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Linalyl acetate prevents olmesartan-induced intestinal hypermotility mediated by interference of the sympathetic inhibitory pathway in hypertensive rat

Soonho Kwon, Yu Shan Hsieh, You Kyoung Shin, Purum Kang, Geun Hee Seol*

Department of Basic Nursing Science, School of Nursing, Korea University, Seoul 02841, Republic of Korea

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ABSTRACT

Olmesartan-associated enteropathy (OAE) is a life-threatening pathological condition, but its underlying mechanisms have not been elucidated. Although intestinal hypermotility is frequently accompanied by chronic diarrhea, there have been no studies of olmesartan-induced hypermotility. Intestinal motility should be well regulated by the enteric nervous system, but degeneration of enteric neurons has been reported in patients with chronic diarrheal diseases, such as irritable bowel syndrome, suggesting a connection between OAE and intestinal hypermotility. In this study, interference with this inhibitory pathway was analyzed in a model of olmesartan-induced intestinal hypermotility (OIH) in rats with nicotine-induced hypertension exposed to chronic immobilizing stress. The effects of the potent inhibitory neurotransmitters norepinephrine (NE) and sodium nitroprusside (SNP), which act via different pathways, were assessed ex vivo, with only NE-modulated frequency and amplitude of spontaneous contractions found to be elevated in OIH rat jejunum. Clinical symptoms frequent in OAE, including atrophy of the intestinal epithelium and weight loss, were observed in these rats. Interestingly, olmesartan significantly elevated heart rate while lowering blood pressure in OIH rats. These abnormal conditions were prevented by adding linalyl acetate (LA), while the blood pressure-lowering effects of olmesartan were maintained. These findings suggest that olmesartan induces intestinal hypermotility by interfering with the sympathetic inhibitory pathway, and reduces epithelial cell size or body weight in hypertensive rats. As LA prevented these effects, combination treatment with olmesartan plus LA may provide better antihypertensive efficacy without inducing OAE.

1. Introduction

Olmesartan medoxomil (olmesartan) is the newest angiotensin receptor blocker (ARBs) used as a first-line agent in the treatment of hypertension, as it has shown clinical efficacy and cost-effectiveness [1]. Although olmesartan selectively inhibits the renin-angiotensin system (RAS), resulting in a low adverse event rate, the incidence of olmesartan-associated enteropathy (OAE) has been gradually increasing since 2012 [2]. The clinical manifestations of OAE, which include severe diarrhea, imbalance of electrolytes, and renal failure, can be life-threatening, but to date the only approach that resolved these clinical complications was to discontinue olmesartan treatment [3,4]. The RAS has been found to contribute to gastrointestinal health [5], suggesting that blockade of the RAS may interfere with this positive effect. Intestinal hypermotility is frequently accompanied by chronic diarrhea [6]. Losartan, another antihypertensive ARB, was recently reported to increase gut contractility in rats [7], suggesting that enteric motor neurons may be influenced by olmesartan. Although intestinal motility is dependent on the balance between stimulatory and inhibitory pathways in the enteric nervous system [8], no study to date has investigated the effects of olmesartan on the inhibitory pathway in the gastrointestinal tract.

Norepinephrine (NE) is a major inhibitory neurotransmitter that regulates intestinal motility [9]. NE, which is released by sympathetic nerve fibers innervating the serosa and smooth muscle, inhibits motoneurons in the myenteric plexus by acting on adrenergic receptors, playing a pivotal role in regulating motility. The degeneration of sympathetic neurons found in chronic diarrheal diseases, such as irritable bowel syndrome, indicates that malfunction of the adrenergic pathway is associated with motility disorders [9]. Nitric oxide (NO), which also inhibits intestinal motility [10,11], is another neurotransmitter with non-adrenergic non-cholinergic properties. Malfunction of the nitrergic nervous system also appears to be responsible in several conditions related to intestinal motility [12]. Olmesartan may

* Corresponding author at: Department of Basic Nursing Science, School of Nursing, Korea University, 145 Anam-ro, Seongbuk-gu, Seoul 02841, Republic of Korea.
E-mail address: ghseol@korea.ac.kr (G.H. Seol).

