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Effects of normal saline and selenium-enriched hot spring water on experimentally induced rhinosinusitis in rats

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ABSTRACT

Objective: This prospective, randomized, and controlled study examined the effects of normal saline and selenium-enriched hot spring water on experimentally induced rhinosinusitis in rats.
Methods: The study comprised two control groups (untreated and saline-treated) and three experimental groups of Sprague Dawley rats. The experimental groups received an instillation of lipopolysaccharide (LPS) only, LPS + normal saline (LPS/saline), or LPS + selenium-enriched hot spring water (LPS/selenium). Histopathological changes were identified using hematoxylin–eosin staining. Leakage of exudate was identified using fluorescence microscopy. Microvascular permeability was measured using the Evans blue dye technique. Expression of the Muc5ac gene was measured using reverse transcription-polymerase chain reaction.
Results: Mucosal edema and expression of the *Muc5ac* gene were significantly lower in the LPS/saline group than in the LPS group. Microvascular permeability, mucosal edema, and expression of the Muc5ac gene were significantly lower in the LPS/selenium group than in the LPS group. Mucosal edema was similar in the LPS/selenium group and LPS/saline group, but capillary permeability and Muc5ac expression were lower in the LPS/selenium group.
Conclusions: This study shows that normal saline and selenium-enriched hot spring water reduce inflammatory activity and mucus hypersecretion in LPS-induced rhinosinusitis in rats.

1. Introduction

Rhinosinusitis has a severe impact on the health-related quality of life in pediatric populations [1]. Although the mainstay of treatment is oral antibiotic therapy, many patients are refractory to even long courses of broad-spectrum therapy. This has led to the exploration of alternative effective treatments, such as functional endoscopic sinus surgery. Although evidence suggests that functional endoscopic sinus surgery is an effective treatment for pediatric chronic rhinosinusitis refractory to antibiotic treatment [2], some authors have expressed concerns about its possible interference with sinus development and midfacial growth [3,4]. For this reason, a more conservative approach is probably advisable before surgery. Among the conservative approaches, nasal saline irrigation is recognized as beneficial in the treatment of children with seasonal allergies, acute sinusitis, or chronic sinusitis [5,6]. However, the development of more effective and tolerable formulations of irrigants other than saline is needed because children may be unwilling to use or may be intolerant to irrigation.

In recent years, the role of selenium in preventing human disease has attracted attention because selenium has significant anti-inflammatory effects [7]. However, determining whether this property could translate into the use of selenium in an ideal nasal irrigant is hindered by a lack of data. The purpose of this study was to compare the effects of normal saline irrigation and selenium-enriched saline irrigation on the lipopolysaccharide (LPS)-induced inflammatory response in the nasal cavity and sinus of a rat model.

2. Materials and methods

2.1. Materials

The LPS used in this study was derived from *Pseudomonas aeruginosa* (L-4524; Sigma–Aldrich, St. Louis, MO). It was dissolved in normal saline solution at a concentration of 1 mg/mL. The source of selenium was a selenium-enriched solution from Gumjin hot spring water, which contains a rich supply of the minerals selenium, vanadium, calcium and magnesium. The chemical analysis of the hot spring water is reported in Table 1.

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Table 1
Chemical composition of hot spring water (thermal water from Gumjin spa).

Parameter	Results (range)	Units of measurement
Acidity	7.33	pH
Sodium (Na)	8500–9500	mg/L
Selenium (Se)	200–500	μg/L
Calcium (Ca)	1400–1700	mg/L
Bromine (Br)	40–56	mg/L
Zinc (Zn)	3–9	mg/L
Magnesium (Mg)	900–1100	mg/L
Potassium (K)	200–230	mg/L
Strontium (Sr)	25–35	mg/L
Vanadium (V)	69–71	μg/L
Germanium (Ge)	1–2	μg/L
Manganese (Mn)	10–40	μg/L
Cobalt (Co)	1–3	μg/L
Titan (Ti)	600–950	μg/L
Copper (Cu)	3–9	μg/L
Lithium (Li)	0.02–0.09	mg/L
Chloride ion (Cl^-)	16,000–19,500	mg/L
Fluorine (F)	1–3	mg/L
Sulfuric acid (SO_4^{2-})	3000–4500	mg/L
Boron (B)	1–2	mg/L

Analyses were carried out by the Korea Institute of Science and Technology, the Korea Basic Science Institute and the Korea Institute of Geosciences and Mineral Resources.

Thirty-one healthy Sprague Dawley rats, weighing 200–250 g and free of pathogens and respiratory diseases according to the health and pathology reports of the supplier, were used. All animals were housed and treated according to the regulations of the Catholic Ethics Committee of the Catholic University of Korea, which conformed to the NIH guidelines for the use of animals in research.

2.2. Methods

All experiments were performed with the rats subjected to 2% xylazine (8 mg/kg) anesthesia. Inhalant anesthesia was avoided to prevent irritation of the nasal mucosa. Both airways of the nasal cavity received an instillation of 0.1 mL of saline containing 0.1 mg LPS once per day for 3 days. The instillate was deposited as a bead of fluid on the external nares and the rats were allowed to aspirate it. Some rats were instilled with saline as a control. We carefully monitored breathing rate and skin color during instillation to prevent respiratory failure.

