Journal of Dental Sciences (2013) **8**, 68–73

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ORIGINAL ARTICLE

Effects of glucosamine on tooth pulpal nociceptive responses in the rat

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Received 12 April 2012; Final revision received 3 September 2012
Available online 26 January 2013

KEYWORDS
C-fiber;
D-glucosamine;
in vitro jaw-nerve preparation;
nociceptor;
tooth pulp

Abstract *Background/purpose:* D-Glucosamine hydrochloride (DGL) has a variety of biological activities and is noted as a nutritional supplement that is effective for improvement and care of various disorders, such as osteoarthritis and atherosclerosis. Although, it has been reported that DGL has a significant pain relief effect in treating osteoarthritis, little is known about its effect on dental pain. The applicability of DGL as a medicament to control pain in pulpalgia has not been reported. In this study, using an *in vitro* rat mandible-inferior alveolar nerve preparation (jaw-nerve preparation), the effect of DGL on nociceptive responses in the tooth pulpal nerve was examined.
Materials and methods: The effect of DGL on nociceptive responses for 20 male Wistar albino rats was evaluated using an *in vitro* jaw-nerve preparation. Bradykinin (BK), used as a chemical nociceptive stimulant, was applied near the exposed tooth pulp. Sixty seconds after BK application, the surface of the exposed pulp was treated with DGL solution or physiological saline (control).
Results: The nerve firing rate was 2.06 ± 0.21 Hz ($n = 10$) after 5 minutes of saline application, and 0.76 ± 0.16 Hz ($n = 10$) after 5 minutes of DGL application. The DGL group showed significantly lower nerve firing rate than the control group.
Conclusion: BK-induced nociceptive responses were significantly suppressed by direct application of DGL. Our results suggest that DGL might have a pain relief effect in dental pain.

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1991-7902/$36
http://dx.doi.org/10.1016/j.jds.2012.09.022

Introduction

D-Glucosamine hydrochloride (DGL), a natural amino monosaccharide, is produced by totally hydrolyzing chitin with hydrochloric acid. Chitin is a polysaccharide found in the outer skeletal tissue of crabs, shrimps and lobsters,[1] and is the main productive source of chitosan.

DGL has a variety of biological activities, such as suppression of the neutrophil functions,[2] progression of adjuvant arthritis, and platelet aggregation,[3] and activation of intestinal epithelial cells[4] and of synoviocytes.[5]

Furthermore, DGL has been used as an effective medicament in various fields of medicine and dentistry.[6,7] For example, DGL is an attractive candidate for adjunctive therapy in arthritis, exhibiting not only chondroprotective action, but also anti-inflammatory actions via the suppression of neutrophil functions.[2] DGL also has a significant antipain effect in treating osteoarthritis,[8–12] which is considered to be a disease with low expectations of the value of treatment.[13,14] Nonsteroidal anti-inflammatory drugs (NSAIDs) are commonly used to treat osteoarthritis but have major adverse effects[15] and might even worsen the symptoms.[16] Several short- and long-term clinical trials in osteoarthritis have shown the significant effect of DGL.[17–19] Therefore, DGL is widely used in an attempt to suppress the pain concerned with disability of osteoarthritis.[20]

In dental fields, DGL promotes tissue regeneration on dental pulp wounds.[21] Evidence for this includes: (1) alkaline phosphatase activity is increased after DGL application[22]; (2) DGL enhances the expression of bone morphogenetic protein-2 mRNA[22]; (3) DGL inhibits interleukin-8 secretion from pulp fibroblast[21]; and (4) DGL induces the initial anti-inflammatory reaction in the pulp tissue.[21]

The present study was undertaken to evaluate the possibility of DGL as a medicament to control pulpalgia, because severe tooth pain is generally observed in the clinical case of pulpalgia.

Materials and methods

The methods described here follow the ethical guidelines and received approval from the Animal Welfare Committee of Nagasaki University (No. 0806090666, 2008-2011).

