Efficacy and Safety of Oral Betaine Glucuronate in Non-alcoholic Steatohepatitis

A double-blind, randomized, parallel-group, placebo-controlled prospective clinical study

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Summary

In a prospective, randomized, double-blind therapeutic trial, 191 patients with non-alcoholic steatohepatitis were treated for 8 weeks daily b.i.d. orally either with betaine glucuronate combined with diethanolamine glucuronate and nicotinamide ascorbate (Ietepar ®) (96 patients) or with undistinguishable placebo capsules (95 patients).

The verum treatment effectively reduced by 25 % hepatic steatosis $p<0.01$) and by 6 % hepatomegaly p <0.05), while placebo did not significantly reduce the disorders. Verum was also more effective than placebo on discomfort in abdominal upper right quadrant. The global efficacy of treatment was rated by the doctor “very good" or “good" in 48 % of verum treated patients and only in 17 % after placebo (P of difference = 9 x 10-6). 52 % of patients self-rated efficacy as “very good" or “good" after verum and only 34 % after placebo (P of difference 0.017). The verum treatment provoked a significant reduction of the increased liver transaminases (ALT, AST and γ-GT) while placebo was ineffective. Adverse events were recorded in 10 % of verum-treated patients and in 7 % under placebo (no significant difference). In both groups the adverse events were mild and transient, did not require treatment discontinuation and were undistinguishable from common symptoms of liver disorders.

In conclusion, the 8-week treatment with betaine glucuronate combined with diethanolamine glucuronate and nicotinamide ascorbate was found effective in non-alcoholic steatohepatitis, a disorder for which the hitherto pharmacological interventions were poorly and inconsistently effective.

Key words Betaine glucuronate, clinical studies, effect on non alcoholic steatohepatitis Diethanolamine glucuronate

Ietepar® . Ietepar® Nicotinamide ascorbate Steatohepatitis

1. Introduction

Non-alcoholic steatohepatitis (NASH) is an increasingly common cluster of abnormalities of the liver in patients in whom heavy alcoholic consumption can reasonably be excluded. In the affluent societies NASH ranks second or third among the most common liver disorders in the hepatological practice [2, 5], and its importance was officially acknowledged in a Consensus Symposium organized by the US National Institutes of Health in Washington, DC (USA) in December 1998.

The term NASH was introduced by Ludwig et al. [7] to describe "the pathological and clinical features of non-alcoholic disease of the liver associated with the pathological features most commonly seen in alcoholic liver disease itself". It has replaced the former term "non-alcoholic hepatic steatosis" because the liver is frequently affected also by necroinflammatory processes, possibly a reaction to the deposition of fat in the hepatocyte or to the toxic agents that have caused NASH.

NASH is characterized by accumulation of triglycerides within hepatocytes, usually in macrovesicular droplets, substantial fibrosis or cirrhosis in 15 *%-50* % of patients, liver enlargement, abnormalities in the liver function tests, discomfort or pain in the upper right quadrant and dyspepsia. It is frequently found in obese patients, in patients with type 2 diabetes or with hyperlipidernia, or with both [4], and in patients with insulin resistance [8]. The cause of fat accumulation in the liver is the consequence of an unbalance between the import and biosynthesis of lipids and the capacity of the hepatocytes to metabolize and to clear triglycerides. This may be due to toxic effects on the cells by nutritional, environmental or pharmacological agents, or to an overload of fats as in obese patients. The natural course of the disease is also unclear and difficult to predict. In the past NASH was considered to be benign [11, 14]. The more recent case reports, however; have recorded an increasingly number of a progressive course leading to cirrhosis and hepatic failure [1]. Therefore a therapeutic intervention should be made already in the early stages of NASH, first of all intervening on the possible underlying disease (e.g. diabetes, hyperlipidernia). Unfortunately there is not yet any specific treatment for NASH that is effective in all patients. Even the benefits of a balanced and restricted diet may be inconsistent [10], as well as those of a lipid-lowering therapy with, e.g., clofibrate [6]. In one clinical study ursodeoxycholic acid was found to be moderately effective probably for its cytoprotective activity [6], but this single agent it is not sufficient to intervene on the multiple etiologic factors involved in NASH.
In a previous study on patients affected by alcoholic steatohepatitis [9] we have proven the efficacy of betaine glucuronate combined with diethanolamine glucuronate and nicotinamide ascorbate. It was therefore interesting to investigate the efficacy of this combination also in NASH.

2. Methods

2.1. Study design

The study was single-center; double-blind, placebo-controlled, on out-patients randomized into two parallel groups by computer-generated blocks of 10 patient&

2.2. Patients

Eligible for the study were patients of both genders aged at least 18 years, suffering from liver enlargement and hepatic steatosis ascertained by ultrasonography.

