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ORIGINAL ARTICLE

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Parenteral arginine impairs intestinal adaptation following massive small bowel resection in a rat model

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Abstract The nitric oxide precursor L-arginine (ARG) has been shown to influence intestinal structure and absorptive function. It is also well known that the route of administration modulates the effects of ARG. The present study evaluated the effects of parenteral ARG on structural intestinal adaptation, cell proliferation, and apoptosis in a rat model of short bowel syndrome (SBS). Male Sprague-Dawley rats were divided into three experimental groups: Sham rats underwent bowel transection and reanastomosis, SBS rats underwent a 75% small bowel resection, and SBS-ARG rats underwent a 75% small bowel resection and were treated with ARG given subcutaneously at a dose of 300 μg/kg, once daily, from days 3 to 14. Parameters of intestinal adaptation, enterocyte proliferation, and enterocyte apoptosis were determined on day 15 following operation. The SBS rats demonstrated a significant increase in jejunal and ileal bowel and mucosal weight, villus height and crypt depth, and cell proliferation index compared with the sham group. The SBS-ARG animals demonstrated lower ileal bowel and mucosal weights, jejunal mucosal DNA and ileal mucosal protein, and jejunal and ileal villus height and crypt depth compared with SBS animals. The SBS-ARG rats also had a lower cell proliferation index in both jejunum and ileum and a greater enterocyte apoptotic index in ileum compared with the SBS-untreated group. In conclusion, in a rat model of SBS, parenteral arginine inhibits structural intestinal adaptation. Decreased cell proliferation and increased apoptosis are the main mechanisms responsible for decreased cell mass.

Keywords Short bowel syndrome · Intestinal adaptation · Arginine · Rat

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Introduction

Short bowel syndrome (SBS) is a clinical condition defined as a state of malnutrition and malabsorption of nutrients and micronutrients resulting from the surgical removal of small bowel and a decrease in the absorptive surface area [3, 4]. Despite a marked improvement in critical care and progress in long-term nutritional support, mortality and morbidity of SBS patients remain high [7]. The single most important factor contributing to individual outcome in patients with SBS is the capacity of the intestinal remnants to adapt [15]. Intestinal adaptation begins within 24–48 h after a bowel resection and includes structural and functional changes in the remaining gut. A large number of nutrients, hormones, and peptide growth factors are known to

stimulate intestinal adaptation. Luminal nutrients appear to be particularly important [24]. It has been reported that prolonged "bowel rest" together with total parenteral nutrition may impair intestinal adaptation and the ability of such patients to achieve nutritional autonomy. Therefore, once the patient stabilizes, slow introduction of enteral feeding is indicated. Dietary constituents may have profound positive or negative effects on intestinal adaptation and should be considered in developing an overall plan for treatment of patients suffering from SBS.

Arginine is a nonessential amino acid processed metabolically by the urea cycle. The physiological significance of arginine metabolism extends far beyond its incorporation as an amino acid into proteins. Arginine plays an important role in many physiologic and biologic processes, including release of several hormones, collagen synthesis during wound healing, immune response, tumor biology, and regulation of inflammation [14, 19]. L-arginine is converted to nitric oxide (NO) and citrulline by the enzyme nitric oxide synthase. NO is an important molecule involved in neurotransmission, vascular homeostasis, immune regulation, and host defense [1]. There is growing interest in the potential roles of arginine and NO as regulators of cell proliferation and apoptosis in general and in the gastrointestinal tract in particular. NO has been shown to promote apoptosis in some cells, whereas it inhibits apoptosis in other cells depending on the amount, duration, and site of NO production and the kind of target cells [6].

The role of arginine following massive small bowel resection is a subject of controversy. Exogenous arginine might stimulate mucosal growth after bowel resection through growth hormone release or production of polyamines. However, Welters et al. [10] have demonstrated that parenteral arginine supplementation in a rat with SBS led to a slowing of the adaptive response, as shown by a decreased intestinal glutamine uptake and decreased protein synthesis. However, morphological adaptation was not examined in this study. A recent study performed in our laboratory showed that arginine-supplemented diets inhibited structural intestinal adaptation in a rat model of SBS [21].