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interfere with either the adrenergic or nitrergic pathway, resulting in unregulated and elevated intestinal motility associated with chronic diarrhea.

Linalyl acetate (LA), a monoterpene ester isolated from essential oils, such as those of *Lavandula angustifolia L.*, *Thymus leptophyllus* and *Chrysactinia mexicana A. Gray*, was found to exhibit antispasmodic effects on intestinal motility [13–15]. LA and essential oils containing LA were shown to have antispasmodic activity in isolated intestines of guinea pigs [14,15] and rabbits [13], indicating that LA treatment of intestinal hypermotility may counteract diarrheal processes. Because LA is a major constituent of lavender, which was shown to have neuroprotective properties [16], we hypothesized that LA may alleviate olmesartan-induced effects induced on the enteric nervous system. To date, however, no study has investigated the possible antispasmodic effects of LA on olmesartan-induced intestinal hypermotility related to diarrhea. Also, it was suggested in our previous study that clary sage, which contains LA as a major ingredient, induced antihypertensive effects by reversing endothelial dysfunction in stress-induced hypertensive rats, indicating that LA may have beneficial effects on the cardiovascular system [17]. This study assessed whether LA, via its antispasmodic properties, can prevent olmesartan-induced intestinal hypermotility, while enhancing the antihypertensive effect of olmesartan.

2. Materials and methods

2.1. Animal experiments

All animal experiments were performed under the guidelines of the Institutional Animal Research Ethics Committee of Korea University and were approved by the committee (KUIACUC-2016-153). Four-week-old male Sprague-Dawley (SD) rats, weighing 150–200 g (Samtaco Inc., Osan City, Korea), were maintained under standard laboratory conditions at 22 ± 1 °C, on a 12 h dark/light cycle, with ad libitum access to chow and water, except for the day before sacrifice. The rats were randomly divided into six groups, a normotension control (NC) group (n = 5), a hypertension vehicle (HV) group (n = 8), a hypertension + olmesartan (HO) group (n = 8), a hypertension + olmesartan + LA 10 mg/kg (HO + L10) group (n = 6), a hypertension + olmesartan + LA 100 mg/kg (HO + L100) group (n = 7), and a hypertension + olmesartan + loperamide hydrochloride (loperamide) 2 mg/kg (HO + LH) group (positive control, n = 6). Loperamide was used as a reference drug for its potent antimotility effects [18] whereas it showed no clinical benefit in treating OAE [2]. Distilled water and polyethylene glycol (PEG) were the vehicles for olmesartan and LA, respectively.

2.2. Induction of hypertension and intestinal hypermotility

Hypertension was induced by immobilization stress (2 h/d) with chronic exposure to nicotine (0.8 mg/kg/d/i.p.) for 21 days followed by an injection of 3 mg/kg nicotine on day 22 day [19]. Rats were subsequently treated with olmesartan or olmesartan plus LA or olmesartan plus loperamide for 5 days. As the model represents hypertensive patients who have started pharmacological treatment, rats were subjected to immobilization stress with 0.8 mg/kg nicotine until sacrificed.

2.3. Measurement of blood pressure and heart rate

Blood pressure (BP) and heart rate were non-invasively measured using a tail occlusion cuff and pulse transducer (CODA HT-4, Kent Scientific Corporation, Torrington, CT, USA). To prevent movement while measuring BP, the rats were restrained in an adjustable animal holder and placed on a warming plate for 10–15 minutes to adjust to the proper temperature. BP and heart rate were measured weekly, with the last measurement taken 3 h after administration of olmesartan or its vehicle to maximize the pharmacological effect [19].

