Effects of L-Arginine and L-NAME on Duodenal Histologic Parameters in Female Wistar Rats

Fatemeh Sabbaghziarani [1], Keywan Mortezaee [1], Golamreza Hassanzadeh [1], Seyed Mohammad Hossein Noori-Mugahi [1*]

1. Department of Anatomy, School of Medicine, Tehran University of Medical Sciences, Tehran, Iran.

Dr. Seyed Mohammad Hossein Noori-Mugahi is the professor of Anatomy Department in Tehran University of Medical Sciences. He teaches histology, and his research fields are nitric oxide, stereology, and medical herbs.

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ABSTRACT

Introduction: Nitric oxide (NO), as a free radical, involves in several physiologic functions in GI tract such as nerve impulse transmission and vascular tonicity regulation. Nitric oxide synthase (NOS) is the enzyme for the production of NO from L-Arginine which in turn inhibits by L-NG-Nitroarginine Methyl Ester (L-NAME). In the current work, we aimed to evaluate morphometric analysis of duodenum under exposure of L-Arginine and L-NAME in female Wistar rats.

Methods: In this study, 5 groups (N=8) of 40 female rats (200-250 g, 8 weeks age) were chosen. Normal saline (2 mL/kg), L-Arginine (200 mg/kg), L-NAME (20 mg/kg) and L-Arginine+L-NAME (with the same doses) were administered intraperitoneal — for 3 days. After 2 weeks, samples were collected, stained with hematoxylin and eosin (H&E) and observed under light microscopy. Duodenal epithelial cell height and number, gland diameter, and submucosal and muscular thicknesses were measured using optical software and analyzed by one-way ANOVA followed by Tukey's post hoc test using SPSS-16. $P \leq 0.05$ was considered statistically significant.

Results: There were no significant changes in mean variables compared to the control group.

Conclusion: The results attested no noticeable changes in regard with the effects of L-arginine and L-NAME on duodenum parameters despite the major roles of NO in GI tract.

1. Introduction

Nitric oxide, as a free radical, is a bio-signaling molecule [1, 2] that involve in many physiological and pathological processes owe to its key role in mammal cellular signaling [3]. Nitric oxide synthase (NOS) is an enzyme, produced naturally by different cell types, for NO synthesis as if three forms of which has been known: Inducible (iNOS), Endothelial (eNOS) and Neuronal (nNOS); all use L-arginine for NO production [4-6]. NOS has the capability of converting L-arginine into NO and L-Citrulline. L-arginine is also metabolized by arginase to form urea and L-ornithine, a precursor of the polyamines [7]. Reaction of NO with O_2 produce N_2O_3 which in turn cause DNA deamination [8], a potentially harmful process. Although NO in high quantity is cytotoxic, it may protect cells against oxidants [9-11]. A number of physiologic functions of NO in several parts of body such as GI tract are as follows: vascular tonosity regulation, nerve impulse transmission, platelet aggregation inhibitor, and body diffense mechanisms [12-14]. Inhibitory effects of NO on GI smooth muscle - and subsequently peristalsis - has been attested in

*** Corresponding Author:**
Seyed Mohammad Hossein Noori-Mugahi, PhD
Address: Department of Anatomy, School of Medicine, Tehran University of Medical Sciences, Tehran, Iran.
E-mail: noorimoo@gmail.com

Table 1. Comparison of the mean±SD of four variables among the study groups.

Groups \ Variable	Mean±SD					Significance
	Epithelial cells height (µm)	Epithelial cells number	Gland diameter (µm)	Submucosal thickness (µm)	Muscular thickness (µm)	
Control	42.23±12.45	66.60±6.30	162.86±26.29	378.85±23.85	336.56±52.70	NS
Normal saline	41.04±9.49	65.6±6.94	167.97±22.03	380.27±26.62	270.70±63	NS
L-Arginine	38.63±2.94	65.2±10.30	181.18±21.22	336.46±105.09	297.15±82.60	NS
L-NAME	48.19±2.26	70.20±4.38	165.27±3.41	453.09±50.76	323.33±90.79	NS
L-Arginine+ L-NAME	39.49±2.73	60.40±7.12	195.40±16.03	322.70±12.02	300.85±49.74	NS

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duodenum, ileum and colon [13]; additionally, it plays a crucial role in homeostasis maintenance through regulation of mucus secretion and mucosal integrity [15]. Gut epithelium, with the high regeneration capacity, is the first defense barrier against microorganisms, exogenous antigens and toxins [16, 17]. Regulatory competence of NO on the variety of cells within intestinal mucosa such as epithelial and endothelial has been certified [13, 14]. On the other hand, function of NO in intestinal disorders is dual: cytotoxic and protective (18). NG-Nitro-L-arginine methyl ester (L-NAME), as an inhibitor of NOS, induces intestinal mucosal damage [19], colonic [20] and duodenal muscle [21] contractions. Based on the mentioned research related to the role of NO in GI tract, we designed the current work for morphologic evaluation of L-Arginine and L-NAME effects on duodenal parameters in female wistar rats.