The purpose of the present study was to evaluate the effects of parenteral supplementation with arginine on structural intestinal adaptation, enterocyte proliferation, and apoptosis following massive bowel resection in a rat model.

Materials and methods

Surgical procedures and animal care were conducted in compliance with the guidelines established by the "Guide for the Care and Use of Laboratory Animals," Rappaport Faculty of Medicine, Technion (Haifa, Israel).

Male Sprague-Dawley rats weighing 250–280 g were divided randomly into three experimental groups of 15 rats each. Group A rats underwent bowel transection and reanastomosis (sham rats), group B animals underwent 75% bowel resection (SBS rats), and group C underwent bowel resection and were treated with ARG given subcutaneously at a dose of 300 μg/kg, once daily, from postoperative days 3 to 14 (SBS-ARG rats). Because the first 2 days are critical for the animals' postoperative recovery, treatment with arginine was delayed until the 3rd postoperative day. The dosage of arginine was chosen in accordance with previously described studies [10, 16].

The animals were housed under standardized conditions (12-h light-dark cycle, controlled room temperature) for 5–7 days. Following an overnight fast, the animals were anesthetized with an intraperitoneal injection of pentobarbital (45 mg/kg). The abdomen was opened through a midline incision. In resected animals, the proximal small bowel was resected from a point 5 cm distal to the ligament of Treitz to a point 10 cm proximal to the ileocecal junction (the standard model of a 75% intestinal resection). The proximal jejunum was anastomosed to the remaining ileum in continuity using interrupted 5-0 silk. Sham animals underwent laparotomy, bowel transection, and reanastomosis 10 cm proximal to the ileocecal junction.

All animals were killed on the 15th postoperative day. The small intestine from the pylorus to the ileocecal valve was removed and divided at the anastomosis. Portions of intestine 1 cm on either side of the anastomosis were discarded because of the surgically-induced hyperplasia occurring in the perianastomotic region. The intestine was split on the antimesenteric border, washed with cold saline, and dried, and each segment was weighed. The mucosa was scraped from the underlying tissue with a glass slide. Mucosal samples were homogenized with TRIzol reagent. The DNA and protein were extracted by the method of Chomczynski [5] and were expressed as micrograms per centimeter of bowel per 100 g of body weight.

Histology

Intestinal samples from the proximal jejunum and distal ileum were fixed in 10% formalin, dehydrated in progressive concentrations of ethanol, cleared in xylene, and embedded in paraffin wax. Deparaffinized 5-μm sections were stained with hematoxylin and eosin. Villus height and crypt depth were measured using a graded eye piece at 10-times magnification by a pathologist blinded as to the tissue origin.

Enterocyte proliferation and apoptosis

Portions of the proximal jejunum and distal ileum were taken as described above. Crypt cell proliferation was

assessed using 5-bromodeoxyuridine (5-BrdU). Standard BrdU labeling reagent (Zymed Laboratories, San Francisco, USA) was injected intraperitoneally at a concentration of 1 ml/100 g body weight 2 h before sacrifice. Tissue slices (5 μm) were stained with a biotinylated monoclonal anti-BrdU antibody system provided in kit form (Zymed Laboratories, San Francisco, USA). An index of proliferation was determined as the ratio of crypt cells staining positively for BrdU per 10 crypts.

Additional 5-μm thick sections were prepared to establish the degree of enterocyte apoptosis. The TUNEL assay for apoptotic cell detection was performed using the In Situ Cell Death Detection kit (Boehringer Mannheim, Germany). The paraffin-embedded sections were briefly dewaxed and rehydrated with xylene and graded alcohol. Tissue sections were microwave-pretreated in 10 mM citrate buffer (pH 6.0) and incubated with TUNEL reaction mixture containing nucleotide mixture with fluorescein-labeled deoxy-UTP and TdT at 37°C for 60 min. After incubation with blocking solution at room temperature for 30 min, the sections were incubated with Converted-POD at 37°C for 30 min. TUNEL-positive color development was obtained by incubating the sections with AES substrate (Zymed Laboratories, San Francisco, USA). The apoptotic index (AI) was defined as the number of apoptotic TUNEL-positive cells per 10 villi.

A qualified pathologist blinded as to the source of intestinal tissue performed all measurements.

