



Original / *Nutrición parenteral*

Betaine; a potential agent for the treatment of hepatopathy associated with short bowel syndrome

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Abstract

Background: The hepatopathy associated with short bowel syndrome (SBS) is a multifactorial disease associated with poor prognosis. Besides intestinal transplantation, no other treatment has been shown effective. The current study evaluated the efficacy of betaine for the treatment of hepatopathy associated with SBS.

Methods: A prospective, unicentric, non-placebo controlled trial was carried out. After initial evaluation, 10g of betaine anhydrous was administered to SBS patients in two divided doses for three months. The hepatic steatosis was assessed through nuclear magnetic resonance (NMR), the inflammatory response by interleukin-6 (IL-6), tumor necrosis factor- α (TNF- α) and ferritin, besides the hepatic lesion through hepatic enzymes and bilirubin. Furthermore, the effect of betaine on homocysteine was evaluated as well as its safety and tolerability in this group of patients.

Results: After three months supplementation, patients showed decreased percentage of hepatic fat ($p = 0.03$) through triphasic NMR examination. There was no significant reduction of serum levels for inflammatory proteins and hepatic lesion markers. Homocysteinemia also did not present significant decrease. The most prevalent side effects were diarrhea and nausea, reported in 62% of the participants; however, these symptoms were transient and not severe enough to justify the treatment interruption. Parenteral nutrition-dependent patients did not present different hepatic lesion degree compared to patients who do not need the prolonged use of it.

Conclusions: Betaine was shown to be a potential agent for the treatment of hepatopathy associated with SBS, which was evidenced by NMR, although the markers for hepatic lesion have not presented significant decrease.

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Key words: *Short bowel syndrome. Hepatic steatosis. Betaine. Magnetic resonance. Homocysteine.*

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BETAÍNA; UN AGENTE POTENCIAL PARA EL TRATAMIENTO DE LA HEPATOPATÍA ASOCIADA CON EL SÍNDROME DEL INTESTINO CORTO

Resumen

Introducción: La hepatopatía asociada con el síndrome del intestino corto (SIC) es una enfermedad multifactorial asociada con un mal pronóstico. Además de trasplante intestinal, ningún otro tratamiento ha demostrado ser eficaz. El actual estudio evaluó la eficacia de la betaína para el tratamiento de la hepatopatía asociada a la SIC.

Métodos: Fue realizado un estudio prospectivo, unicéntrico, no controlado con placebo. Después de la evaluación inicial, 10 g de betaína anhidra fue administrado a pacientes con SIC en dos dosis divididas durante tres meses. La esteatosis hepática se evaluó a través de resonancia magnética nuclear (RMN), la respuesta inflamatoria por la interleucina-6 (IL-6), factor de necrosis tumoral- α (TNF- α) y la ferritina, además de la lesión hepática por medio de enzimas hepáticas y de la bilirrubina. Además, el efecto de la betaína sobre la homocisteína fue evaluada así como su seguridad y tolerabilidad en este grupo de pacientes.

Resultados: Después de la administración de la betaína por tres meses, los pacientes mostraron disminución de la porcentaje de grasa hepática ($p = 0,03$) demostrado por examen de RMN trifásico. No hubo una reducción significativa de los niveles séricos de proteínas inflamatorias y marcadores de lesión hepática. La homocisteína también no presentó disminución significativa. Los efectos secundarios más frecuentes fueron diarrea y náuseas, presentado en 62% de los participantes, sin embargo, estos síntomas fueron transitorios y no lo suficientemente graves como para justificar la interrupción del tratamiento. Pacientes dependientes de nutrición parenteral no presentaron diferentes grados de lesión hepática en comparación con los pacientes que no necesitan el uso prolongado de la misma.

Conclusiones: La betaína demostró ser un agente potencial para el tratamiento de la hepatopatía asociada a la SIC, que se evidenció mediante RMN, a pesar de los marcadores de lesión hepática no presentaron disminución significativa.

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Palabras clave: *Síndrome del intestino corto. Esteatosis hepática. Betaína. Resonancia magnética. Homocisteína.*

Introduction

Short Bowel Syndrome (SBS) is a malabsorption condition caused by the reduction of the functional intestinal mass, which is essential for the digestion and absorption of nutrients required for the maintenance of nutritional and hydro-electrolytic status. This disorder is characterized by weight loss, malnutrition, intestinal malabsorption, steatorrhea and acid diarrhea caused by intestinal surface loss and decreased intestinal transit time¹.

