Effect of experimental traumatic occlusion on blood flow in the temporomandibular joint of the rat

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Fluorescent microspheres (FM) were used to visualize and semi-quantify blood flow in the temporomandibular joint (TMJ) during experimental unilateral traumatic occlusion of the maxillary and mandibular molar teeth in 30 young rats. At different postoperative observation periods varying from 1 to 30 days FM were injected systemically, and the number of FM were counted in serial sections from the TMJ in a fluorescent microscope. Blood flow was related to the number of FM found in the fibrous connective tissue and bony condyle of the TMJ. A percentage increase in blood flow was found at 15 to 20 days on the experimental side, compared with the contralateral side. Furthermore, there was an increase in blood flow in both TMJs in the experimental animals compared with an unoperated control material of 10 animals. The study thus indicates that a local unilateral occlusal trauma initiates blood flow responses not only unilaterally but also in the TMJ on both sides in rats. □ *Blood flow; temporomandibular joint; traumatic occlusion*

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The blood supply of the temporomandibular joint (TMJ) has been well documented in rodents (1), primates (2), and in human fetal (3) and adult material (4). Studies of cellular kinetics within the joint structures in induced stress have been undertaken in the rat by several authors (5–8). However, few characteristics of the TMJ apart from its morphology in health and disease have been investigated, partly owing to technical difficulties, and so far little information has accumulated on the blood flow of the TMJ under normal and experimental conditions. Various methods have been used to measure blood flow in oral tissues, including the hydrogen polarographic approach in the dental pulp (9), plastic microspheres in the periodontal ligament (10), and fluorescent microspheres (FM) in the periodontal ligament and pulp (11, 12). In the present study FM were used to visualize and semi-quantify changes in blood flow in the TMJ fibrous connective tissue and superior aspect of the bony condyle during experimental unilateral traumatic occlusion of the maxillary and mandibular molars in rats.

Materials and methods

A total of 30 Møll–Wistar rats (140–150 g) 55 ± 6 days old were anesthetized with subcutaneous injection of fentanyl/fluanisone midazolam (Hypnorm®/Dormicum®), 0.3 ml/100 g body weight. The occlusal surface of the first maxillary right molar was raised 1 mm with the composite material P-30 after acid etch with Scotchbond etching gel for 30 sec, followed by water rinsing and air drying. The right TMJ was defined as the experimental side, whereas the contralateral TMJ served as operated control. During the experimental period the animals received a standard diet. One, 5, 10, 15, 20, and 30 days later five animals in each group were reanesthetized, the heart was surgically exposed, and 0.2 ml FM (Fluoresbrite Plain Microspheres, Polysciences Inc., Warrington, U.K.), suspended in 10% Ficol-70 in 0.9% saline, was injected directly into the apex of the left ventricle in the course of 20 sec. The diameter of the microspheres was 9.5 μm ± 15%, which means most will be lodged in the precapillary arterioles in the

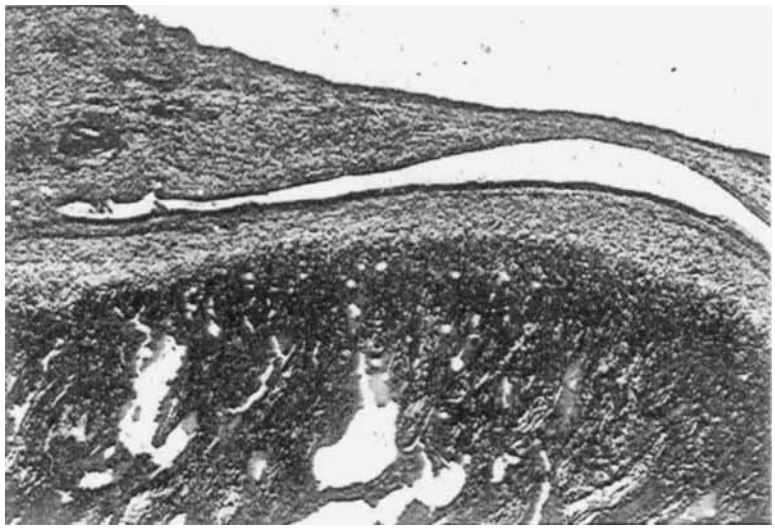

Fig. 1. Fluorescent microspheres in the fibrous connective tissue of the temporomandibular joint and in the superior aspect of the bony condyle.

