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Original

Microbiota benefits after inulin and partially hydrolyzed guar gum supplementation – a randomized clinical trial in constipated women

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Abstract

Introduction: Prebiotics positively affect gut microbiota composition, thus improving gut function. These properties may be useful for the treatment of constipation.

Objectives: This study assessed the tolerance and effectiveness of a prebiotic inulin/partially hydrolyzed guar gum mixture (I-PHGG) for the treatment of constipation in females, as well as its influence on the composition of intestinal microbiota and production of short chain fatty acids.

Methods: Our study enrolled 60 constipated female health worker volunteers. Participants reported less than 3 bowel movements per week. Volunteers were randomized to treatment with prebiotic or placebo. Treatment consisted of 3 weeks supplementation with 15 g/d I-PHGG (fiber group) or maltodextrin (placebo group). Abdominal discomfort, flatulence, stool consistency, and bowel movements were evaluated by a recorded daily questionnaire and a weekly interview. Changes in fecal bacterial population and short chain fatty acids were assessed by real-time PCR and gas chromatography, respectively.

Results: There was an increased frequency of weekly bowel movements and patient satisfaction in both the fiber and placebo groups with no significant differences. Total *Clostridium* sp significantly decreased in the fiber group ($p = 0.046$) and increased in the placebo group ($p = 0.047$). There were no changes in fecal short chain fatty acid profile.

Conclusions: Consumption of I-PHGG produced clinical results comparable to placebo in constipated females, but had additional protective effects on gut microbiota by decreasing the amount of pathological bacteria of the *Clostridium* genera.

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Key words: Guar gum. Inulin. Gut microbiota. Short-chain fatty acids. Constipation.

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BENEFICIOS EN LA MICROBIOTA INTESTINAL DESPUÉS DE LA SUPLEMENTACIÓN CON INULINA Y LA GOMA GUAR PARCIALMENTE HIDROLIZADA – UN ENSAYO CLÍNICO ALEATORIZADO EN MUJERES CON ESTREÑIMIENTO

Resumen

Introducción: Los prebióticos influyen positivamente en la composición de la microbiota intestinal, mejorando así la función intestinal. Estas propiedades pueden ser útiles para el tratamiento del estreñimiento.

Objetivos: Este estudio evaluó la tolerancia y la eficacia de una mezcla de prebiótico inulina con la goma guar parcialmente hidrolizada (I-PHGG) para el tratamiento de mujeres con estreñimiento, así como su influencia en la composición de la microbiota intestinal y la producción de ácidos grasos de cadena corta.

Métodos: Nuestro estudio contó con la participación de 60 mujeres voluntarias con estreñimiento y profesionales de la salud. Las participantes informaron tener menos de tres evacuaciones por semana y fueron asignadas aleatoriamente a tratamiento con prebióticos o placebo. El tratamiento consistió en 3 semanas de suplementación con 15 g/d I-PHGG (grupo de fibras) o maltodextrina (grupo placebo). Malestar abdominal, flatulencia, consistencia de las heces, y los movimientos intestinales se evaluaron mediante un cuestionario de registro diario y una entrevista semanal. Cambios en la población de bacterias fecales y los ácidos grasos de cadena corta fueron evaluados por PCR en tiempo real y cromatografía de gases, respectivamente.

Resultados: Hubo un aumento en la frecuencia de las evacuaciones intestinales por semana y la satisfacción del paciente, tanto en la fibra y el grupo placebo, sin diferencias significativas. El total de *Clostridium* sp disminuyó significativamente en el grupo de fibras ($p = 0,046$) y aumentó en el grupo placebo ($p = 0,047$). No hubo cambios en el perfil fecal de ácidos grasos de cadena corta.

Conclusiones: El consumo de I-PHGG ha producido resultados clínicos comparables a placebo en mujeres con estreñimiento, pero ofreció otros efectos protectores sobre la microbiota intestinal al disminuir la cantidad de bacterias patológicas de lo género *Clostridium*.

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Palabras clave: Goma guar. Inulina. Microbiota intestinal. Ácidos grasos de cadena corta. Estreñimiento.