SBS has high mortality and morbidity with severe consequences on the patients' quality of life. Survival rate can reach 65% in 6 years in those patients with bowel remnant longer than 50 cm in length. The introduction of prolonged parenteral nutrition in SBS patients, in the decade of 60s, propitiated an increased survival; however, it also contributed to the development of complications such as septicemia, hyperglycemia, thrombosis and hepatic disease². The main cause of death in this group of patients is the sepsis related to central catheter infection and hepatic failure^{3,4}.

SBS and prolonged parenteral nutrition lead to hepatic and biliary changes. The most common alteration in adult patients is the hepatic steatosis, which afflicts about 40-50% of the SBS patients, while in pediatrics patients it is the cholestasis (40-60%)⁵. Other evidenced hepatic alterations are steatohepatitis, fibrosis and cirrhosis, and other possible biliary changes besides the cholestasis are biliary sludge, cholelithiasis and acalculous cholecystitis⁶.

Liver disease worsens the prognosis for SBS patients. In a cohort study evaluating 218 pediatric SBS patients from 1989 to 2006, 26% showed hepatopathy, of which 32% passed away⁷. In a retrospective study that evaluated 42 patients treated with home total parenteral nutrition (TPN) between 1974 and 1997, 14.3% developed end-stage liver disease and died 10.8 ± 7.1 months after initial bilirubin elevation⁸. In a French cohort study, out of 65% patients that presented hepatopathy associated with SBS, 8.6% developed cirrhosis and 22% died directly because of the liver disease SBS related⁹.

Several treatments are proposed for hepatopathy associated with SBS such as the use of metronidazole and gentamycin^{10,11}, glutamine¹², ursodeoxycholic acid^{13,14}, cholecystokinin^{15,16} and omega-3^{17,18}.

In spite of the diverse options for treatment, the gold standard therapeutic remains the intestinal transplant. In 1995, the world 1-year survival for small bowel transplant was 69% and for combined small bowel and liver transplant 66%¹⁹. While the 5-year survival rate for isolated small bowel transplant was 50% and for combined small bowel and liver transplant 40%²⁰.

The diagnosis of hepatic steatosis is one of the complicating factors for accurate estimative of this disease prevalence since it requires liver biopsy, which is an invasive procedure with potential of complications. Therefore, new diagnostic tools are needed for detection of steatosis. In a study that assessed hepatic steatosis in pa-

tients undergoing liver resection, different imaging methods were compared to liver biopsy results. Nuclear magnetic resonance spectroscopy (NMRS) showed higher sensibility (91%) than nuclear magnetic resonance (NMR) (90%), computed tomography (74%) and ultrasonography (65%). NMR presented the best specificity (91%), followed by NMRS (87%), ultrasonography (77%) and computed tomography (70%). The positive predictive values for NMR and NMRS were 90% and 80%, respectively. While the negative predictive values for NMR and NMRS were both 91%²¹.

Betaine is a methylated aminoacid derived from glycine, which formula is $(\text{CH}_3)_3\text{N}^+\text{CH}_2\text{COO}^-$, also known as trimethylglycine, it represents an essential biochemical compound in methionine/homocysteine cycle. Betaine acts as a methyl group (CH_3) donor, helps to maintain the cell osmolarity and protects against protein denaturation. Human studies show that betaine is rapidly absorbed in the small bowel, particularly in the duodenum and distributed, with a plasma concentration peak after 1-2 hours uptake²².

In a pilot study, ten volunteers with non-alcoholic steatohepatitis were given 20g of betaine, two divided doses daily for 1-year, and showed an improvement in serum hepatic enzymes levels and liver fibrosis stage assessed by biopsy²³. However, in a posterior randomized, prospective and placebo-controlled trial evaluating 55 patients, there was no improvement in the pattern of hepatic steatosis assessed through biopsy, as well as serum values for insulin, glucose, inflammatory proteins and hepatic enzymes²⁴.

In another prospective, randomized, double-blind and placebo-controlled trial, 191 patients received either betaine or placebo for 8 weeks. The treatment reduced by 25% hepatic steatosis ($p < 0.01$) and by 6% hepatomegaly ($p < 0.05$) when compared to the placebo group²⁵.