rat. To ensure a sufficient number of FM in the small areas under investigation, a relatively large quantity was used in suspension for each injection (4×10^6), giving 150 to 900 FM for each tissue sample. This, together with the pneumothorax created by opening of the thorax during microsphere injection, caused a drop in systemic blood pressure which must be taken into account when referring to the absolute FM values. It was assumed, however, that the drop in blood pressure was equal on the two sides of the head and that the difference in blood flow in the experimental TMJ compared with the contralateral side will be valid. The microspheres were injected directly into the left ventricle of the heart to avoid the systematic error involved in passing a catheter into the same site via the common carotid artery, which will invariably cause different arterial blood flow to the two sides of the head (11).

Ten rats from the same litters as the experimental animals served as unoperated parallel controls and received FM at the corresponding times as described for the experimental groups.

The animals were killed with an overdose of anesthetics 1–2 min after the injection of FM. The TMJ, with the mandibular condyle, discus, and articular fossa of the temporal bone, was carefully dissected out in one piece, fixed in 10% buffered formalin, demineralized in 4 *N* formic acid and 0.5 sodium formate at 4°C for 10 days, and finally sectioned sagitally at 40 μm in a cryostat. The sections were mounted serially and examined unstained in a fluorescent microscope. The FM were clearly visible in the fluorescent microscope, and the total numbers were counted in the fibrous connective tissue attached to the collum immediately inferior to the condyle and to the discus and in the superior third of the bony condyle on the experimental and contralateral sides (Fig. 1). The same procedure was carried out on both sides on the 10 unoperated control animals. Special care was taken to ensure that FM were counted in the same areas in all specimens from the total material. To evaluate possible changes in blood flow, the number of FM was expressed as the mean percentage increase or decrease of the experimental side compared with the contralateral side at the different postoperative stages (Fig. 2). The experimental and contralateral total FM values per tissue sample were furthermore compared with the corresponding FM values from the unoperated control animals. This was undertaken to ascertain whether the contralateral side and the experimental side were affected by the exper-

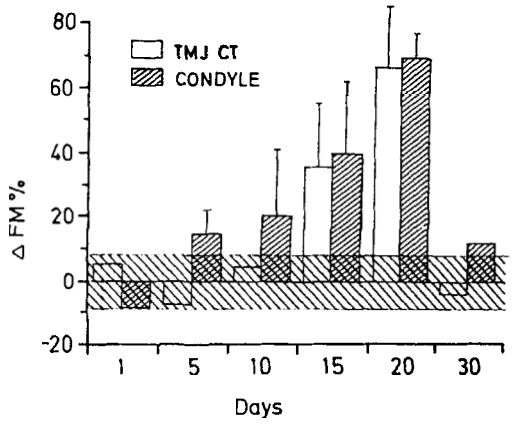

Fig. 2. Mean percentage change in the number of fluorescent microspheres (FM) in the fibrous connective tissue of the temporomandibular joint (TMJ CT) and bony condyle on the experimental side compared with the contralateral side. The contralateral FM values were defined as unity at zero (a hypothetic axis parallel with the abscissa). Shaded area represents ± 1 SD of mean contralateral FM values. Bars indicate 1 SD of mean experimental FM values. Note the percentage increase in FM in the TMJ fibrous connective tissue and bony condyle compared with the contralateral side at 15 and 20 days. There were five animals in each group.

imental procedure. These methods should give a semi-quantitative indication of blood flow changes in the aforementioned regions under the experimental conditions.

