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Dietary nitrate protects submandibular gland from hyposalivation in ovariectomized rats via suppressing cell apoptosis

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ABSTRACT

Xerostomia, a major oral symptom of menopause, is a subjective feeling of dry mouth associated with oral pain and difficulties in deglutition and speech, which significantly reduces patient's quality of life. Dietary nitrate, which can be converted to nitric oxide, has multiple physiological functions in the body, including antioxidant activity and vasodilatation; however, its protective effect against xerostomia remains poorly understood. The present study aimed to evaluate the effects of dietary nitrate on estrogen deficiency-induced xerostomia. We established an ovariectomized (OVX) rat model, which included five groups: sham-operated, OVX, OVX + 0.4 mM nitrate, OVX + 2 mM nitrate, and OVX + 4 mM nitrate (n = 6). After ovariectomy, animals in the nitrate treatment groups received appropriate amounts of sodium nitrate dissolved in distilled water for 3 months. The results showed that nitrate treatment reduced body weight and water intake, and increased serum nitrate and nitrite levels. Furthermore, nitrate uptake increased saliva secretion as evidenced by saliva flow rates and aquaporin 5 expression, and alleviated histological lesions as evidenced by reduction of the fibrotic area and cell atrophy in the salivary glands. Although protective effects of nitrate against estrogen deficiency-induced xerostomia were observed at all doses, treatment with 2 mM nitrate was more effective than that with 0.4 mM and 4 mM nitrate. Terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) and caspase-3 expression analyses showed that nitrate also protected cells from apoptosis, possibly through upregulation of Cu-Zn superoxide dismutase (Cu-Zn SOD) known to inhibit oxidative stress-related apoptosis. Our findings indicate that nitrate could improve functional activity of the salivary glands in OVX rats by suppressing apoptosis and upregulating Cu-Zn SOD expression, suggesting that dietary nitrate may potentially prevent hyposalivation in menopausal women.

1. Introduction

Menopause, generally occurring in women aged 45–55 years, is a process of dynamic decline of ovarian function [1]. Xerostomia, a major oral symptom of menopause, is a subjective sensation of dry mouth associated with oral pain and functional deterioration of deglutition and speech [2]. Menopause-induced xerostomia is generally thought to be associated with estrogen deficiency [3–5], and is effectively relieved by estrogen supplementation in menopausal women [6,7]. However, long-term use of estrogen supplements may increase the risk of breast tumors, stroke, and heart disease [8], and recent clinical studies indicate that approximately half of menopausal women refused long-term estrogen treatment to reduce menopausal symptoms [9–11]. The mechanism underlying menopause-induced xerostomia is unclear. Recent studies revealed that atrophy of salivary gland cells due to estrogen deficiency is a key event in menopause-induced xerostomia [12,13]. Atrophy usually results from apoptosis and inadequate nutrient

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supply, which is often a consequence of estrogen deficiency associated with deregulation of vasodilatation [12]. Moreover, estrogen is known for its antioxidant activity through which it exerts protective effects on the central nervous system and heart [14–16]. Furthermore, antioxidant effects of estrogen were shown in the salivary glands of female rats, where it regulated the expression of Cu-Zn superoxide dismutase (Cu-Zn SOD) and reduced reactive oxygen species (ROS) generation [17].

For many years, dietary nitrate and nitrite have been thought of as the precursors of carcinogenic N-nitroso compounds [18,19]. However, increasing evidence suggests that adequate intake of dietary nitrate is likely to be safe and beneficial [20,21]. Dietary inorganic nitrate can be absorbed in blood, recycled in salivary glands, converted to nitrite by oral bacteria, and further reduced to bioactive nitrogen oxide (NO) in the acidic environment of the stomach to maintain systemic nitrate (NO_3)/nitrite (NO_2)-NO homeostasis in the body [22,23]. Dietary nitrates have multiple physiological functions, including decrease in blood pressure and increase in microvascular density and vasodilatation [24,25]. Furthermore, in male rats, nitrate administration inhibited ROS generation [25], and nitrite caused dose-dependent antioxidant effects in a hypertension model [26,27]. These data clearly indicate that nitrate may serve as an antioxidant to effectively protect cells against ROS-induced damage. As nitrate levels in salivary glands are reduced in xerostomia [26], we hypothesized that dietary nitrate can alleviate xerostomia symptoms associated with menopause. In the present study, we tested this hypothesis by feeding ovariectomized rats with sodium nitrate and analyzing their salivary glands for cell atrophy and apoptosis.

2. Materials and methods

2.1. Animals

All experimental procedures were approved by the Animal Care and Use Committee of the Capital Medical University (AEEI-2016-056). Surgeries were performed under anesthesia with intraperitoneal injection of 1% sodium pentobarbital. Female Sprague-Dawley rats (10-week-old, 230–250 g) were provided by the Capital Medical University Experimental Animal Center. and housed in polypropylene cages at 25 ± 1 °C and 55% ± 5% humidity, under light-controlled conditions (12-h:12-h light/dark photocycle). Animals were allowed to adapt to this environment with *ad libitum* access to distilled drinking water and regular pelleted food for at least 7 d before the experiment.