Subjects and methods

This study was approved by the Research Ethics Committee in Ribeirão Preto Clinics Hospital, Faculty of Medicine-University of São Paulo (HCFMRP/USP), SP, Brazil, (Process nº 9416/2010). Before beginning data collection, all volunteers were informed about the purpose of the study, procedures they would be submitted to and potential risks. Informed consent form was presented to all participants, and the research protocol was started only after elucidation of possible questions and volunteers' signature.

All the SBS patients followed by the Service of Nutrology at the HCFMRP/USP, who did not fit the exclusion criteria listed below, were invited to participate of this study:

- Age below 18-years;
- Oral intake inability;
- Alcohol use of > 20 g/day;

- Presence of metallic prosthesis;
- Presence of Hepatitis B or C.

A prospective, intervention, unicentric, non-placebo controlled trial was carried out in SBS patients followed by the Service of Nutrology at the HCFMRP/USP.

At the beginning of the study, information was collected, such as age, gender, weight, height, time of diagnosis for SBS, intestinal remnant length, TPN dependence and frequency of hospitalization to receive parenteral nutrition therapy.

First blood sample was taken after 12 hours fasting, the day before the start of supplementation with betaine in order to analyze aspartate transaminase (AST), alanine transaminase (ALT), alkaline phosphatase, γ -glutamyl transpeptidase (γ -GT), total bilirubin and fractions, total proteins, albumin, lipid panel, tumor necrosis factor, interleukin-6, methionine, vitamin B₁₂, iron, Unbound iron binding capacity (UIBC), ferritin and homocysteine. Second blood sample was taken 180 days after the start of betaine supplementation and the same parameters measured before were evaluated.

The measurements for hepatic enzymes, lipid panel, total bilirubin and fractions, albumin, INR, vitamin B₁₂, iron, UIBC, ferritin and homocysteine were performed through automated ultraviolet kinetic enzymatic method by using Wiener equipment.

Serum TNF- and IL-6 levels were measured by using IMMULITE kit (Siemens®), a chemoluminescent method involving antigen-antibody reaction in solid phase.

Determination of methionine levels in plasma was performed through high-performance liquid chromatography-HPLC Shimadzu® LC10AD (Shimadzu, Tokyo, Japan), using fluorescence detector Shimadzu RF535. Concentrations were obtained according to aminoacids standard curve.

For hepatic parenchyma evaluation, high-field 3.0 Tesla (Vision, Siemens, Malvern, PA) magnetic resonance scans were performed with a phase-array coil for the abdomen. The examination protocol included the following sequences: T1-weighted sequence, in-phase, with breath-hold, spoiled gradient echo (SGE) (TR = 140-175 msec, TE = 4.2 msec, flip angle = 80°, slice thickness = 8 mm, gap = 20%, 21 slices in 20 seconds breath-hold), T-weighted sequence, out-phase with breath-hold, SGE (TR = 140-175 msec, TE = 2.1 msec, flip angle = 80°) and T2-weighted echo train spin echo sequence (TR = 4000 msec, TE = 90 msec) or half-Fourier acquisition single-shot turbo spin echo (HASTE) (TR = infinite, TE = 90 msec). SGE images were subsequently acquired immediately after bolus injection of 0.1 mmol/kg gadolinium chelate in 45 seconds - known as arterial phase, 90 seconds (with fat suppression) - called the portal phase, and 5-10 minutes-called delayed phase.

For the interpretation of images all examinations were prospectively read by two radiologists independently for reliability analysis. The examiners evaluated

the tests regarding: 1) presence and pattern of fibrosis, 2) presence and pattern of fatty infiltration, 3) reinforcement pattern of hepatic parenchyma, and, 4) other morphological aspects.

The objective measurement of hepatic fatty infiltration was obtained by calculating the hepatic fat fraction²⁶. A pair of images in- and out-phase obtained in the portal vein section was used for signal intensity (SI) measurement; four measurements were taken per slice. Region of interest (ROI) size was > 1 cm², and adjusted by each hepatic segment, avoiding vessels or artifacts image contamination. The mean SI values of all ROIs were determined at the same locations for in-phase and out-phase MR images. Mean hepatic fat fraction was calculated by using the following equation:

$$\text{Hepatic Fat Fraction} = (\text{SI}_{\text{in-phase}} - \text{SI}_{\text{out-phase}}) / 2\text{SI}_{\text{in-phase}}$$

After performing the initial protocol examination, the supplementation with 10g/day betaine anhydrous (Betafin® BF 20) was started, in two divided doses, which were diluted in citric fruits juice in order to lessen the bitter taste of betaine.