2. Materials & Methods

This study is an interventional experimental research. Forty female Wistar rats, weighing 200-250 gr with an average age of eight weeks were purchased from Laboratory Animal Center, Faculty of Pharmacy at Tehran University of Medical Sciences. In this study, animal care conditions were 12 h periods of light and darkness, 23±2ºC temperatures and enough food and water. All animal experiments were carried out according to the guidelines of the Iranian Council for Use and Care of Animals and were approved by the Animal Research Ethical Committee of Tehran University of Medical Sciences. Then, five rats were kept in each cage and assigned to the following groups: Control, Sham (2ml/kg normal saline), L-Arginine (200mg/kg), L-NAME (20 mg/kg), and combination of L-Arginine+L-NAME with the same dose (Sigma, Germany) via intra perito-

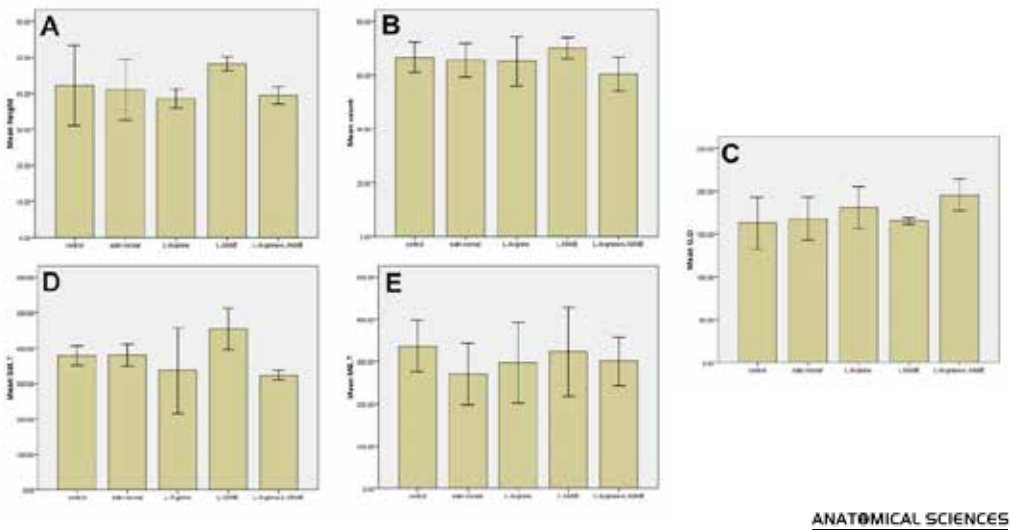

Figure 1. The mean value of different variables. A and B: Epithelial cell height and number, C: Gland diameter (G.D), D: Submucosal thickness (SM.T), and E: Muscular thickness (MS.T).

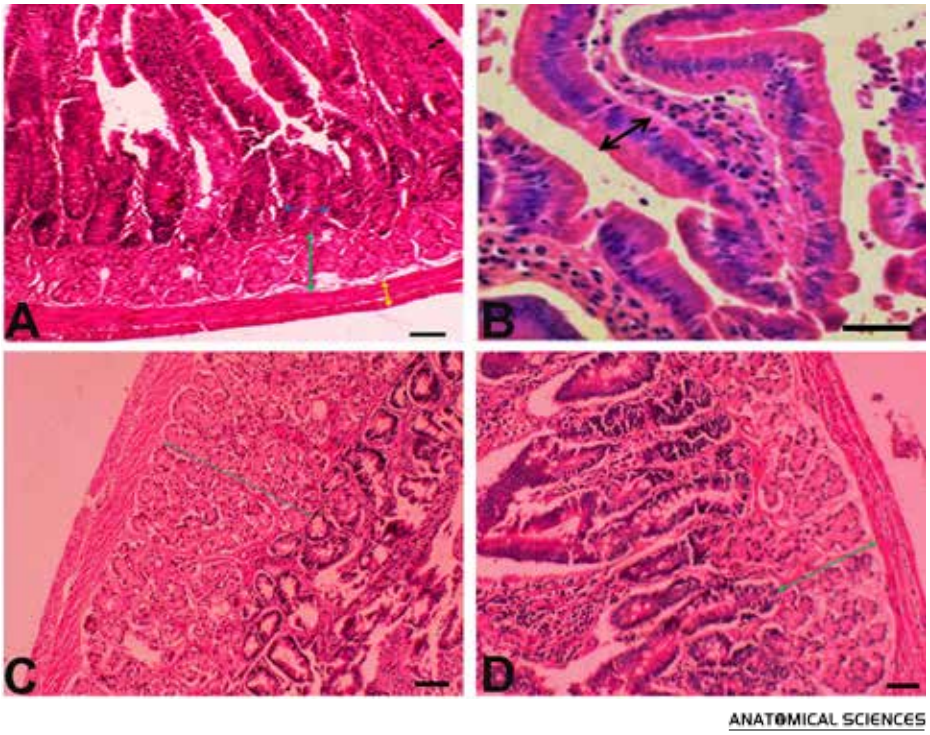

Figure 2. Conspicuous changes in transverse duodenal sections. A: Control; B and C: L-NAME, with the highest epithelial height and submucosal thickness respectively; and D: L-Arginine + L-NAME with the lowest submucosal thickness (H&E; Scale bar for A, C and D is 100µm and for B is 50 µm) – the black, blue, green, and yellow arrows were for epithelial cell height, gland diameter, submucosal thickness, and muscular thickness parameters respectively.