Preparation

Twenty male Wistar albino rats (body weight about 200 g) were used in the present study. The animals were deeply anesthetized with thiamylal sodium (60 mg/kg, i.p., Isozol; Nippon-Iko Pharmacy, Toyama, Japan). An *in vitro* jaw-nerve preparation[23] was slightly modified to enable easy direct access to the dental pulp (Fig. 1). The mandible was divided into the right and left halves at the central suture using a pair of scissors, and surrounding masticatory muscles were completely removed using surgical scissors. Then, the inferior alveolar nerve was identified at the foramen mandibulae on one side and isolated from the surrounding tissue. The mandible on one side was removed together with the inferior alveolar nerve by cutting the temporomandibular joint. Finally, about 20 mm of inferior alveolar nerve trunk was obtained from the foramen mandibulae and proximal end of the nerve was ligated with cotton thread. A small hole at the center part of the incisor tooth was carefully made to expose the dental pulp using a dental bur (round bur #3; Meisinger, Neuss, Germany).

Drug solutions

Modified Krebs-Henseleit solution consisted of 110.9 mM NaCl, 4.8 mM KCl, 2.5 mM $CaCl_2$ 1.2 mM $MgSO_4$, 1.2 mM KH_2PO_4, 22.4 mM $NaHCO_3$, and 20 mM glucose was used for perfusion.

Bradykinin acetate salt (BK; Sigma, St. Louis, MO, USA) was dissolved in sterile physiological saline (Otsuka Pharmaceutical Co, Tokyo, Japan). A 10^{-4} M BK solution was used to stimulate tooth pulpal nociceptors chemically.

DGL was supplied by Koyo Chemical Co. (Osaka, Japan). The molecular weight of the DGL used in this experiment was about 215 Da. A 10% (w/v) solution was prepared by dissolving DGL powder in sterile physiological saline.

Chamber design

As shown in Fig. 1, a chamber (total volume: 147 μL) was made with a plastic plate (thickness: 2 mm). The chamber room was separated into two pools (test pool: 84 μL; oil pool: 63 μL) by a thin plastic plate (thickness: 1 mm), at the center of which a small hole (diameter: 1.5 mm) was drilled to pass the trunk of the inferior alveolar nerve. A hard rubber bed was attached to the bottom of the test pool to prevent damage of the preparation.

Recording procedures

The mandible was placed in the test pool and the inferior alveolar nerve was passed through a hole at the center of the partition plate, and placed in the recording chamber (oil pool) and fixed to the wall of the chamber with the cotton thread. The hole at the center of the partition plate was capped by Vaseline. The inferior alveolar nerve in the oil pool was desheathed by slipping off the epineurium, and was slightly pulled to give a tension for insertion of the recording electrodes. The oil pool was filled with liquid paraffin to immerse the inferior alveolar nerve. The test pool was perfused (0.3 mL/s) with modified Krebs-Henseleit solution. The solution was saturated with a gas mixture of O_2:CO_2 (95%:5%).

To compare the data quantitatively, the fluid was heated to maintain the temperature at 31 °C with a feedback-controller; this temperature was used because, in rats, it is the mean temperature of peripheral tissue, such as surface facial skin or oral structures.

To record single fiber responses, a tungsten microelectrode (tip impedance 10–12 MΩ at 10 kHz; A-M Systems, Carlsborg, Washington, USA) was inserted into the inferior alveolar nerve trunk using a micromanipulator (MP-1; Narishige, Tokyo, Japan). The action potential was fed into a high-impedance, low-noise amplifier (DAM-80; WPI Instruments, New Haven, CT, USA) and displayed on a computer through the CED 1401 interface (Cambridge Electronic Design Ltd., Cambridge, UK). Spike analysis was