Statistical analysis

The data are expressed as mean ± SEM. Statistical analysis of parameters of adaptation, enterocyte proliferation, and apoptosis was performed using the nonparametric Kruskal–Wallis ANOVA test followed by the corrected Mann–Whitney test, with $p < 0.05$ considered statistically significant.

Results

Body weight changes

There were no perioperative problems in group A (sham), and after an initial weight loss, animals regained their preoperative weights by the 7th postoperative day. The SBS rats (group B) grew slower than sham animals from days 3 to 15 and had significantly lower final body weights. Rats receiving arginine demonstrated an additional decrease in body weight from days 7 to 15 compared with SBS-untreated animals (Fig. 1).

Parameters of intestinal adaptation

Adaptation in the residual bowel in the resected rats (group B) was manifested by a significant increase in bowel and mucosal weight in jejunum and ileum (Fig. 2), mucosal DNA and protein in jejunum and ileum (Fig. 3), villus height in jejunum and ileum, and crypt depth in ileum (Fig. 4) compared with sham animals (group A). The reported changes are consistent with results of our previous studies [23–25] and with data presented by other investigators [9–11]. Following parenteral arginine administration (group C), SBS rats demonstrated a significant decrease in ileal overall bowel

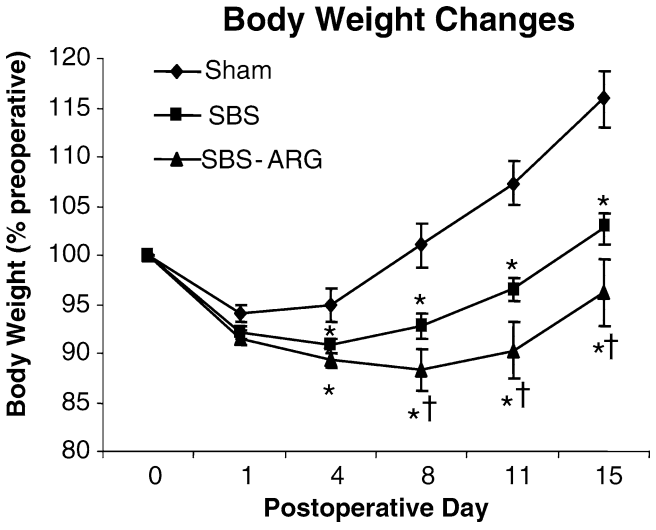

Fig. 1 Body weight changes expressed as percent of preoperative weight (mean ± SEM) in control (*sham*) and untreated resected rats (*SBS*) or SBS rats treated with parenteral arginine. (*SBS* short bowel syndrome rats, *ARG* arginine, *$p < 0.05$ SBS vs. sham rats, †$p < 0.05$ SBS–ARG vs. SBS rats)

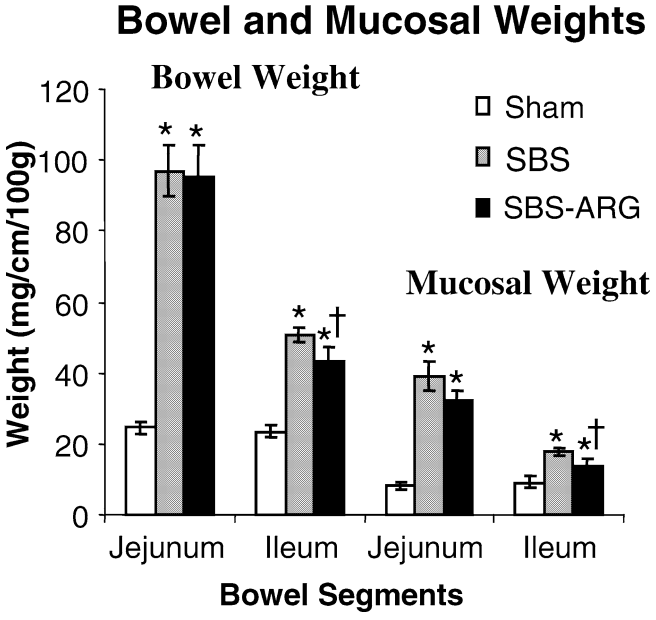

Fig. 2 Effect of bowel resection and parenteral arginine on the macroscopic appearance of the remaining small intestine. Values are mean ± SEM. (*SBS* short bowel syndrome rats, *ARG* arginine, *$p < 0.05$ SBS vs. sham rats, †$p < 0.05$ SBS–ARG vs. SBS rats)