By the end of three months, the same protocol evaluation was repeated and new symptoms related to possible side effects were analyzed.

Data were processed with the softwares STATISTICA 6.0, INSTANT (GraphPad Software V2.01-Copyright 1990-1993) and Kyplot version 2.0. Differences between variables were considered significant when $p < 0.05$.

The individual response to supplementation was calculated through cross multiplication considering the basal value as 100%, and then the subjects separated into two groups: below and above the basal. Groups were compared by Mann-Whitney test. When all participants response was homogeneous, comparison of numerical variables in both stages of the study (basal and after supplementation with betaine) was performed by using the paired t test.

Results

Fifteen SBS patients followed by the Service of Nutrology of HCFMRP/USP were invited to participate in the study. Five out of those declined, and two were excluded one by having entered the intestinal transplant protocol and the other for having undergone intestinal transit reconstruction. A total of 8 patients participated in the study, 3 women (37.5%) and mean age of 58.6 years. The average time of SBS patients was 75.1 months and the average of bowel remnant length was 59.4 cm, 62.5% had no ileocecal valve. Three participants (37.5%) were TPN-dependent.

The results are summarized in table I. Regarding the hepatic lesion examination, decreased levels were found for total bilirubin (62.5% of patients), direct bilirubin (75% patients), AST (50% of patients), ALT (75% of

Table I
Results before and after supplementation with betaine

Parameter	Before	After	p	Cases with reduction
Total Bilirubin	0.85	0.64	0.0368	62.5%
Direct Bilirubin	0.35	0.25	0.0667	75%
AST/ ALT	22.37/ 26.37	18.12/ 18.5	0.1336/ 0.0667	50%/ 75%
Alkaline Phosphatase	269.87	208.75	0.3827	62.5%
γ-GT	77.75	31.5	0.0667	87.5%
Ferritin	350.5	184.08	0.0106	100%
IL-6	9.24	4.54	0.0651	87.5%
TNF-α	13.15	9.77	0.0667	75%
Hepatic steatosis assessed by NMR	14.1	13.0	0.0368	62.5%

patients), alkaline phosphatase (62.5% of patients) and γ-GT (87.5% of patients). After considering only individuals who had values above the reference at the beginning of the study, total bilirubin, direct bilirubin, AST, ALT, alkaline phosphatase and γ-GT, had respectively, reduction to normal values, 50% (1/2), 100% (1/1) 100% (1/1), 100% (1/1) 62.5% (5/8) 0% (0/2). Only the total bilirubin showed significant difference.

Comparison between the phases of NMR and spectrophotometry were discarded due to interference from reading the software by hepatic iron impregnation in 62.5% of patients. Triphasic evaluation of NMR showed that 62.5% of participants presented reduced percentage of hepatic fat, with significant difference ($p = 0.0368$) before and after the supplementation with betaine, although no significant difference have been found for serum concentration of the hepatic enzymes –AST, ALT, γ-GT and alkaline phosphatase.

All patients had decreased levels of ferritin, and compared the two points of investigation there is a significant p ($p = 0.0106$) by paired t test. The supplementation with betaine decreased the ferritinemia to normal levels in 50% of participants, who had high levels before the treatment.

There was a 24 μmol/L mean increase in methionine values and 2.5 μmol/L decrease in homocysteine levels, although no significant difference has been found.

A mean decline of 25.7% in TNF-α and 51.1% of IL-6 levels was observed, however, with no statistic difference.

With respect to the side effects, diarrhea was observed in 62.5% of the cases and nausea in 37.5%. In general, the side effects were transient and lasted about four days. Only two patients presented persistent symptoms for more than a week, but those were not severe enough to impede the intervention continuation.

Discussion

Five out of eight participants showed decreased hepatic fat percentage, which was evaluated by using the

triphasic method of NMR. Between the three participants who worsened, two presented no serum methionine increase, which was expected since betaine is methyl group donor for the formation of methionine. The fact that these individuals do not show increased serum methionine suggests they did not use the supplementation properly or show any metabolism abnormality of betaine which prevented its action on hepatic fat. Other studies have also used the elevation of serum methionine as an indirect marker of betaine supplementation²⁷.

