Morphological Study of the Articular Disc and Capillary of the Retrodiscal Tissue in a Type 2 Spontaneous Diabetes Mellitus Rat Model

By

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Summary: The objective of this study was to evaluate morphological changes at the articular disc of the temporomandibular joint and capillary of the retrodiscal tissue in a rat model for type 2 spontaneous diabetes mellitus (DM) (i.e., Goto-Kakizaki [GK] rats) compared to normal Wistar rats. A total of 20 experimental rats were used in this study; the rats were categorized into the normal (n = 10 male 8-week-old Wistar rats) and DM (n = 10 male 8-week-old GK rats) groups. Hematoxylin-eosin stained specimens were obtained from 5 rats from each group. Image analyses of the hematoxylin-eosin stained specimens were conducted using light micrographs, which allowed comparisons of the thickness of the anterior, central, and posterior parts of the articular disc. Afterwards, the microvascular corrosion cast specimens were obtained from 5 rats from each group. The diameter of the capillary of the retrodiscal tissue was determined by analyzing scanning electron micrographs of the microvascular corrosion cast specimens. Student’s t-test was used to test for statistical significant differences between the 2 groups. Differences were considered significant when p < 0.01. We found that the thickness of the anterior, central, and posterior parts of the articular disc, and the diameter of the capillary of the retrodiscal tissue was significantly lower in the DM vs. normal group. Therefore, we consider that DM-associated the hyperglycemia causes atrophy of the articular disc and microangiopathy of the capillary of the retrodiscal tissue in GK rats.

Introduction

It has been clarified that diabetic microangiopathy occurs in the capillary in various oral organs (the filiform papillae on the lingual dorsum,¹ lingual gingiva of the mandibular first molar,² palatal gingiva of the maxillary first molar,³ palatine mucosa,⁴ and acinar surrounding the submandibular gland⁵) in the Goto-Kakizaki (GK)⁶ rat, which is a model for type 2 spontaneous diabetes mellitus (DM). However, to date, no studies have been focused on the histology of the articular disc, which consists of the fibrous cartilages and capillary of the retrodiscal tissue, in the GK rats. Therefore, we investigated and compared the morphological changes between the DM and normal rats in the articular disc of the temporomandibular joint (TMJ) and the capillary of the retrodiscal tissue.

Materials and methods

Experimental animals

A total of 20 experimental rats (Shimizu Laboratory Supplies Co., Kyoto, Japan) were used in this study. The DM and normal groups were comprised of 10 male Goto-Kakizaki (GK) rats (8 weeks old, body weight 220–180 g) and 10 male Wistar rats (8 weeks old, body weight 180–220 g).
weight 250–230 g), respectively. Hematoxylin-eosin stained sections were prepared from 5 rats from each group, and microvascular corrosion cast specimens were prepared from the remaining 5 rats from each group.

This experiment was approved by the Osaka Dental University Animal Research Committee (approval numbers 10-07002, 11-03015, 12-06001, and 13-02031), and complied with the guidelines for animal experiments.

**Measurement of fasting blood glucose and hemoglobin A1c levels**

All rats fasted for 20 hours, followed by measurements of their body weights. The rats were placed under anesthesia by isoflurane inhalation (Forane®, Abbott Japan, Tokyo, Japan) and intraperitoneally injected with heparin sodium (1000 units; Novo Heparin Injection 5000®, Mochida Pharmaceutical, Tokyo, Japan). After 30 minutes, the rats were euthanized with an intraperitoneal injection of excess sodium pentobarbital (Nembutal®, Dainippon Sumitomo Pharma, Osaka, Japan), the thorax was opened, and mouse was closed. Blood was collected from the left ventricle with a syringe (5-mL Terumo Syringe®, Terumo, Tokyo, Japan), and fasting blood glucose and hemoglobin A1c (HbA1c) levels were measured.

Fasting blood glucose levels were measured in rat serum samples with the HK-G-6-PDH (Quick Auto Neo GLU-HK, Sino Test, Tokyo, Japan) according to instructions of the manufacturer. Sera were obtained by centrifugal separation of the collected blood samples. In this study, the DM group n = 10 GK rats) was expected to have fasting blood glucose levels of > 150 mg/dL, as reported by Goto and Kakizaki.

HbA1c levels were measured by latex agglutination (RAPIDIA®, AUTO HbA1c-L, Fujirebio, Tokyo, Japan) according to the National Glycohemoglobin Standardization Program guidelines.

**Preparation of specimens**

**Hematoxylin-eosin stained specimens**

Hematoxylin-eosin stained specimens were obtained from 5 rats in each group to observe the histological morphology of the articular disc. We inserted a cannula through the left ventricle into the ascending aorta and then infused physiological saline into the ascending aorta. Blood was thrown from the right atrium. A 4% (w/v) paraformaldehyde solution (Formaldehyde Solution®, Kishida Chemical Co., Ltd., Osaka, Japan) was infused into the ascending aorta. The TMJ, including the mandibular head, articular disc, and mandibular fossa were removed, and then fixed by soaking in 4% paraformaldehyde at 4 °C for 24 hours. These specimens were decalcified in 10% (w/v) ethylene diamine tetraacetic acid disodium salt solution (2-hydrate®, Kishida Chemical Co., Ltd.) for 10 days using a microwave rapid sample processor (ML-77, Azumaya Corp., Tokyo, Japan).