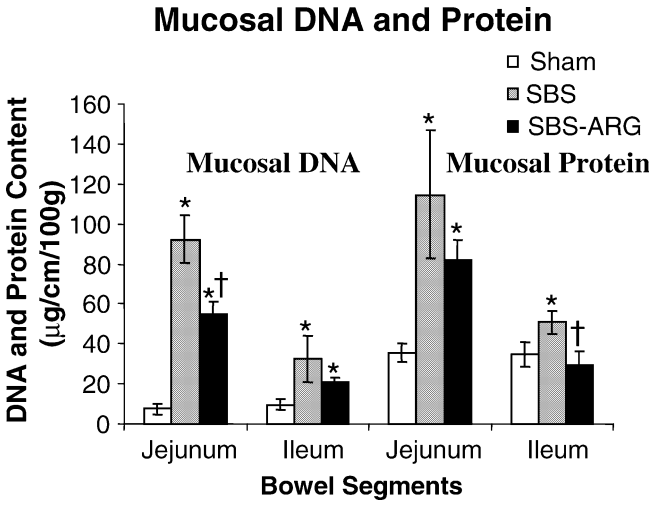

Fig. 3 Effect of bowel resection and parenteral arginine on mucosal DNA and protein content. Values are mean ± SEM. (*SBS* short bowel syndrome rats, *ARG* arginine, *$p < 0.05$ SBS vs. sham rats, †$p < 0.05$ SBS–ARG vs. SBS rats)

(43 ± 4 vs. 51 ± 2, $p < 0.05$) and mucosal weight (14 ± 2 vs. 18 ± 1, $p < 0.05$), mucosal DNA content in jejunum (55 ± 7 vs. 92 ± 12 μg/cm/100 g, $p < 0.05$) and protein content in ileum (30 ± 6 vs. 51 ± 6 μg/cm/100 g, $p < 0.05$), villus height in jejunum (712 ± 128 vs. 1009 ± 64 μm, $p < 0.05$) and ileum (493 ± 58 vs. 622 ± 65 μm, $p < 0.05$), and crypt depth in jejunum (167 ± 2 vs. 235 ± 25 μm, $p < 0.05$) and ileum (159 ± 13 vs. 201 ± 14 μm, $p < 0.05$) compared with SBS-untreated animals (group B).

Cellular proliferation and apoptosis

Figure 5 demonstrates changes in enterocyte proliferation and enterocyte apoptosis following bowel resection and exposure to parenteral arginine. As expected, a significant increase in enterocyte proliferation occurred in both jejunum (61%, $p < 0.05$) and ileum (51%, $p < 0.05$) in rats following massive bowel resection compared with sham animals. The SBS rats treated with parenteral arginine demonstrated a significant decrease in cell proliferation index in jejunum (45%, $p < 0.05$) and ileum (60%, $p < 0.05$) compared with SBS-untreated animals.

Evaluation of enterocyte apoptosis showed a significant increase in AI in jejunum (twofold, $p < 0.05$) after massive small bowel resection compared with sham animals. Administration of parenteral arginine resulted in a significant increase in cell apoptosis in ileum (43 ± 11 vs. 23 ± 23 TUNEL-positive cells per 10 villi, $p < 0.05$) compared with SBS-untreated animals. The SBS-ARG rats also demonstrated a significantly greater AI in jejunum and ileum compared with sham animals.

Discussion

After massive small bowel resection, the remaining gut undergoes compensatory structural and functional changes to compensate for the loss of surface area. Morphological changes include villus and crypt hyperplasia, increased enterocyte proliferation, and increased migration of enterocytes along the villi. At a functional level, the active nutrient transport by isolated enterocytes increases in parallel with the increase in mucosal mass. In recent decades research has focused on identifying numerous factors that stimulate epithelial cell proliferation and differentiation and that may potentially have a therapeutic role in treating patients suffering from SBS.