Introduction

Constipation is a very common condition in the general population, both in adults and children.¹ According to a study in the United States, the prevalence of constipation in adults is 2-28% and is higher among females.^{2,3} Constipation uses significant healthcare resources and its control is a target for reducing healthcare expenditures.

Among the causes for constipation in adults is inadequate dietary fiber intake.⁴ Insoluble fibers, such as bran, have been traditionally used to control adult constipation with satisfactory results.⁵⁻⁷ However, soluble and prebiotic fibers have several advantages and have also been studied for constipation control.^{8,9}

The concept of prebiotics as “a non-digestible food ingredient that beneficially affects the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon” arose from the observation that inulin and fructooligosaccharides selectively stimulate the growth of bifidobacteria, which are considered beneficial for human health.⁸⁻¹²

If prebiotics modulate gut microbiota, they might also influence gut function by reducing intestinal pH, increasing stool weight and bowel movements, and exerting an osmotic effect that increases water content in the colon. These properties may be useful for the treatment of constipation.¹³

Dietary fiber and non-digestible oligosaccharides are the main growth substrates of gut microorganisms¹⁴ As bacterial metabolism in the human colon is primarily anaerobic, these substrates are fermented and can potentially form short chain fatty acids (SCFA) that serve as local fuels and may regulate cellular processes.^{12,15}

Partially hydrolyzed guar gum (PHGG) is a water soluble and non-gelling fiber associated with reduced constipation in females and relief of abdominal pain in patients with irritable bowel syndrome.^{16,17} Currently, there is available a mixture of fibers that combines PHGG with the prebiotic activity of inulin. The mixture of these two fibers could maximize their individual effects and provide benefits for health and bowel function, growth of beneficial bacteria, and increased fermentation activity in the intestine. Together, these properties may be beneficial for constipated patients.

This study evaluated the efficacy and tolerance of the combination of oral inulin with PHGG as prebiotics in female patients with constipation. We also analyzed its influence on the composition of intestinal microbiota and production of SCFA.

Patients and methods

Ethical issues

The current study was carried out following the ethical recommendations of the Declaration of Helsinki and the Ethical Committees “Comitê de Ética em Pesquisa do Hospital das Clínicas de São Paulo” and

“Comitê de Ética do hospital São Joaquim and Hospital São José da Real e Benemerita”, which approved the study protocol. All subjects gave written informed consent and were selected and followed by a research team at Ganep at Hospital da Real e Benemerita Associação Portuguesa de Beneficência de São Paulo.

Subjects

Adult (18-65 years) female volunteers (n = 60) with at least 3 months of constipation defined as less than 3 bowel movements per week were recruited among health workers from the Hospital da Real e Benemerita Beneficência Portuguesa de São Paulo. None of the recruits used laxatives or enemas. Criteria for exclusion were constipation secondary to pharmacological effects, use of drugs that influence intestine motility, narcotic or alcohol use, being pregnant or lactating, lactose intolerance, habitual consumption of lactic acid bacteria-containing food or prebiotics, any historical or current diagnosis of large bowel disease such as inflammatory bowel disease and colon cancer, and history of neurologic disorder.

Experimental Design

This study was a randomized, double-blind, placebo controlled trial. After four days of treatment adaptation with increased consumption of fiber or placebo, treatment consisted of three weeks supplementation with 15 g/d of a mixture of inulin and PHGG (fiber group) or 15 g/d of maltodextrin (placebo group) divided in three sachets (5 g each). Subjects were required to record daily the sachet consumption and advised to maintain their daily physical activity, life style, and sleeping habits.

Gastrointestinal response including abdominal distension and pain, flatulence, stool consistency (classified according to Lewis 1997), and bowel movement were also recorded daily.¹⁸

Fresh stools were collected individually in plastic bags and immediately sent to laboratory in an ice bucket to be weighed and blended, fractionated, and stored for further determination of the fecal microbiota and SCFA content. All laboratory procedures were performed by researchers at the Metanutri team from the Department of Gastroenterology of the University of São Paulo Medical School.

Efficacy of treatment

The efficacy and tolerance of treatment were based on self-recording of gastrointestinal response and a weekly interview. At the interview, physicians and dieticians evaluated the frequency of bowel movement and subject satisfaction in relation to bowel movements.