**Microvascular corrosion cast specimens**

The microvascular architecture of the retrodiscal tissue in the TMJ was investigated in 5 rats from each group. A cannula was inserted through the left ventricle into the ascending aorta and then physiological saline was infused into the ascending aorta. Blood was thrown from right atrium. The preparation and injection of the acrylic resin was conducted according to the method described by Suwa et al. The polymerization inhibitor (hydroquinone) was maintained in commercial methyl methacrylate (Acrylic Ester M®, MMA, Mitsubishi Rayon, Tokyo, Japan). Hydroquinone was removed using the sodium hydroxide solution method according to polymer synthesis.

Commercial methyl methacrylate (1 L) was placed in a 3 L beaker, and 1 L of 0.5% (w/v) sodium hydroxide solution was added. This mixture was then stirred for 5
minutes with a magnetic stirrer (HVE-S, As One, Osaka, Japan). After stirring, the liquid was placed in a 3 L separation funnel (Separatory Globe Funnel with Teflon Stopcock, Sanyo, Tokyo, Japan), and allowed to settle. The sodium hydroxide solution (an aqueous solution that became brown with the addition of sodium salt of hydroquinone) and the methyl methacrylate (oil) were separated. The brown-colored aqueous solution was removed using a separation funnel. This procedure was repeated 3 times. We confirmed that the brown-colored aqueous solution became transparent.

The remaining oil (1 L) was placed in the 3 L beaker. In addition, 1 L of the distilled water was placed in the oil and then stirred for 5 minutes with the magnetic stirrer to adjust the neutrality. This procedure was repeated 3 times. We checked the neutrality with a pH meter (BASIC pH meter, Denver Instrument GmbH, Göttingen, Germany). Finally, 100 g of anhydrous magnesium sulfate (Nacalai Tesque, Kyoto, Japan) was added to the oil to remove any remaining water. We filtered the solution to remove anhydrous sodium sulfate. The commercial methyl methacrylate (liquid monomer), which was used to remove hydroquinone, was moved to a dark glass bottle and maintained at 4°C.

We prepared a low-viscosity acrylic resin by mixing polymethyl methacrylate (solid polymer; Sigma-Aldrich Japan, Tokyo, Japan) and liquid monomer at a 1:9 ratio. High-viscosity acrylic resin was prepared from a 3:7 ratio of polymer to monomer. We added 0.5% polymerization promoter (benzoyl peroxide, Kishida Chemical), 5% plasticizer (di-n-butyl phthalate, Kishida Chemical), pigment (Cromophtal Red, Ciba Japan, Tokyo, Japan), and 0.5% polymerization initiator (N, N-dimethylaniline, Kishida Chemical) to these mixtures to initiate polymerization. Firstly, we injected the low-viscosity acrylic resin (45 mL, 5 mL/minute) into the ascending aorta of rats using a precision syringe pump (KDS200, Muromachi Kikai, Tokyo, Japan). We then used the same pump to inject 5 mL of high-viscosity acrylic resin at 1 mL/minute into the aorta. The injected carcasses were allowed to polymerize at 42°C for 24 hours in a water bath (Thermo Regulator, CTR-320®, Iwaki).

Specimens were collected from the polymerized animals. The TMJs were removed, and then the soft tissues were removed by soaking the specimens in 10% aqueous sodium hydroxide for 24 hours at 42°C. The specimens were then washed in an ultrasonic cleaner with hot (42°C) running water. Each specimen was air-dried, mounted on a metal stage with silver paste and conductive tape, and coated with gold using an ion-sputtering coating device (JFC-1500, JEOL, Tokyo, Japan). Digital images of the microvascular architecture of the retrodiscal tissue were obtained by a scanning electron microscope (acceleration voltage, 5 kV; work distance, 40 mm; JSM-5500®, JEOL).

**Image and statistical analyses**

The thickness of the anterior, central, and posterior parts of the articular disc were measured in the digital images of the hematoxylin-eosin stained specimens (n = 5 from each group) using an image analysis and measurement software (BZ-2 Analyzer, Keyence) (Fig. 1).

Ten capillaries of the retrodiscal tissue were randomly selected from the digital images of the microvascular corrosion cast specimens (n = 5 from each group). The capillaries of the retrodiscal tissue were measured using a measurement software (Image-Pro Plus® 5.1J, Nippon Roper, Tokyo, Japan).