Arginine (2-amino-5-guanidinovaleric acid) is a nonessential amino acid that is metabolically processed by the urea cycle; it is one of the most versatile amino acids in animal cells [14]. It was first isolated from lupin seedlings in 1886 and subsequently was found to be a

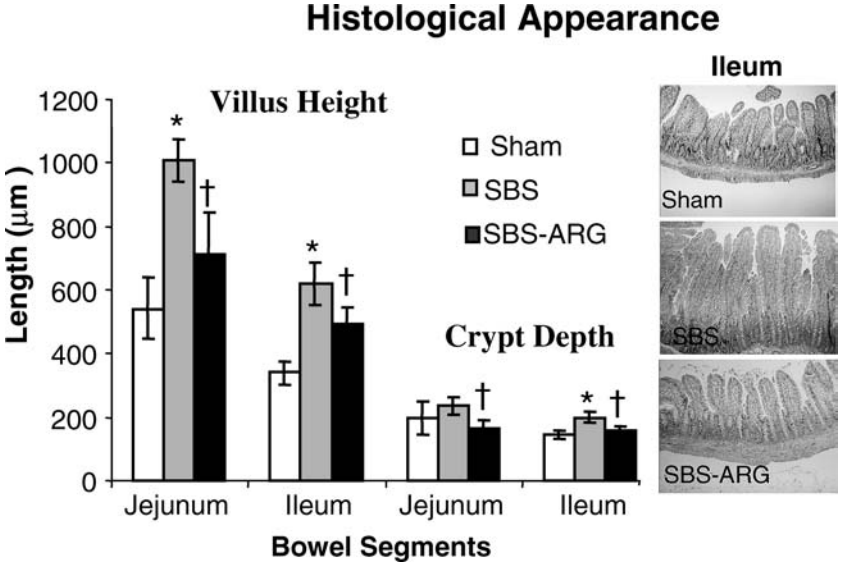

Fig. 4 Effect of bowel resection and parenteral arginine on the microscopic appearance of the remaining small intestine. Values are mean ± SEM. (*SBS* short bowel syndrome rats, *ARG* arginine, *$p < 0.05$ SBS vs. sham rats, †$p < 0.05$ SBS–ARG vs. SBS rats)

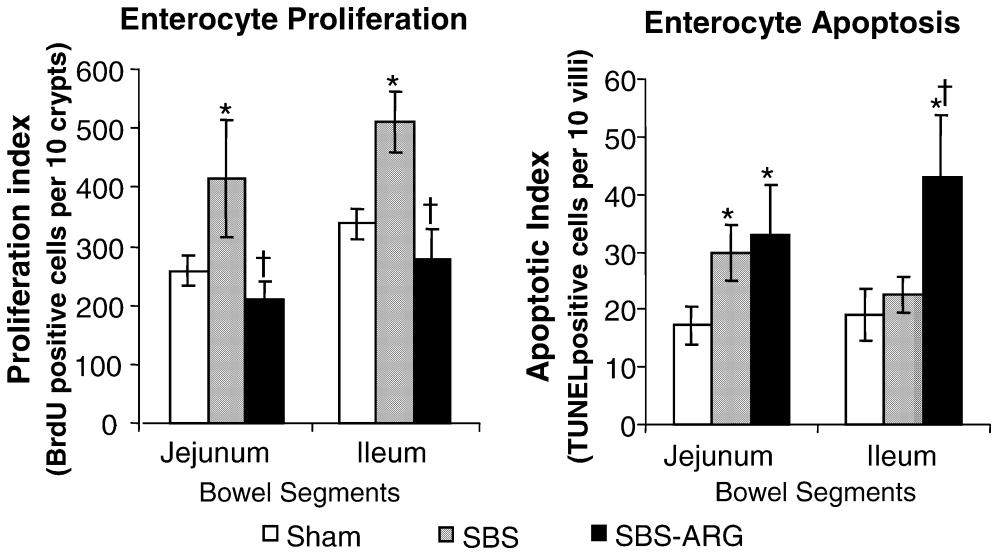

Fig. 5 Effect of bowel resection and parenteral arginine on crypt cell proliferation and enterocyte apoptosis in jejunum and ileum. 5-BrdU incorporation into proliferating jejunal and ileal crypt cells was detected with a goat anti-BrdU antibody, and TUNEL assay was used to determine enterocyte apoptosis. Values are mean ± SEM. (*SBS* short bowel syndrome rats, *ARG* arginine, $*p < 0.05$ SBS vs. sham rats, $\dagger p < 0.05$ SBS–ARG vs. SBS rats)