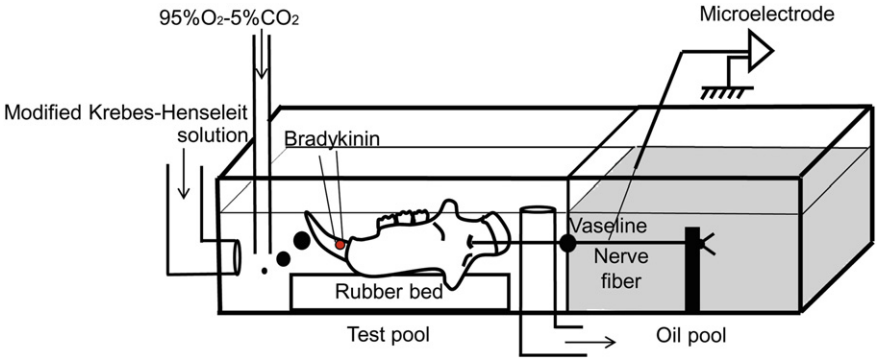

Figure 1 Schematic presentation of the chamber set up for the jaw-nerve preparation (see text for details).

carried out using Spike 2 software for Windows (Version 2.01).

To test chemical nociceptive sensitivity in the pulpal nerve, we selected only C-fiber responses for quantitative analysis and observed one unit/rat to prevent sensitization of the nociceptor. As criteria for C-fiber in the tooth pulp, two characteristics were adopted: (1) it is reported that C-fiber terminals are located in the center core of the pulp[24]; (2) spontaneous discharge of the C-fiber had a mean frequency of 1.6 ± 0.5 Hz (range, <2.0 Hz) as reported by Xiao and Bennett.[25] According to these criteria, we recorded unit responses from the center part of the tooth pulp having initial firing frequency of <2.0 Hz.

BK was used as a chemical nociceptive stimulant and applied into the test chamber near the exposed tooth pulp at a rate of 0.1 mL/s. Sixty seconds after BK application, the surface of the exposed pulp was treated with DGL solution or with physiological saline (control).

Statistical analysis

The obtained data were evaluated using StatView software Version 5.0 (SAS Institute Inc. Cary, NC, USA). Values are expressed as means $\pm$ SE, and the difference between the control and DGL groups was compared using an unpaired Student *t* test. A P value of <0.05 was considered to be significant.

Results

A total of 20 single unit responses were obtained. Initially, a majority of the units were spontaneously firing at 0.1–2.0 Hz. This low spontaneous activity was a good indicator showing that the tissue damage level induced by surgical removal of the jaw or making a small hole on the incisor was not so severe for electrophysiological recordings. The firing rate just after preparation in the control group was 0.91 ± 0.12 Hz ($n = 10$), and that in the DGL group was 0.96 ± 0.16 Hz ($n = 10$). There were no significant differences in the initial firing rate before BK application between the control and DGL groups (Fig. 2)

Fig. 3 shows a typical example of pulpal unit responses to BK applications and the effect of saline (A) and DGL (B) on the BK-induced excitatory responses. Histograms below raw data show the number of impulses/10 seconds. Upward arrows indicate the time of BK (filled) and DGL (open) application. It was observed that BK application significantly increased spike responses, indicating that nociceptive responses were evoked. After DGL application, BK-induced excitatory responses were gradually decreased; however, saline application had no effect.

Fig. 4 shows a summary of the time course of the effect of DGL on the BK-evoked responses. It was observed that the firing rate in the control group (open circle) kept increasing after saline application. By contrast, the firing rate in the DGL group (filled circle) was decreased after DGL application. The frequency in the control group was 2.06 ± 0.21 Hz ($n = 10$) after 5 minutes of saline application, while that in the DGL group was 0.76 ± 0.16 Hz ($n = 10$) after 5 minutes of DGL application. The DGL group showed significantly lower frequency than the control groups (asterisks; $P < 0.05$).