All measurements were calculated and represented as means ± standard deviations, and Student’s t-test was used to determine statistical significant differences between the 2 groups. Differences were considered...
Fasting blood glucose levels
The fasting blood glucose levels in the DM group (239.4 ± 24.8 mg/dL) were significantly higher than that of the normal group (112.9 ± 19.4 mg/dL; p < 0.01; Fig. 2).

HbA1c levels
The HbA1c levels in the DM group (4.9 ± 0.3%) were significantly higher than that of the normal group (4.2 ± 0.3%; p < 0.01; Fig. 3).

Specimen findings
Hematoxylin-eosin stained specimens
The TMJs from both groups were composed of the mandibular condyle of the mandibular bone and the mandibular fossa of the temporal bone. The articular disc composed of the fibrous cartilage was observed between the mandibular condyle and fossa. The mandibular condyles were smaller and the articular discs were thinner in the DM group than the normal group. The superior and inferior articular cavities were observed from the large gaps in the DM group (Figs. 4A and B).

Microvascular corrosion cast specimens
Reticulation was observed in the microvascular architecture of the retrodiscal tissue in both the DM and normal groups (Figs. 5A–D).

Image and statistical analyses
Thickness of the articular disc
The thicknesses of the anterior, central, and posterior parts of the articular disc in the DM group (196.2 ± 16.4 µm, 27.3 ± 3.2 µm, and 273.2 ± 20.7 µm, respectively) were significantly lower than that of the normal group (295.9 ± 26.9 µm, 40.9 ± 5.5 µm, and 440.0 ± 21.5 µm, respectively; p < 0.01; Fig. 6).

Diameters of the capillary retrodiscal tissue
The diameters of the capillary retrodiscal tissue in the DM group were significantly lower (16.6 ± 6.3 µm) than that of the normal group (43.4 ± 22.0 µm; p < 0.01; Fig. 7).

Discussion
Many previous studies have been conducted in streptozotocin-induced11–13 DM or GK rats.1–4 Streptozotocin destroys the β cells in the pancreatic islet, causing type 1 diabetes and increased fasting glucose levels (421–573 mg/dL).13, 14 However, many cases of type 2 DM are
Articular disc of TMJ in type 2 DM being reported as borderline type DM in Japanese adults with low fasting glucose levels. Therefore, GK rats (fasting glucose levels, 239.4 ± 24.8 mg/dL), which are considered a model for type 2 spontaneous DM, were used as experimental animals for this present study.

It has been reported that the morphological changes of the connective tissue papillae and capillary loops of the filiform papilla on the lingual dorsum, proper mucous membrane and capillary of the lingual gingiva of the mandibular first molar, palatal gingiva of the maxillary first molar, palatine mucosa, acinus, and capillary of the submandibular gland in GK rats. The TMJ in DM rats was only reported in a study titled, “Effects of Experimental Abnormal Occlusion on the Mandibular Condyles of Spontaneous Diabetic Rats (GK rats)”.

However, no study to date has focused on the morphology of the artic-
ular disc, consisting of fibrous cartilages and the capillary in the retrodiscal tissue in GK rats. Therefore, we investigated and compared the morphological changes in the articular disc and capillary of the retrodiscal tissue in the TMJ between GK and normal rats.

**Articular disc**

Golub et al. (1978) reported that hyperglycemia stimulated collagenase synthesis in gingival tissues of type 1 DM model rats. Furthermore, Schneir et al. (1998) reported that hyperglycemia decreased collagen synthesis in the above mentioned rats. In an *in vitro* study, it has been reported that dermal fibroblast proliferation was suppressed in GK rats when the cells were cultured in media containing high glucose. In an *in vivo* report, Kanemura et al. (2007) concluded that hyperglycemia causes regressive change in the mandibular gingival connective tissue papillae of GK rats. Uemura et al. (2009) concluded that hyperglycemia causes regressive change in the connective tissue papillae on the lingual dorsum in GK rats.

According to the image and statistical analyses of the hematoxylin-eosin stained specimen, the thicknesses of the anterior, central, and posterior parts of the articular disc in the DM group were found to be significantly lower than in the normal group. Therefore, we concluded that hyperglycemia is caused by an atrophic change in the articular disc of the TMJ in GK rats.

**Capillary of retrodiscal tissue**

It was reported that the proliferation of vascular endothelial cells was suppressed when placed *in vitro* in a media containing high glucose. The diameter of the capillary in the retrodiscal tissues in the DM group was significantly thinner than the normal group. Therefore, we suggested that hyperglycemia caused microangiopathy in the retrodiscal tissue of the TMJ and above the capillary of various oral organs.

Based on our results, we concluded that hyperglycemia in DM rats was caused by an atrophic change in the articular disc, which led to diabetic microangiopathy in the retrodiscal tissue of the TMJ.

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of the Japanese Society for the Temporomandibular Joint held (Sapporo-shi, Japan) on July 14–15, 2012. We would like to thank the staff of the Osaka Dental University facilities for their support with the animal experiments and image processing.

References