major amino acid in the basic proteins of many mammals' cells and tissues [25]. Physiological and nutritional studies during the last 70 years started a new area of arginine research. It has been shown that arginine is required for the synthesis of NO, polyamines, proline, glutamate, and creatinine [8]. Arginine is classified as a dispensable (nonessential) amino acid for healthy adult humans and as an essential amino acid for young, growing mammals [18]. Arginine has been shown to influence metabolism in mammalian cells directly or through stimulation of the secretion of hormones such as insulin, growth hormone, glucagon, and prolactin.

In the last two decades, arginine has attracted major interest since it has been identified as the natural substrate of NO and is now recognized to play a major role in many regulation processes [1]. Arginine and NO are critical to the normal physiology of the gastrointestinal tract. Pretreatment with L-arginine ameliorates survival and improves mucosal barrier function after intestinal ischemia-reperfusion injury in rats [17]. Dietary supplementation with arginine accelerates ulcer healing in experimental ulcerative ileitis [23] and stimulates small intestinal mucosal recovery following experimental radiation enteritis [12]. There are few and controversial studies concerning the effects of arginine supplementation on intestinal adaptation in experimental models of SBS. It has been reported that ornithine alpha-ketoglutarate, which is a precursor of arginine, enhances intestinal adaptation after massive resection in the rat and improves muscle glutamine and protein content [11]. Hebiguchi and colleagues reported that arginine supplementation in the diet resulted in a significant increase in ileal villus height compared with control animals; they concluded that arginine stimulates mucosal growth after massive bowel resection, probably through stimulation of growth hormone secretion [13]. Arginine may also influence intestinal adaptation through production of polyamine [9] or NO. There is growing evidence suggesting the central importance of apoptosis in controlling the enterocyte mass following massive small bowel resection. Therefore, alteration in enterocyte apoptosis through production of NO may be considered an additional mechanism by which arginine may affect postresection bowel growth.

In the present study, we evaluated the effect of parenteral arginine supplementation on morphological intestinal adaptation following massive bowel resection in rats. We used a 75% bowel resection model and studied the adaptive response in the remaining jejunum and ileum. Short-bowel animals demonstrated increased bowel and mucosal weight, increased mucosal DNA and protein, lengthening of the villi, and deepening of the crypts, reflecting a promote adaptive response. These changes have been previously described in the small intestine by many investigators studying the morphological changes following massive bowel resection. Observed increase in the enterocyte proliferation rate may be considered a main mechanism responsible for compensatory hyperplasia and the resultant increased intestinal cell mass.

Unlike our previously reported results [20, 22], apoptosis increased in jejunum but did not change significantly in ileum, which may be considered a mechanism for maintaining mucosal structure following bowel resection. The present study demonstrates that parenteral arginine inhibits structural intestinal adaptation following massive small bowel resection in rats. This conclusion is supported by the observed decrease in mucosal weight of the remnant bowel. Reduced mucosal DNA and protein suggest decreased cell metabolism, which is consistent with the reduced epithelial cell proliferation and the decrease in villus height and crypt depth, all of which are important indices of decreased absorptive surface area in this group of animals. The most significant differences were observed in the terminal ileum, since hyperplasia in proximal jejunum was less prominent. This experiment does not address whether impaired structure is associated with impaired function.

However, decreased absorptive surface area is supposed to be accompanied by decreased nutrient absorption. Decreased body weight in rats receiving arginine may suggest a malnutrition state. These data are consistent with our previous experimental work, which showed an inhibitory effect of enteral arginine on morphological intestinal adaptation [21]. This impaired adaptive response was accompanied by a decrease in enterocyte proliferation and increased enterocyte loss via apoptosis. It is not clear whether the increased apoptosis reflects increased enterocyte mass or represents an adaptive mechanism that maintains cellular balance.

In summary, in a rat model of SBS, parenteral arginine administration inhibits intestinal adaptation. Possible mechanisms may include decreased enterocyte proliferation and increased enterocyte loss via apoptosis.

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