Results

The results are presented in Figs. 2, 3, and 4. Observations from the experimental, contralateral, and unoperated control FM values are presented as arithmetic mean ($\bar{x}$) ± 1 SD. SD bars are plotted when there are non-overlapping intervals between the experimental and contralateral/control materials.

Unoperated control animals

Mean FM numbers observed in 20 TMJs from 10 rats was 210 ± 25 for the fibrous connective tissue and 310 ± 75 for the superior aspect of the bony condyle. We found no significant difference in the mean numbers of FM between the two sides at any time interval.

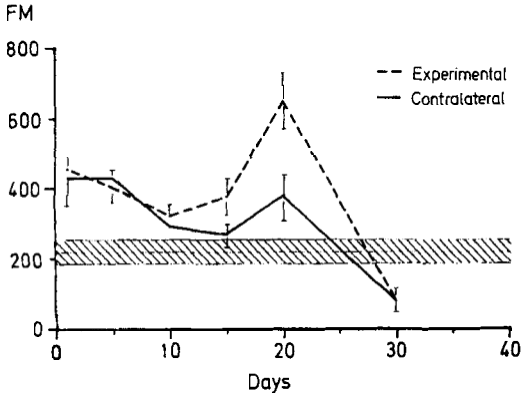

Fig. 3. Mean number of fluorescent microspheres (FM) in the fibrous connective tissue of the temporomandibular joint on the experimental and contralateral sides compared with the corresponding mean number of FM from the unoperated controls (210 ± 25, horizontal line and shaded area). Bars indicate ± 1 SD of mean FM values. Notice the similar behavior of blood flow changes on the experimental and contralateral sides compared with the unoperated controls.

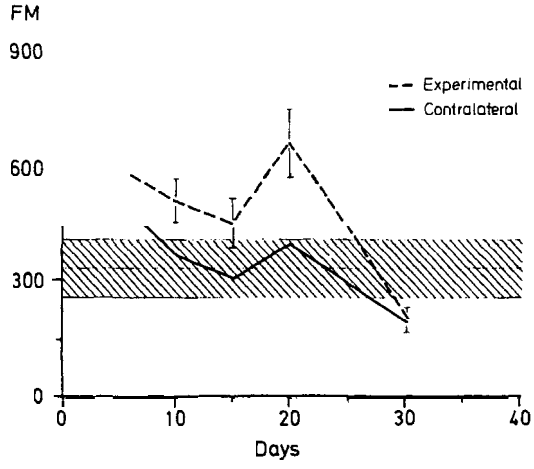

Fig. 4. Mean number of fluorescent microspheres (FM) in the superior aspect of the bony condyle on the experimental and contralateral sides compared with the corresponding mean number of FM from the unoperated control animals (310 ± 75, horizontal line and shaded area). Bars indicate ± 1 SD of mean FM value. Note the similarity in behavior of the experimental and contralateral sides compared with the unoperated controls.

Experimental animals

Fibrous connective tissue of the TMJ. In the early postoperative stages we found only small percentage changes between the experimental and contralateral sides in the connective tissue of the TMJ. At 15–20 days a marked percentage increase in FM was found on the experimental side compared with the contralateral side; thereafter the blood flow seemed to equalize on the two sides (Fig. 2).

Comparison of the mean numbers of FM in the experimental and contralateral sides with the corresponding numbers of FM in the unoperated control material showed that there was a bilateral increase in blood flow at day 1 in the TMJ fibrous connective tissue in the experimental animals, compared with the unoperated controls; thereafter there was a decrease towards day 15 and then an increase at day 20. Although the increase in blood flow was most marked on the experimental side, both the experimental and contralateral sides gave evidence of being affected by the experimental traumatic occlusion compared with the unoperated control animals (Fig. 3).