2.2. Establishment of the ovariectomized rat model

Thirty rats were randomly assigned to five groups (n = 6 rats per group): sham-operated (SHAM), ovariectomized (OVX), OVX + 0.4 mM nitrate (0.4 mM nitrate), OVX + 2 mM nitrate (2 mM nitrate), and OVX + 4 mM nitrate (4 mM nitrate). Surgery was performed as follows. After anesthesia, an incision was made in the lower abdomen midline and the ovaries were revealed. In the SHAM group, the ovaries were not removed, and fat tissue around the ovaries was resected instead. In the other groups, bilateral resection of the ovaries was performed. The abdominal incision was then closed and rats were allowed to heal for 2 weeks after the operation. Then, the animals of the nitrate groups received sodium nitrate dissolved in distilled water for 3 months right after the

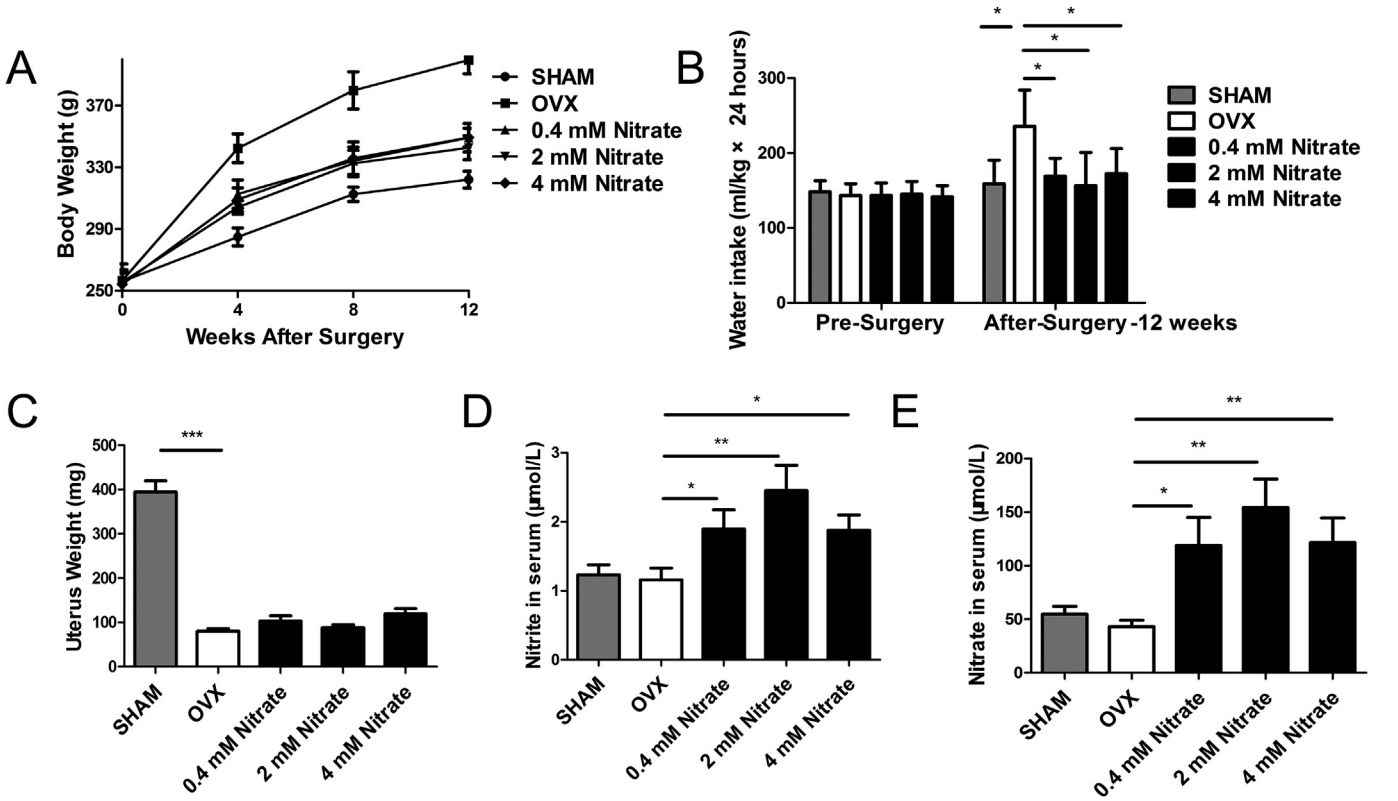

Fig. 1. Effect of nitrate on body and uterus weight, water intake, nitrite and nitrate levels. (A) Body weight curve during nitrate feeding. (B) Water intake pre-OVX and 12 weeks after OVX. (C) The weight of uterus after nitrate feeding. (D and E) Nitrite and nitrate levels after 12-week nitrate feeding. (Data are expressed as the mean ± SEM, n = 6, *P < 0.05; **P < 0.01; ***P < 0.001 versus OVX group).

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