Discussion

Several studies have shown that DGL has pain relief effects through anti-inflammation and wound healing.[2,3,21,26,27] The molecular weight of DGL is about 215, and it is thought to dissolve easily at the applied site although this

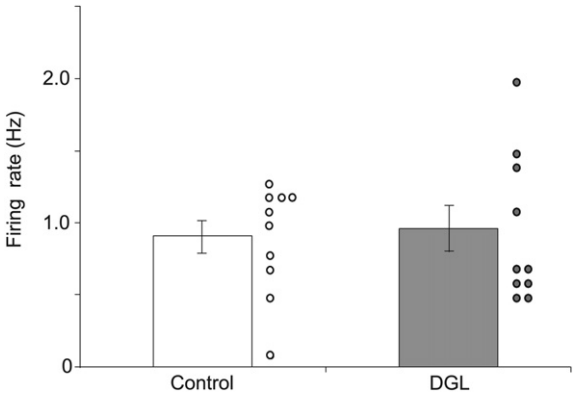

Figure 2 The firing rate (Hz) just after preparation for the control and DGL groups. There were no significant differences ($P > 0.05$). Dots show the firing rate. Vertical bars indicate standard errors.

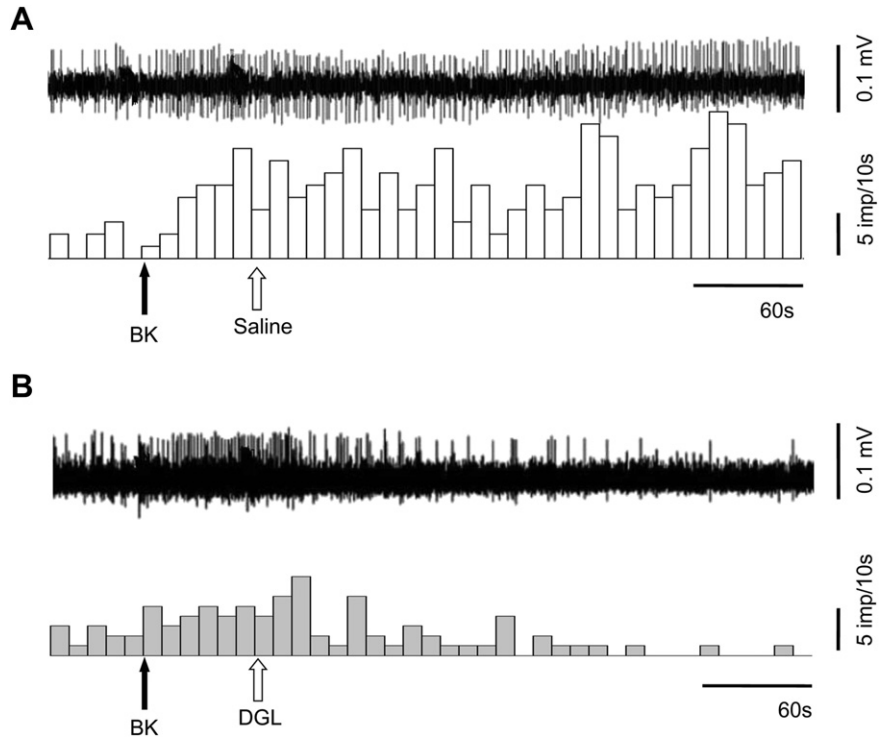

Figure 3 Typical examples of pulpal unit responses to BK applications and the effect of (A) saline and (B) DGL on the BK-induced responses. Arrows indicate the time points of BK (filled arrows) and saline or DGL (open arrows) applications.

agent may also be brought into contact with the tooth pulp tissue when used as part of a dressing material.

Very mild postoperative inflammation is characteristic after dressing tooth pulp with a high concentration of DGL.[28] In this study we used a 10% (w/v) solution prepared by dissolving DGL powder in sterile physiological saline. DGL appeared to induce no inflammatory reaction at this concentration, because no enhancement of the spike responses was observed after DGL application, as shown in Fig. 4.