Table I

List of genera of bacteria from the gut analyzed in our study and, their respective controls and primers used to the analysis

Genera	Control strain	Reference n°	Primers (forward and reverse)
<i>Bifidobacterium sp</i>	<i>B. longum</i>	ATCC 15707	F:5' -CTC CTG GAA ACG GGT GG-3' R:5' -GGT GTT CTT CCC GAT ATC TAC A-3'
<i>Lactobacillus sp</i>	<i>L.acidophilus</i>	ATCC 4356	F:5' -AGC AGT AGG GAA TCT TCC A-3' R:5' -CAC CGC TAC ACA TGG AG-3'
<i>Bacteroides sp</i>	<i>B. fragiles</i>	ATCC 43859	F:5' - TAGTTTGTTGGCGGGGTAAC -3' R:5' - GGCTGGTTCAGACTCTCGTC -3'
<i>Clostridium sp</i>	<i>C.perfringens</i>	ATCC 13124	F:5' -GTG AAA TGC GTA GAG ATT AGG AA-3' R:5' -GAT YYG CGA TTA CTA GYA ACT C-3'
<i>Escherichia coli</i>	<i>E. coli</i>	ATCC 25922	F:5' GTT AAT ACC TTT GCT CAT TGA 3' R:5' ACC AGG GTA TCT AAT CCT GTT 3'

ATCC: American Type Culture Collection.

Quantification of fecal bacteria

We studied the non-pathogenic bacteria of the genera *Bifidobacterium* and *Lactobacillus* and the pathogenic bacteria of the genera *Bacteroides* and *Clostridium* and the species *Escherichia coli*. Bacterial DNA was extracted and purified from stool samples (200mg) using the kit Qiamp DNA Stool Mini Kit (Qiagen, USA). Its integrity was determined on 2% agarose gel and its concentration in a spectrophotometer (Nanodrop, USA). DNA samples remained at -20°C freezer for later quantitative analysis by real-time polymerase chain reaction (real-time PCR). Total DNA of bacteria strains cultured in blood agar at 37°C and under anaerobic conditions (table I) was extracted and purified using the gDNA mini ChargeSwict bacteria (Invitrogen, USA) to be used as positive control of real-time PCR.

Changes in fecal bacterial population were assessed using real-time PCR through the absolute quantification of bacterial 16S ribosomal DNA (rDNA) genes by using specific primers to the 16S site of ribosomal DNA (Table 1). The number of genes copies was determined using Platinum® SYBR Green qPCR Supermix UDG (Invitrogen, USA) and the reactions were developed according to the manufacturer's protocol. In absolute quantification, the number of interest genes copies was determined based on the number of copies of curve pattern constructed by cloning into plasmid DNA. Cloning of 16S rDNA of different genera and species of studied bacteria was performed following the protocol of the kit TOPO TA Cloning Kit for Sequencing (Invitrogen®, USA). After cloning, the DNA plasmid containing the cloned 16S rDNA was extracted and purified. Quantification of plasmid DNA was determined using Nanodrop ND-1000 spectrophotometer. The concentration found was used to determine the calculation of molecules/mL. The plasmid DNA was stored at -20° C and was used to determine the standard curve of real-time PCR by serial dilution of different genes of interest.

Fecal SCFA Content

The quantification of fecal SCFA (acetic, propionic, isobutyric, butyric, isovaleric, and valeric acid) was developed by gas chromatography. Fecal samples (1 g) were diluted in water at 4°C and mixed for 5 minutes. The suspension was adjusted to a pH of 2 to 3 with 5M hydrochloric acid and the sample was centrifuged for 20 minutes at 5,000 rpm and 4°C. After centrifugation, the supernatant was collected and analyzed using a gas chromatograph (GC-Shimatzu model 2010F, Japan) equipped with auto injector (Shimadzu Model AOC-20i, Japan), split/splitless injector (Shimadzu model SPL-2010, Japan), flame ionization detector (FID-2010 model Shimadzu, Japan), and Cilicia capillary column (30 m x 0.25 mm internal diameter and 0.25 mm in film; Nukol, 30 m x 0.25 mm ID, 0.25 µm film- Supelco, Japan). A pattern of SCFA (volatile acid standard mix-Supelco, Japan) was used to enable the identification and analysis of the peaks generated by gas chromatography. The concentrations of various SCFA by analysis by gas chromatography were corrected in relation to dry weight of the samples. The initial temperature of the oven was 100°C and this was raised to 185°C at 4°C/min. The temperature of the injection port and detector was 240°C and 250°C, respectively. The flow rate of carrier gas (H2) was adjusted to split injection.

