contra-lateral direction. By using this technique we obtained maximal access to the upper joint chamber.

Both joints of the rats of the control group were injected with 0.2 ml of a physiological saline solution with two drops of heparin and 10% Gd-DTPA using the same technique.

**MR imaging (MRI)**

After the injections, the rat still under anaesthesia was brought to the MR scanner. MR experiments were performed on a 7 T/200 mm horizontal-bore MR spectrometer. The image acquisition protocol consisted of proton density weighted multi-slice spin echo images for anatomical localization and T1 weighted multi-slice spin echo images for checking the quality of injection in the TMJ.

Body temperature was kept constant at approximately 37°C using a water bath. Body temperature was measured using a Luxtron fluorescent thermometer, type 712.

Imaging parameters were image matrix size of 512 × 512 pixels, field of view (FOV) of 60 mm × 60 mm, slice thickness of 1 mm and 1 signal average per phase-encoding step. The values of repetition time (TR) and echo time (TE) were TR/TE = 2000/14 ms for the proton density weighted images and TR/TE = 500/14 ms for the T1 weighted images. Total scan time per rat was approximately 30 min.

Each MR image sequence set consisted of 10 slices overlaying the left and 10 slices overlaying the right TMJ, orientated on a fast scout image. Injection accuracy was determined by visual control of the images. First the proton density images were used for detection of the TMJ, then T1 weighted images were used to check the injection accuracy. Due to the T1 shortening caused by the Gd-DTPA, the injected autologous blood will appear significantly enhanced on the nuclear magnetic resonance (NMR) images.

**Histopathological and histobiochemical evaluation**

After sacrifice the rats were decapitated. The skin of the heads was removed and the heads were frozen at minus 20°C. The heads of the rats were then prepared by the lab. The left joint was prepared for histopathological research and the right side for histobiochemical research.

The heads of the rats were sliced while frozen. In this way, the left TMJs were harvested for histopathological evaluation along with the surrounding soft tissues. The specimens were then placed on a rudder in a 4% neutral formaldehyde solution for 1 week. Hereafter the specimens were decalcified in 20% formic acid and 5% sodium-citrate for 14 days. They were dehydrated and embedded in paraffin. Sections in the sagittal plane (6 μm) were deparaffinized and stained with Haematoxylin—Eosin (HE) and with Safranin O (SO).
From each TMJ, three slices were selected for analysis. The specimens were assessed using a light microscope. The HE coloured specimens were assessed histopathologically for the correct intra-articular injection as was confirmed by the presence of erythrocytes in the upper joint compartment of the TMJ. We also evaluated any damage to the TMJ due to traumatic injection. Finally we assessed the specimens for synovial inflammation, defined by hyperplasia of the synovial lining cells, capillary dilatation, fibrin deposition and synovial adhesions as described by Muto et al. (1998).

The SO coloured specimens were assessed on the loss of staining, as this represents the loss of proteoglycan and is an early sign of degeneration of the intra-articular cartilage.

RESULTS

Verification of the site of injection with MRI (Fig. 1) in all cases, except 1, showed contrast material in the upper joint chamber of the TMJ. Fig. 1 also showing high contrast in the right masseter muscle, indicating a surplus of injected solution in this region.

The injection site at the right TMJ of rat 1 from experimental group I was doubtful. This joint was once more injected and again verified with MRI. The images showed a correct injection this time.

A characteristic feature on MR images was the presence of contrast enhancement only in the upper chamber of the TMJ and not in the lower chamber.

One rat died after 3 h because of a possible fatal injection or overdose of anaesthetic drugs and was excluded from the experiment and replaced by a spare rat. The site of injection of one rat from group V was considered doubtful on MRI and this rat was also replaced by a spare.

Fig. 1 — MR imaging (proton density weighted image). This is a coronal slice of a rat used in our pilot study. The test rat was injected in the right TMJ with autologous blood with heparin and Gd-DTPA. The arrows are pointed at the right TMJ clearly showing the contrast in the right upper chamber of the TMJ. The left side represents the natural situation without blood in the TMJ. Also note the surplus of contrast in the right masseter muscle region.

Fig. 2 — Haematoxylin-eosin staining. Upper left: control group 24 h post-injection. Both upper and lower joint chambers are totally lucent, showing no signs of inflammation (×25 magnification; bar size 400 μm). Upper right: 6 h post-injection. The upper joint chamber is completely filled with blood (×25 magnification; bar size 400 μm). Lower left: 24 h post-injection. The upper joint chamber is partially filled with some erythrocytes and a number of plasma cells (×25 magnification; bar size 400 μm). Lower right: 3 days post-injection. Showing no signs of inflammation, the joint chambers are totally lucent (×25 magnification; bar size 400 μm).
Histopathology

The TMJs of rats consist of an upper and a lower joint chamber divided by an articular disc. The disc has a close relationship with the condylar cartilage (synfibrosis). The temporal fossa is concave and the mandibular condyle is a convex structure, each with a joint surface consisting of fibrocartilage. We orientated the specimen on specific markers, the auditory duct and the tissue of the parotid gland. In this way we could distinguish anterior from posterior.