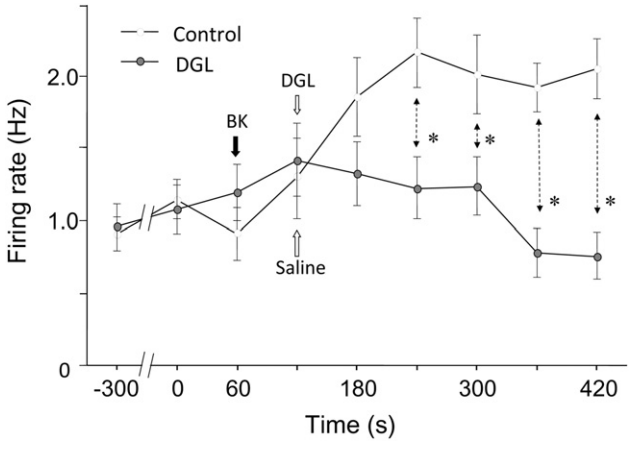

Figure 4 Effect of DGL on the BK-induced responses. Arrows indicate the time points of BK and DGL applications, respectively. The DGL group showed significantly lower frequency than the control groups after 240–420 seconds. *$P < 0.05$. Vertical bars indicate standard errors.

The effect of DGL on dental pain has been reported rarely. The present study was undertaken to evaluate the effects of DGL directly applied into exposed dental pulp, showing that DGL suppressed nociceptive responses evoked by BK application in the tooth pulp.

An *in vitro* preparation has the advantages controlling quantitatively all environmental variables surrounding the oral tissue and allowing direct application of chemical solutions onto the receptive field.[22] Therefore, we investigated the analgesic effect of DGL on the tooth pulp "directly", although many clinical trials have shown the effect of DGL by oral administration.[17–19]

The precise mechanisms of antinociceptive action of DGL are still unknown; however, glucosamine-induced antinociception is presumed to be caused by anti-inflammatory actions. Neutrophils, which are thought to be primary defenders in bacterial infections, are implicated in the destructive, inflammatory responses, and glucosamine suppresses the functions of the neutrophils, thereby possibly exhibiting anti-inflammatory actions.[2] Furthermore, in rats, glucosamine is reported to suppress the progression of adjuvant arthritis by inhibiting the chronic phase of inflammatory reaction and also suppress the production of inflammatory mediations (NO and PGE_2).[3] Therefore, glucosamine is potentially a novel anti-inflammatory agent.

In addition, it has been reported that DGL reduces the elementary current amplitude and increases the mean channel open time.[29] Because DGL has a weak binding site in the channel itself,[29] the channel cannot be closed.[29] Voltage-gated sodium channels, which are necessary for electrogenesis and nerve impulse conduction, can be

dynamically regulated after nerve injury or peripheral inflammation and play important roles in modulating neural excitability.[30,31] DGL might have an antinociceptive effect by the binding to sodium channels, resulting in a longer open time.

It has been reported that DGL has a significant pain relief effect in treating osteoarthritis,[11] in spite of negative results for temporomandibular joint disorder.[12] Osteoarthritis, developing as a result of progressive destruction of articular cartilage, is the most common joint disease and the leading cause of pain and physical disability in the elderly population. High blood pressure and diabetes are major risk factors for cardiovascular disease in this population. They have a high prevalence of multiple diseases that must be managed at the same time and often require the use of different drugs. Conventional pharmacological approaches to symptom management in osteoarthritis involve NSAIDs. However, there are accumulating data showing that any of these pharmaceutical drugs frequently produce insufficient benefit, with an associated risk of untoward side effects.[32,33] For example, NSAIDs can increase blood pressure. Glucosamine appears to be an attractive alternative, because it is a naturally occurring compound in the articular cartilage, and DGL is a symptom modifying drug with good evidence for favorable long-term effects on disease progression. DGL has a significant pain relief effect in treating osteoarthritis. Therefore, it is widely used in an attempt to suppress the pain and to treat disability of osteoarthritis.

The results of our study show that DGL might have a pain relief effect in dental pain. These findings led us to examine the application of DGL to dental analgesia. Furthermore, a recent systematic review showed that glucosamine appears to be just as effective as ibuprofen for the management of temporomandibular joint osteoarthritis.[34]

Use of glucosamine to treat disorders in the orofacial area is thought to be a possible alternative with a promising future. Also, further experiments may be necessary to evaluate the analgesic effect of DGL in cellular level and animal models.

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