Statistical analysis

Quantitative data from bowel microbiota and SCFA were evaluated on a log basis using the Student T test to compare T0 (before treatment) and T1 (after treatment) within and between treatment groups (fiber and placebo). The variation between T0 and T1 was also compared between groups using the Wilcoxon test. The frequency of bowel movement and subject satisfaction were evaluated by Fisher exact test for each evaluated period (weekly) and by qui-quadrante for total studied period (3 weeks). For all comparisons p ≤ 0.05 was considered statistically significant.

Table II
Descriptive data of patients distributed in fiber and placebo groups

Group	Age (y)	BW (kg)	BH (cm)	BMI (kg/m ²)	SBP (mmHg)	DBP (mmHg)	HR (b/min)	T (C°)
Fiber	36.1	63.7	160.5	24.75	117.6	79.2	83.1	36.1
Placebo	40.2	66.0	160.6	25.45	117.0	77.4	81.4	36.2
p value	0.2772	0.8995	0.9641	0.8919	0.9429	0.4985	0.3935	0.2902

BW: Body weight; BH: Body height; BMI: Body mass index; SBP: Systolic blood pressure; DBP: Diastolic blood pressure; T: Temperature. Student *t* test.

RESULTS

Patients

Sixty constipated woman were enrolled (fiber *n* = 28; placebo *n* = 32); 2 of them left voluntarily in the study (both from fiber group) and 12 were excluded due to loss of follow up (fiber *n* = 4; placebo *n* = 8). Randomization was considered adequate since no differences in relation to the means of descriptive characteristics ($p \geq 0.2772$, table II) were found between groups. All enrolled constipated woman were clinically followed and 32 of them submitted to fecal quantification of differential bacteria and short-chain fatty acids (fiber group *n* = 14; placebo group *n* = 18).

Fecal bacteria

By comparing selective bowel bacteria before and after treatment, the fiber group decreased and placebo group increased the amounts of total *Clostridium* sp; these differences between groups were significant (table III). We did not find differences in the amounts of fecal *Bifidobacterium*, *Lactobacillus*, *Bacteroides*, or *Escherichia coli* (table IV) between the groups and studied periods.

SCFA fecal concentration

The mean of the absolute amount of fecal acetic, isobutyric, butyric, and valeric acids did not differed between fiber and placebo groups in any evaluated period (table IV). Fecal isovaleric acid increased in placebo group (before *versus* after treatment), but this difference was not significant between the treatments (placebo *versus* fiber) (table IV).

Treatment efficacy

There was an increased frequency of weekly bowel movements and patient satisfaction in both treatment groups, with no differences between groups (tables V and VI, respectively).

Discussion

Our trial shows that a fiber mixture of inulin with agar gum can benefit the treatment of female constipation by reducing the amount of pathogenic bacteria *Clostridium* sp.

Among the 100 bacterial species included in the *Clostridium* genera there are several important human

Table III
Fecal volume of selective bacteria (cells/ul) in constipated women treated with fiber or placebo

Bacteria sp	Treatment	Mean \pm standard deviation		p1 value	p2 value (BT; AT)
		BT	AT		
<i>Bifidobacterium</i>	Fiber	6.63 \pm 0.97	6.80 \pm 1.28	0.405	(0.378; 0.644)
	Placebo	6.32 \pm 0.93	6.67 \pm 0.48	0.053	
<i>Lactobacillus</i>	Fiber	4.92 \pm 1.17	4.88 \pm 1.15	0.876	(0.092; 0.285)
	Placebo	4.24 \pm 1.04	4.49 \pm 0.88	0.233	
<i>Bacteroides</i>	Fiber	10.10 \pm 0.45	10.38 \pm 0.41	0.158	(0.717; 0.292)
	Placebo	9.98 \pm 1.15	10.20 \pm 0.54	0.494	
<i>Clostridium</i>	Fiber	5.23 \pm 0.67	4.76 \pm 0.92	0.046*	(0.769; 0.045*)
	Placebo	5.14 \pm 0.92	5.50 \pm 0.91	0.047*	
<i>Escherichia</i>	Fiber	5.70 \pm 1.27	5.54 \pm 1.46	0.592	(0.531; 0.410)
	Placebo	5.41 \pm 1.29	5.11 \pm 1.41	0.183	

p1 = Before treatment (BT) *versus* after treatment (AT) within groups.

p2 = Fiber *versus* placebo in both studied periods (BT and AT).