Only one patient showed hepatic enzymes levels changed, although six of them present more than 10% of fat in hepatic parenchyma. It confirms the low sensibility of serum hepatic enzymes for the screening of hepatopathy associated with SBS²⁸, and corroborates the difficult to diagnose this pathology since the onset of symptoms and elevation of liver injury enzymes occur in advanced stages of the disease. Thus, more sensitive screening methods are needed.

As expected, there was a significant triglyceridemia raise after betaine supplementation because of the increased hepatic excretion of triglycerides²⁹, which represents one possible explanation for its beneficial effect on steatosis. Nevertheless, not all studies have observed such behavior of triglycerides²⁴.

Despite the non-significant p -value, reduced homocysteine levels were found in 87.5% of volunteers, as expected since betaine donates a methyl group to homocysteine for the methionine synthesis, which has also been observed in other trials²⁴. The lack of statistical significance can be explained by the small sample size and future confirmation may be required. In case decreased levels of homocysteine associated with betaine are confirmed, it can be important for patients with SBS because of thrombophilia, in which hyperhomocysteinemia may be involved.

Besides the significant ferritin decrease, a decline of 87.5% and 62.5% was noticed in IL-6 and TNF-α level, respectively, however, there was no statistical significance. Reduced IL-6 levels, but not TNF-α, have also been shown after supplementation with 20 g betaine during 12 months²⁴. The lessening of inflammation

can be explained by the reduced fat percentage in hepatic parenchyma, which assuages the chronic inflammatory process or could represent one reason by which betaine can contribute to decrease the hepatic fatty accumulation, since chronic inflammatory processes can be involved in steatosis development. The reduced chronic inflammatory process in this group of patients may contribute to prevent cachexia since IL-6 is involved in its pathogenesis³⁰.

Ferritinemia decrease can also mean improvement of the hemochromatosis profile; however it cannot be ascertained by NMR, and requires confirmation by biopsy.

Interestingly, it was not possible to evaluate the hepatic steatosis degree by the NMR biphasic method due to the interference caused by the iron accumulation in hepatic parenchyma. It may suggest the limited use of this method in cases of hepatic steatosis associated with hemochromatosis, but that can be compensated by the triphasic evaluation. There are no studies that show the hemochromatosis as a variant of liver disease associated with SBS. Although the participants present lesions suggestive of hemochromatosis, liver biopsy would be needed to confirm them.

There was no difference in mean percentage of hepatic fat found in the TPN-dependent (13.7%, SD: 8.5) and non-dependent (13.8%, SD: 4.4) participants, although it was expected that TPN-dependent patients had higher percentage of liver injury by being exposed to more aggressive factors and having smaller bowel remnant length (36.7 cm for TPN-dependent and 73 cm for non-dependent participants). One possible explanation is the frequency of TPN administration to patients, which confers lower exposure to the toxic factors of TPN and may contribute to the hepatic steatosis genesis.

Diarrhea and nausea represent the main side effects responsible for the interruption of betaine use, since SBS patients already experience these symptoms and their serious complicating effects such as dehydration, electrolyte disturbances, and malnutrition. Even so, only two participants showed symptoms persistence for more than 14 days, and at no time it was necessary to interrupt treatment because of their side effects. Other side effects reported in other studies, such as vomiting and body odor, were not observed in this casuistic.

Few highlights must be mentioned. The small sample size restricts the results; however, because it is a rare disease, most of the studies with SBS patients present this limitation. Non-placebo controlled trials can restrict the interpretation of the results, but the population size did not allow the possibility of conducting a placebo-controlled study. The method used to assess hepatic steatosis is not the gold standard, but previous studies²¹ showed a good correlation with evaluation by biopsy.

This study evaluated for the first time the effectiveness of betaine in the treatment of hepatopathy associated with SBS, and showed it may be an option for adjuvant treatment of this disease. Betaine decreased the

percentage of hepatic fat and serum levels of ferritin, and can be a choice to attenuate the chronic inflammatory status, factor that contributes to the cachexia in these patients. Contrary to the expected, no significant reduction in homocysteinemia was found. Also, unexpectedly, no differences were found in the hepatic lesion degree between patients dependent or not on parenteral nutrition.

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Conflict of interest

The authors declare no relevant conflicts of interest. G.T.A. is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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G.T.A. was the primary investigator and involved in data research, wrote the manuscript, and analyzed data. F.A.D. was responsible for the laboratory analysis. H.V. and J.E.J. was involved in study design, conceptualizing of the project and was involved in study design. B.J. H.V. also reviewed/edited the manuscript.

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