Thirty-one rats were allocated randomly to three treatment groups of seven animals each with 10 animals allocated to the control groups. One control group of three rats received no LPS or saline instillation (normal group), and the other control group of seven rats was instilled with 0.1 mL of normal saline once per day for 3 days (saline group). All experimental groups received an instillation of LPS (0.1 mL) once per day for 3 days. The LPS group received an LPS instillation alone, the LPS/selenium group additionally received an instillation of 0.1 mL of a selenium-enriched saline solution, and the LPS/saline group additionally received an instillation of 0.1 mL of normal saline. Evans blue dye (E2129-10G; Sigma–Aldrich) was injected into the femoral vein at 20 mg/mL per kilogram of body weight 30–60 min before death. The rats turned blue immediately after infusion of the dye, confirming its uptake and distribution throughout the body. The rats were exsanguinated 30 min after injection of dye and residual blood cells were flushed from the vascular system by perfusion of 100 mL of normal saline solution through an intra-aortic catheter. The nasal cavity was then lavaged with 0.1 mL of formamide for 5 min to collect the extravasated Evans blue dye. After collecting the extravasated Evans blue dye, the head was removed and cleaned of skin and fur. A coronal incision was then made 1 mm posterior to the eyes to extract the maxilla (including the sinonasal cavity) for tissue processing. Half of the harvested bone was used for reverse transcription polymerase chain reaction (PCR) analysis and half was used for staining. For staining, the bone was fixed in 10% paraformaldehyde for 24 h, decalcified in a rapid decalcifying solution (CalciClear Rapid; National Diagnostics, Atlanta, GA), embedded in a paraffin block, and cut into 4–5 μm thick sections perpendicular to the plane of the hard palate. The mucosa of the maxillary sinus and nasal cavity was stained with hematoxylin and eosin to determine histopathological changes.

2.3. Interpretation

The degree and location of the Evans blue dye extravasations in the nasal cavity and sinus mucosa were examined on unstained slides using confocal scanning microscopy (543 nm, Bio-Rad Radiance Plus; Bio-Rad, Hercules, CA). To quantify the amount of extravasated dye, absorbance of the supernatant at 630 nm was measured using a model Du-530 spectrophotometer (Beckman Coulter, Fullerton, CA). The thickness of the mucosa was defined as the maximum thickness of the mucosa overlying the nasal septum and was measured at a magnification of ×400. The mean mucosal thickness was calculated using three sections per group. The other half of the harvested bone was homogenized, frozen in liquid nitrogen, and stored at −70 °C. RNA was then extracted using an extraction kit (iNtRON Biotechnology, Gyeonggi-do, Korea) according to the manufacturer's instructions. Polymerase activation for Muc5ac was performed at 95 °C for 15 min followed by 32 cycles at 94 °C for 1 min, 55 °C for 1 min, and 72 °C for 1 min. Polymerase activation for glyceraldehyde 3-phosphate dehydrogenase (GAPDH) was performed at 94 °C for 4 min, followed by 35 cycles at 94 °C for 1 min, 60 °C for 1 min, and 72 °C for 1 min. The primer sequences were obtained from GenBank and were designed using Gene Runner software (Hastings Software, Las Vegas, NV). The sequences of the primers were as follows: Muc5ac: forward, 5′-CATAGCCTCCTCTTGTTC-3′ and reverse, 3′-ATTCCTGTAGCAG-TAGTGAG-5′; and GAPDH, forward, 5′-GCTGGTGCTGAG-TATGTCGT-3′ and reverse, 3′-GAATGGGAGTTGCTGTTGAA-5′. GAPDH was used as a constitutive control. The products were separated by agarose gel electrophoresis and visualized using ethidium bromide staining. The bands were digitized using a Universal Hood system (Bio-Rad). The mean Muc5ac-to-GAPDH band photodensity ratio was calculated for each group.

2.4. Statistics

Group means were compared using the Kruskal–Wallis test. Results are presented as the mean score ± standard. A P value < 0.05 was considered significant.

3. Results

3.1. Changes in the microvascular permeability of the mucosa of the nasal cavity and sinus

Results are shown in Figs. 1 and 2. In the control groups (untreated and saline-treated), little Evans blue was extravasated into the mucosa and the mean absorbance levels were 0.07 ± 0.03 and 0.10 ± 0.04, respectively. In the LPS group, a significant amount of dye was observed in the mucosa, which was observed as intense red under 543 nm wavelength of fluorescence microscope. Its absorbance was 0.20 ± 0.01, significantly higher than those of control groups ($P = 0.001$). In the LPS/saline group, a similar amount of dye was observed in the mucosa, with an absorbance of 0.17 ± 0.03. The difference absorbance values for the LPS/saline and LPS groups were not significant ($P = 0.08$). In the LPS/selenium group, a small amount of dye was observed in the mucosa, and the absorbance was