Table IV
Fecal SCFA content (area) of constipated women treated with fiber of placebo

SCFA	Treatment	Mean \pm standard deviation		p1 value	p2 value (BT; AT)
		BT	AT		
Acetic	Fiber	5.96 \pm 0.85	5.86 \pm 0.43	0.666	(0.530; 0.860)
	Placebo	5.81 \pm 0.46	5.88 \pm 0.41	0.328	
Isobutyric	Fiber	4.97 \pm 0.42	4.92 \pm 0.61	0.581	(0.133; 0.762)
	Placebo	4.65 \pm 0.73	4.81 \pm 0.61	0.077	
Butyric	Fiber	5.65 \pm 0.87	5.42 \pm 0.63	0.315	(0.295; 0.999)
	Placebo	5.35 \pm 0.74	5.42 \pm 0.65	0.478	
Isovaleric	Fiber	5.20 \pm 0.59	5.06 \pm 0.78	0.131	(0.355; 0.682)
	Placebo	4.99 \pm 0.73	5.14 \pm 0.65	0.031*	
Valeric	Fiber	5.21 \pm 0.77	4.95 \pm 0.67	0.200	(0.215; 0.912)
	Placebo	4.90 \pm 0.66	4.98 \pm 0.5673	0.258	

p1 = Before treatment (BT) versus after treatment (AT) within groups.
p2 = Fiber versus placebo in both studied periods (BT and AT).

pathogens, such as *C. botulinum*, *C. difficile*, and *C. perfringens*. *Clostridium difficile* is a major etiological agent of diarrhea and is now recognized as the primary cause of hospital acquired colitis in patients receiving antibiotics, chemotherapeutics, or other drugs that alter normal flora.¹⁹ *C. perfringens*, used as our control bacteria, produces different enterotoxins associated with diseases including food poisoning and necrotic enterocolitis.^{20,21} About 5-20% of antibiotic-associated diarrhea cases and sporadic non-food borne diarrhea may be due to *Clostridium perfringens* type A, mainly in geriatric patients and those negative for *C. difficile*²²⁻²⁴. Therefore, the reduction in *Clostridium* bacteria with oral intake of an inulin/PHGG mixture indicates potential benefits for gut health.

Currently, dietary fibers such as inulin and PHGG are associated with the promotion of endogenous microbial population growth, such as *Bifidobacteria* and *Lactobacilli*.² These bacteria are beneficial for

intestinal health due to a variety of effects, including inhibition of pathogenic bacterial growth.² However, we did not find changes in the amount of *Bifidobacteria* and *Lactobacilli*. Therefore, we suggest that the decrease of *Clostridium* bacteria by prebiotics observed by us could be due to increased levels of other beneficial bacteria, which we did not evaluate in this study, or by other mechanisms independent of the changes in beneficial microbiota.

The fermentation of prebiotics by microorganisms of the gastrointestinal tract leads to the production of SCFA. The SCFA produced in the colon, specifically acetic acid (2C), propionic acid (3C), and butyrate (4C), are the main source of energy for the enterocyte. They also stimulate cell proliferation of the epithelium, increase visceral blood flow, and promote water and sodium absorption. SCFA have antibacterial properties that prevent growth of pathogenic bacteria, such as reduction of luminal pH that stimulates the growth of

