Can Hyaluronidase Be an Alternative Postoperative Anti-edema Agent to Dexamethasone? Preliminary Results of an Animal Study

Q3 *Onur Koç, DDS, PhD,* and Nuray Er, DDS, PhD†*

Purpose: Recombinant human hyaluronidase (rHuPH20) is widely used as a spreading factor, which enhances the absorption of subcutaneously injected medicines. The anti-inflammatory and antiedema effects of the enzyme were demonstrated in previous studies. In the present study, the antiedema effect of rHuPH20 was compared with that of dexamethasone in a traumatic rat paw edema model.

Materials and Methods: Twenty-four Sprague-Dawley rats (weight 200 to 450 g) were divided into 3 groups: control (group 1), rHuPH20 (group 2), and dexamethasone (group 3). Traumatic edema was induced in the right hind paws of the rats using Feeney's weight-drop model. After edema induction, 0.4 mL of rHuPH20 (100 U/kg = 0.88 μg/kg dose) and 0.4 mL of dexamethasone (0.5 mg/kg dose) were injected into the right hind paws of the rats in groups 2 and 3. The paw volumes were measured before edema induction and at 3, 6, 12, 24, 48, and 72 hours after induction using a plethysmometer. The Mann-Whitney *U* test was used for the statistical analyses. Probabilities < .05 were accepted as statistically significant.

Results: The between percentage change in the edema mean values of groups 1 and 3 showed no significant difference at all time points; however, group 2 showed significantly less change in the edema mean values at 3, 6, 12, 24, and 48 hours after edema induction ($P < .05$) compared with group 1. The change in the edema mean value for group 2 was significantly less than that for group 3 at 3, 6, 12, 24, and 48 hours after edema induction ($P < .05$).

Conclusions: Local rHuPH20 injection more effectively reduced the edema that was induced traumatically in rat paws than did dexamethasone. However, further clinical studies are needed regarding the use of rHuPH20 as a postoperative antiedema agent in place of dexamethasone.

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Edema is an exacerbated accumulation of serous fluid in the interstitial space. It manifests clinically as swelling and is an inevitable complication after several surgical procedures. During the early healing period, it can cause severe pain and dehiscence at the operative site. Eventually, it will reduce the functionality of the neighboring tissues and prolong patients' recovery time. Various methods and medications, such as cold therapy, low-level laser therapy, and steroid and nonsteroidal anti-inflammatory agents, have been widely used for the reduction of postoperative edema.[1,2]

Cold therapy has been commonly used after several surgical interventions and is intended to reduce edema

Q2 *■■■, Department of Oral and Maxillofacial Surgery, Faculty of Dentistry, University of Hacettepe, Ankara, Turkey.

†■■■, Department of Oral and Maxillofacial Surgery, Faculty of Dentistry, University of Hacettepe, Ankara, Turkey.

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Address correspondence and reprint requests to Dr Koç: Department of Oral and Maxillofacial Surgery, Faculty of Dentistry, Hacettepe University, Sihhiye, Ankara, Turkey; e-mail: onurkoc101@gmail.com

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and pain. Many studies have been conducted on the efficacy of this therapy.[2,3] Low-level laser therapy has been widely used postoperatively because of its anti-inflammatory effects. The use of low-level laser therapy can help to control edema by reducing the presence of inflammatory mediators.[4] Nonsteroidal anti-inflammatory agents inhibit the cyclooxygenase enzyme in the arachidonic acid synthesis pathway, resulting in decreases in cytokine synthesis and inflammatory reactions.[5] Corticosteroids, such as prednisolone, methylprednisolone, and dexamethasone, have been widely used to control postoperative edema after various oral surgical operations.[6,7] Corticosteroids exhibit a strong antiedema effect through the inhibition of phospholipase A2 enzyme, which is active at a higher level of the arachidonic acid pathway.[8] Nevertheless, better methods for edema control are still under development by researchers to manage the unfavorable outcomes of surgical procedures.