Bony condyle. Blood flow in the superior aspect of the bony condyle showed the same fluctuations as for the connective tissue, with a marked percentage increase at 15 to 20 days on the experimental side compared with the contralateral side (Fig. 2).

Comparison of the mean number of FM on the experimental and contralateral sides with the corresponding number of FM in the unoperated control material showed the same tendencies as for the connective tissue—namely, a substantial bilateral increase in blood flow of the TMJ at the beginning of the experiment and at 20 days for the experimental side (Fig. 4). No FM were observed either in the cartilaginous part of the condyle or in the articular discus (Fig. 1).

Discussion

This study indicates that unilateral experimental traumatic occlusion initiates bilateral blood flow responses in the TMJ of the rat, including the fibrous connective tissue and the bony condyle. In a previous study in our laboratory unilateral experimental traumatic occlusion was found to initiate blood flow responses bilaterally in the periodontal ligament and the pulp (12).

Previous experimental studies on the effect of occlusion on the TMJ are scarce and mostly histologic, examining the cellular elements in cartilage and bone. Furstman (13) found an increase in thickness of the articular discus after extractions of molar teeth. The changes were bilateral even though the extractions were unilateral. Assuming that an increase in cellular proliferation with concomitant formation of extracellular matrix is dependent on abundant blood supply, his findings coincide well with our own. Lindsey (14), investigating cell proliferation in the rat mandibular condyle by means of tritiated thymidine incorporation after use of a unilateral bite-raising splint, found more cell proliferation on the side of the splint than on the opposite side.

In the present study we found a percentage increase in blood flow as expressed by the number of FM at 15 and 20 days postoperatively in the TMJ connective tissue and bony condyle, respectively, on the experimental side, compared with the contralateral side. When the number of FM in the bony condyle and TMJ on the experimental and contralateral sides were compared with the unoperated control material, however, a bilateral increase in the number of FM was found at day 1 and also at day 20. This agrees well with a previous study in our laboratory (12), in which maxillary molars were subjected to a unilateral traumatic occlusion; the greatest bilateral increase in blood flow in both the periodontal ligament and the pulp was observed after 1 day, but also with a peak around 15 to 20 days when compared with the unoperated control material. The bilateral increase in blood flow found early in experimental traumatic occlusion both in the TMJ and in the periodontal ligament and pulp (12) is in accordance with previous studies showing that when stimuli of different types are directed to experimental tissues with intact innervation, a prompt increase in blood flow is produced (17).

Increase in blood flow in response to load application has also been observed in other sites (15). An increase in blood flow is dependent on vasodilation, absence of gross vascular leakage, and absence of stasis. Vasodilation is shown to be initiated by stimulation of sensory nerves with the release of neuropeptides like substance P (SP) and calcitonin gene-related peptide (CGRP) (16, 17). Concomitant nerve proliferation and blood flow increase have been demonstrated in the periodontal ligament in response to experimental orthodontic tooth movement (18) and traumatic occlusion (12, 19). That both TMJs showed blood flow changes compared with the unoperated controls was an interesting finding. Owing to the primary occlusal trauma the mandible will probably change to a new pattern of occlusal activity, which in itself can cause trauma to the TMJ both on the experimental and on the contralateral side and hence induce an increase in blood flow on both sides. This could possibly explain the late increase in the number of FM found in the present and previous (12) studies.

From the results of the present study it may be deduced that experimental traumatic occlusion in rats causes a relative increase in blood flow in the TMJ on the experimental side compared with the contralateral side.

Furthermore, that the unilateral experimental traumatic occlusion initiated a blood flow increase in both TMJs in the experimental animals compared with the unoperated controls suggests that traumatic occlusion is not a purely local phenomenon but probably a more general reaction in teeth, jaws, and TMJ, induced by a local stimulus.

The explanation can only be speculative but factors like muscular hypertrophy, increased functional/metabolic demands, and increased loading on the articular tissues with a resultant inflammatory reaction must be borne in mind.

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