Table V
Frequency of bowel movements in women treated with fiber or placebo

Bowel movement (n°)	Fiber			Placebo		
	Week 1	Week 2	Week 3	Week 1	Week 2	Week 3
0	0	1	0	0	0	0
1	1	0	0	3	2	1
2	3	3	1	4	3	1
3	5	3	3	7	1	4
4	3	2	3	1	6	1
> 5	10	13	14	9	12	16
Mean \pm SD	5.32 \pm 4.66	5.82 \pm 4.14	5.95 \pm 2.50	4.41 \pm 3.06	5.17 \pm 3.21	6.70 \pm 3.83
Fiber versus placebo (p)	Week 1		Week 2		Week 3	
	+0.90 (3.91)		+0.65 (3.68)		-0.74 (3.27)	

Table VI
Number and percentage of constipated woman reporting constipation relief after 3 weeks treatment with fiber or placebo

Treatment	0*	1*	2*	3*	Total
Fiber	6 (27.3%)	19 (4.5%)	6 (27.3%)	9 (40.9%)	22 (100.0%)
Placebo	4 (16.7%)	5 (20.8%)	5 (20.8%)	10 (41.7%)	24 (100.0%)
Total	10 (21.7%)	6 (13.0%)	11 (23.9%)	19 (41.3%)	46 (100.0%)
Fiber versus placebo (p)	0.372				

*) 0 = Always unsatisfied; 1 = Satisfied only 1 week; 2 = Satisfied in 2 weeks; 3 = Always satisfied.

Lactobacilli and *Bifidobacteria* and direct suppression of pathogenic bacteria.¹³ However, we did not observe changes in the fecal concentration of SCFA between the treatments in the present study.

Different factors related to stool collection without professional supervision could contribute to lack of observed changes in SCFA. Small stool sample weight could lead to sampling errors, as the entire bowel does not contain a homogenous distribution of SCFA. Samples transportation was performed after patients' calling to inform us of its collection, and we could not control the time that samples remained waiting to be adequately stored. In addition, SCFA are volatile and can be rapidly lost at physiological pH values, and perhaps our stool samples should have been stabilized by acidification prior to freezing.²⁵ On the other hand, it is also possible that there was an increased production of SCFA that were consumed by enterocytes and resident bacteria.

In agreement with our study, previous authors showed that the ingestion of inulin was able to shorten the orofecal transit time and improve stool consistency in subjects suffering from constipation, but did not change concentrations of the abundant SCFA (acetate, propionate) and fecal contents of *Lactobacillus acidophilus* and *Bifidobacterium lactis*.²⁶ These authors did not evaluate the stool composition of pathogenic bacteria.

As long we had controlled the treatment with inulin and PHGG by using placebo, from our data it is not possible to infer if the mixture of two fibers can potentiates its individual effect. In a previous study of 188 adult irritable bowel syndrome patients, PHGG was as effective as a high fiber diet in improving pain and bowel habits, and was better tolerated by patients. Another study in childhood constipation found comparable benefits between a fluid fiber mixture and isolated lactulose.^{7,27}

Interestingly, although placebo treatment did not change the amount of pathogenic bacteria, it improved bowel movements and patient satisfaction. Maltodextrin was chosen as placebo control because it is an easily absorbed and digested carbohydrate that escapes bacterial fermentation in the colon and does not interfere in microbial ecology of the gastrointestinal tract, gut metabolism, and function. Therefore, our data suggest that improvement of bowel movements and

patient satisfaction after treatment with placebo may be due to the placebo effect.

The placebo effect has been widely documented by randomized, placebo-controlled drug studies. The main theories proposed to explain the placebo effect include the conditioning theory (placebo effect as a conditioned response) and the mentalistic theory (patient's expectation as the primary cause of the placebo effect).²⁸ Brain imaging has demonstrated that placebo can mimic the effect of the active drugs by activating the same brain areas.²⁹ This is the case for placebo-dopamine in Parkinson's disease and for placebo-analgesics.^{30,31}

Considering that our study included women from a health care institution with greater awareness of the potential benefits of using fiber for the treatment of constipation, it is possible that our participants experienced a placebo effect. Beneficial effects of fiber versus placebo were found in studies performed in children, who are less susceptible to the placebo effect.^{32,33}

In conclusion, a mixture of inulin and PHGG gave comparable clinical results to placebo in the treatment of female constipation, but had an additional beneficial effect on gut microbiota by decreasing the amount of pathological bacteria of the *Clostridium* genera.

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