The specimens 6 h post-injection (Fig. 2) showed upper joint chambers completely filled with erythrocytes — clear evidence of correct injections. We also noted pigmentation of the synovia with haemosiderin. The synovia were very cell rich with the presence of plasma cells and polymorpho nuclear granulocytes.

The specimens 24 h after injection (Fig. 2) showed an upper joint chamber filled with debris, which consisted of some erythrocytes and a number of plasma cells. The lower joint chamber was totally lucent without signs of any intra-articular injection. The synovia showed no signs of lymphocytic infiltrate. The specimen 2 days after injection showed fewer erythrocytes and fewer plasma cells. The synovial lining cells were somewhat more numerous, but there were no signs of inflammation. The specimens 3 and 4 days after injection were considered to be identical and showed remnants of an intra-articular injection in the upper joint chamber. The synovial lining cells and the cartilage were considered to be unaltered. There were no signs of inflammation.

Histological evaluation showed the presence of cartilage on the condylar process and only a thin layer of cartilage on the temporal fossa. There was no difference in SO staining between the experimental and control groups (Fig. 3).

DISCUSSION

We have succeeded in the development of a model for the induction of a haemarthrosis in the TMJ of young mature rats. We have developed the distraction technique by manipulating the lower jaw in order to obtain optimal entrance to the upper joint chamber. Moreover we have developed and tested a method to confirm the haemarthrosis with MRI.

On histopathological evaluation we found a complete haemarthrosis in the experimental group I after 6 h. In the following groups II–V, this haemarthrosis gradually disappeared, until in the experimental groups after 3 and 4 days the haemarthrosis had completely disappeared. There were only initial signs of inflammation. Therefore a further analysis of the synovitis as described by Muto et al. (1998) was not performed. There was also no noticeable damage of the cartilage and the interposing articular disc on histopathological examination. There were no clues for any fibrous or osseous ankylosis.

These results are more or less in accordance with those found by Niibayashi et al. (1995). They found after intra-articular injection of autologous blood into the knee joints of rats, a higher number of mononuclear cells after 8 h, 1 and 2 days. After 7 days there was a complete recovery. The results of Roosendaal et al. (1999) are in contrast with our experimental data. They created a haemarthrosis in the knee joints of Beagle dogs. A synovial inflammation was seen after 4 and 16 days.

As an analogy with the study by Hooiveld et al. (2003, 2004) of haemarthrosis in the knee joints of Beagle dogs, we also had intended to perform biochemical research on the cartilage of the TMJ. Due to the limited size of the TMJ of rats, there was not enough cartilage that could be harvested. Moreover the harvested cartilage was contaminated by the underlying bone. Therefore we had to conclude that our rat-model is not appropriate to perform biochemical analysis on the cartilage. Another purpose was to evaluate the loss of SO staining as this is a representative for the loss of proteoglycans in the cartilage (Roosendaal et al., 1999). Analysis showed no loss of SO staining in the cartilage of the temporal fossa.

Since MRI showed contrast only in the upper chamber of the TMJ of the rats, without signs of contrast in the lower chamber, we concluded that there is no connection between the two chambers and no contact of blood with the condylar process cartilage. Due to the limited size of
the TMJ of rats, it seems technically not possible to create an intra-articular haemarthrosis in this lower chamber by injection of blood. This was confirmed by histopathological evaluation.

Oztan et al. (2004) created an intra-articular haematoma by a surgical procedure to approach the TMJ of Guinea pigs. They had no indications for the development of ankylosis. We did not perform a surgical approach to the TMJ of rats because it had to be assumed that this would affect the results negatively and would not be in concordance with the formation of an intra-articular haematoma after trauma in human beings. A suggestion would be to induce standardised trauma to the condylar process of rats in order to create a haemarthrosis in the lower joint compartment.

There is convincing evidence that the creation of an intra-articular haematoma can lead to damage of the cartilaginous surface (Nishiyama et al., 1995; Roosendaal et al., 1999; Hooiveld et al., 2003, 2004). This has been shown by Hooiveld et al. (2003, 2004) by creating intra-articular haematomas in the knee joints of Beagle dogs. This damage to the cartilaginous surface was lasting when the creation of the haematoma was combined with a forced loading protocol. This model could be superposed on the TMJ of a larger animal, since the TMJ of rats has been proven to be too small in our study. Evidence of damage may be found by biochemical and/or histochemical analysis.

**CONCLUSION**

We conclude that we have established a reproducible animal model for the induction of a haemarthrosis in the upper chamber of the TMJ of rats. Histopathological analysis showed no signs of intra-articular damage. We would like to emphasize the unique character of this study in which we compared MRI with histopathological analysis of the same TMJs. The results of this experimental study warrant the continuation of analogous studies with additional biochemical analysis in larger animals. Yet, the implications for the treatment of human traumatic TMJ haemarthrosis cases are speculative.

**References**