Owing to their anti-inflammatory effects, protease and peptidase enzymes have been used to reduce postoperative edema.[9] Recombinant human hyaluronidase (rHuPH20) is an enzyme produced from human hyaluronidase genes using recombination technology. It breaks down the skeletal structure of the extracellular matrix (ECM)[10] by degrading hyaluronan (HA). HA is a nonsulfated linear glycosaminoglycan macromolecule. A key property of HA is its high water-binding capacity. This common component of the ECM is responsible for the stability of connective tissue. HA also provides lubrication, molecule filtration, and cell behavior regulation in the extracellular space.[11] The disruption of the compact connective tissue allows the fluids within the interstitial space to spread into a larger area. The resulting increases in capillary and lymphatic contact accelerate the entry of the fluids into the systemic circulation.[11,12] In summary, owing to the disruption of the ECM barrier, the dispersion and permeability capacity for fluids contained in the interstitial space is increased. In the present study, we tested this feature of rHuPH20 to control post-traumatic edema.

The antiedema effect of hyaluronidase was demonstrated in an animal study that showed that the hyaluronidase-releasing gel form of the enzyme decreased post-traumatic edema in rats.[13] The aim of the present study was to compare the antiedema effect of rHuPH20 with that of the most potent steroid anti-inflammatory agent, dexamethasone,[14] in a rat model.

Materials and Methods

The animal assays ethics committee of Hacettepe University approved the present study (approval no. 2014/36-8). A total of 24 male Sprague-Dawley rats (weight 200 to 450 g) were included in the present study. The rats were housed individually in metal cages with sawdust bases at the animal growing and feeding unit at the Faculty of Medicine Experimental Animals Laboratory of Hacettepe University. The rats were fed a standard laboratory diet, provided water ad libitum, and maintained under a 12-hour light/dark cycle. The rats were anesthetized with an intraperitoneal injection of 90 mg/kg ketamine (Ketasol; Richter Pharma Ag, Wels, Austria) and 10 mg/kg xylazine (Alfazyne; Alfasan International BV, Woerden, The Netherlands).

Before the treatments, each rat was weighed, and the doses of the medicines were adjusted for the test groups accordingly. The formation of traumatic edema was established using Feeney's weight-drop model.[15,16] In this model, a cylinder of 150 g was dropped on the right hind paw of the rats from a height of 40 cm.

The rats were randomly divided into 3 groups, which each contained 8 rats, as follows. In group 1, traumatic edema was induced in the rats, and no further treatment was applied (Fig 1). In group 2, traumatic edema was induced, and the right hind paw was injected with 0.4 mL rHuPH20 (Hylenex; Halozyme Therapeutics, San Diego, CA) at a dose of 100 U/kg[17] (or 0.88 μg/kg) diluted with saline to a 0.4-mL volume in accordance with the rats' body weight through a 26-gauge needle (Fig 2). In group 3, traumatic edema was induced, and the right hind paw was injected with 0.4 mL of dexamethasone (Dekort; Deva Holding Co, Istanbul, Turkey) at a dose of 0.5 mg/kg[18] diluted with saline to a 0.4-mL volume in accordance with the rats' body weight through a 26-gauge needle (Fig 3).

In all groups, the paw volume was measured using a plethysmometer[4] (May PLMO 1-A Commat; Pharmacology & Physiology Instruments, Ankara, Turkey). A plethysmometer is a computer-connected, highly sensitive device that can measure small volume changes through the calculation of increased volumes when an object is placed in the water container of the device. The baroreceptor linked to the base of the container measures small changes in volume in milliliters.

Increases in the edema volume of the rats were calculated by subtracting the basal volume (before treatment) from the final volume at the time of measurement. Edema was expressed as the percentage of change in the right hind paw volume of the rats: $(V_2 - V_1)/V_1 \times 100$, V_2 is the volume of the paw at the measurement, and V_1 is the volume of the paw before edema formation.[4] All edema mean values of the groups at the determined hours are presented as the arithmetic mean ± standard deviation and statistically evaluated using SPSS, version 21 (IBM Corp, Armonk, NY), software. The differences between the