neal (IP) injection at days 3-5 after evaluation. On day 18, rats were anesthetized with ether. After abdominal opening, samples were harvested, fixed in 10% formalin, processed, and sectioned by microtome; then, slices 5, 8, 11, 14, and 17 (5 µm thickness) from each section were chosen, stained with H&E and investigated by a light microscope (Olympus CX31, Japan). Five parameters (epithelial cell height and number, gland diameter, and submucosal and muscular thicknesses) of the duodenum were measured via micrometric criteria by Image tool III software.

Statistical analysis was performed by one-way ANOVA followed by Turkey's post hoc test using SPSS 16 (Microsoft, IL, USA) to evaluate the level of significance between different groups. $P \leq 0.05$ was considered statistically significant.

3. Results

Table 1 provides information about the changes in the mean±standard deviation (SD) of five variables. As it could be understood from the data, there were not dramatic alterations in all groups as compared with control. The epithelial cell height and number and also submucosal thickness had the highest raise in L-NAME group with 48.19±2.26 µm, 70.20±4.38 µm and 453.09±50.76 µm respectively; as contrast, the epithelial cell height had the lowest rate in L-Arginine group with 38.63±2.94 µm. L-Arginine+L-NAME group had the lowest rate in epithelial cell number and submucosal thickness with the order of 60.40±7.12 µm and 322.70±12.02 µm, and the highest rate in gland diameter with 195.40±16.03 µm. Muscular thickness and gland diameter had the down and upward changes. On the other hand, there were no regular pattern in submucosal thickness and epithelial cell number in treatment groups, as compared with the control. Figures 1 (A-E), and Figure 2 also indicate duodenal wall characteristics in different groups.

4. Discussion

In the current work, the effect of L-arginine and L-NAME on duodenum features was analyzed, and there were no significant changes in mean variables including epithelial cell height and number, submucosal thickness, gland diameter, and muscular thickness in comparison to the control group; this results were consistent with our previous work on some colonic parameters such as mucosal thickness and gland diameter [23].

Clinical and experimental studies have reported various effects of NO and its precursor on GI tract. L-Arginine plays a major role in intestinal physiology. There is an evidence in favor of using dietary L-Arginine as a supplementary for having protective function on intestinal cells by contribution in repair of damaged cells and tissues through a mechanism mediated by NO and polyamines which in turn take part in growth, differentiation and function of intestinal mucosal cells. It has also attested that L-Arginine has the stimulatory effects on intestinal cell protein synthesis, and also the regulatory action in protein turnover which in turn is the basis for cell growth [23]. More recent study elicited a slight increase of protein synthesis and cell proliferation regarding with L-Arginine [4]. Human studies attested that although NO inhibition has no impacts on rectal motor and sensory nerves, it could increase the distal ileum and proximal colon motility. in the other work, NO administration - in the dose-dependent manner - could increase mucus gel thickness which in turn inhibit the probable toxic injuries; this function may be provide through maintaining mucosal blood flow and inhibiting leukocyte-endothelial cell interactions [22]. When we compare these results with ours, we could say that NO has different actions on various parts of GI tract and its functions might be explained by using differ doses and also time period.

Longitudinal muscle relaxation of duodenum through enteric nerve stimulation mediated solely by NO - as a nonadrenergic noncholinergic (NANC) inhibitory neurotransmitter - via cGMP dependent and - independent mechanisms according to the region and to the species; as it has been proven, NOS is present in GI track external muscle nerve fibers. On the other side of view, L-NAME has the capability to abolish NANC relaxation [24]; another function of L-NAME which has been demonstrate, is its action on cell proliferation reduction. Furthermore, increase in systemic blood pressure and a decrease in resting gastric mucosal blood flow is another action of L-NAME through down regulation of cGMP pathway resulting in leaker mucosal barrier [22]. in our current experiment, L-NAME had the highest rate for the three of the discussed variables - epithelial cell number, submucosal thickness and also epithelial cell height -; on the other hand, the first two variables, explained about L-NAME, had the lowest rate in L-arginine + L-NAME group although none of the results were significant; when we add all of these data together, we could say the different probable effects related to the actions of L-NAME on different parts of GI tract, and also when we referring to the low amount regarding to the L-arginine+L-NAME group, we could propose that there was the L-arginine can diminish the effects of L-NAME on duodenum.

To sum up, although NO has many different functions on GI tract, the resulting data revealed no remarkable effects for L-arginine and LNAME on the duodenal characteristics which in turn requires more works to be done to obtain more information.

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Authors' Contributions

With regard to the work design, statistical analysis, and manuscript writing, All authors had equal role.

Conflict of Interest

The authors declare no conflict of interest.

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