2.4. Preparation of rat jejunal tissue

Rats were fasted for 24 h before sacrifice [6]. To maximize plasma concentrations of olmesartan and its BP-lowering effect, rats were anesthetized with isoflurane and sacrificed through cervical dislocation 3.5 h after the last administration of olmesartan or its vehicle [20,21]. A surgical incision was made in the abdomen, and the jejunum was dissected out and quickly immersed in Tyrode's solution (136.9 mM NaCl, 2.68 mM KCl, 11.9 mM $NaHCO_3$, 0.42 mM NaH_2PO_4, 5.55 mM glucose, 1.8 mM $CaCl_2$, and 1.05 mM $MgCl_2$) after removing mesenteries. The tissues were cut into 15–20 mm long pieces, which were mounted longitudinally in a 6 ml myograph tissue bath (Mechano-transducer 620 M; DMT, Aarhus, Denmark), maintained at 37 °C with continuous aeration with 95% O_2, 5% CO_2. The motility of each tissue was recorded using LabChart Pro v8.0.7 (AD Instruments, Australia). The segments were equilibrated for 40 minutes by stretching to a baseline tension of 0.5–1.0 g. After attaining rhythmical, spontaneous contractions, the jejunal segments were treated with experimental drugs, and their motility was assessed. Spasmodic effects were induced by 10 μM acetylcholine (ACh), reaching a plateau within 2 minutes. Subsequently, 100 μM sodium nitroprusside (SNP) or 1 μM norepinephrine (NE) was administered to induce relaxation [22]. The concentration of each experimental drug was chosen to induce submaximal effects [23–25]. Between each cycle, the segments were washed 3 times and equilibrated for 40 minutes. To assess intestinal motility, only segments showing spontaneous contractions were analyzed. Because the range of spontaneous contraction varied in each tissue, the mean frequencies and amplitudes were expressed as percentages of responses modulated by experimental drugs. As the tissue segments were precontracted by a spasmodic drug and relaxed by adding a spasmolytic drug, relaxation was expressed relative to the maximal point of contraction.

2.5. Preparation of rat aorta

After the jejunum was dissected out, the thoracic aorta of each rat was harvested. Connective tissue was carefully removed so as not to damage the vascular endothelium, and each aorta was cut into 2-mm rings. These aortic rings were immersed in an organ bath system containing 5 ml of Krebs solution (118.3 mM NaCl, 4.78 mM KCl, 25 mM $NaHCO_3$, 1.22 mM KH_2PO_4, 11.1 mM glucose, 2.5 mM $CaCl_2$, and 1.2 mM $MgCl_2$), and each ring was quickly mounted with two tungsten wires. The chambers were maintained at 37 °C while being continuously provided mixed gas (95% O_2, 5% CO_2). The same transducer used to measure intestinal motility was connected to a personal computer and isotonic contractions were recorded. The rings were equilibrated at a resting tension of 1.0 g for 50 minutes before adding experimental drugs. Vasoconstriction was induced by adding 10 μM phenylephrine (PE), and, when vasoconstriction plateaued, 10 μM SNP or ACh was added to assess vasodilating ability. The concentration of each experimental drug conformed to that previously reported [26]. Between each contracting-relaxing session, the chambers were washed 3 times with fresh Krebs solution and equilibrated for another 50 minutes.

2.6. Measurementof rat intestinal tissue or serum nitrite

Jejunal tissue segments not used to assess motility were frozen in liquid nitrogen and their proteins extracted using a Protein Extraction Kit (iNtRON Bio, Sungnam, Korea). The lysates were centrifuged at 13,000 g for 10 min at 4 °C, and the protein concentrations of the tissue lysate supernatants measured using protein assay kits (Thermo Fisher Scientific, Waltham, MA). As NO has a short half-life, nitrite concentrations in serum and tissue lysate samples were determined by adding 0.1% N-(1-naphthyl) ethylenediamine dihydrochloride and 1% sulfanilamide to each serum or tissue lysate sample placed in 96-well microplates, and measuring absorbance at 540 nm using an optical microplate reader.