Table 1.' **Characteristics of patients at enrollment.**

GROUP	BG	PLACEBO
Number (M/F)	96 (62/34)	*95(72/23)*
Age ave ± SD	*57±15*	*60±14*
Duration of steatosis		
< 1 year	*42(44%)*	*32(34%)*
1-5 years	*35 (36%)*	*28 (29 %)*
> 5 years	*19 (20%)*	*35 (37%)*
Obese	*23(24 %)*	*20 (21 %)*
Diabetes	*34 (35 %)*	*39 (41 %)*
Hyperlipemic	*29 (30 %)*	*41(43 %)*

Exclusion criteria were history of past or present alcohol abuse, history of past or present viral hepatitis, mononucleosis, cytomegalovirus or spirochete infection, Wilson disease, Crohn's disease, pregnancy or nursing, uncooperative patients or patients not able or not willing to release the informed consent. Enrolled were 191 patients, 96 resulted to have been assigned to the betaine glucuronate group and 95 to the placebo group. The characteristics of the patients at enrollment are shown in Table 1 and were comparable in the two groups.

2.3. Treatments

The patients received daily for 8 weeks two capsules (one at breakfast and one at the evening meal) administered with a glass of water. The capsules had the following composition.
BG. Capsules containing betaine glucuronate (CAS 107-43-7; CAS 6556-12-3)150 mg, diethanolamine glucuronate (CAS 111-42-2; CAS 6556-12-3)30 mg, nicotinamide ascorbate (CAS 1987-71-9) 20 mg Ietepar®, produced and marketed by Rotta Research Group, Monza, Italy). PLAC. Placebo capsules, identical in appearance to the BG capsules but without the active ingredients.

No special diet restrictions were prescribed, besides the advise to take low-fat meals and to reduce food intake.

2.4. Procedure

Before enrollment the medical history was recorded and the inclusion and exclusion criteria were checked. The patients were informed on the aims, procedures and the risks involved in the study and were asked to participate with a written informed consent.

At the beginning of the study (Visit 1 - Week 0) and at the end of the study visit 3 - Week 8) the following was recorded.

a) Complete physical examination, body weight, height, systolic and diastolic blood pressure and heart rate.
b) Routine hematology, i.e. hemoglobin, hematocrit, counts of erythrocytes, of leukocytes (with differential count) and of platelets.
c) Blood chemistry, including prothrombin time, total, direct and indirect bilirubin, total proteins, albumin, alpha, beta and gamma globulins, urea, glucose, total and high-density lipoproteins (HDL), cholesterol, triglycerides, alkaline phosphatase, alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma glutamyltranspeptidase (γ-GT) and creatinine.
d) Urinalysis, including pH, proteins, bilirubin, urobilinogen, hemoglobin, nitrites, ketones, glucose and sediment. The analyses were performed in the Hospital's Central Laboratory.
e) Scoring of hepatic steatosis by ultrasound scan (US) according to Saverymuttu et al. [13]. The severity of fatty infiltration was estimated on the hyperechogenic intensity and scored in 0, 1, 2 and 3 for normal, mild, important or massive.
f) Scoring of liver enlargement (hepatomegaly) by dipping the liver area. The scores were 0, 1, 2 and 3 for absent, mild, moderate or severe.
g) Identification of "obese" patients (body mass index larger than 30).
h) Scoring of symptoms, i.e. dyspepsia, right upper quadrant discomfort or right upper quadrant tenderness or pain. The scores were: 0, 1, 2 and 3 for absent, mild, moderate or severe.

Four weeks after the start of the study a second visit (Visit 2) was performed with the following examinations.

a) Complete physical examination.
b) Scoring of hepatomegaly.
c) Scoring for symptoms.

At the end of treatment the investigator and the patient expressed their judgment on the global efficacy of the therapeutic intervention as: none, fair, good and very good.

The safety of treatment was evaluated recording any adverse event either reported by the patient, or found at the visits 2 or 3 by the physical examination, or evidenced by the laboratory and instrumental tests.

2.5. Statistical methods

Comparison between groups was made by ANOVA. When normality of distribution did not hold it was made by suitable non-parametric tests (Mann-Whitney, Chi-square). The within patient comparisons was made by Wilcoxon's U test. Safety and efficacy between groups was evaluated on the intention-to-treat patients. Efficacy within patients was evaluated on the evaluable patients.

2.6. Ethical considerations

BG and the doses used are authorized for marketing for the specific indication in Italy and in several other Countries. The study was registered at and authorized by the Italian Health Authorities, was conducted in Italy in the department of Internal Medicine of the Hospital "S. Orsola - Malpighi" of the University of Bologna (1taly) was performed in accordance with EC Guidelines for Good Clinical Practice [3] and in compliance with the Declaration of Helsinki (Hong Kong revision 1989). The patients were informed verbally and in writing about the aim, procedures and risks involved in the study and gave their written informed consent to participate. The study was submitted to an approved by an independent Ethical Committee. All patients were insured against possible damages arising from their participation in the study.

3. Results

3.1. Efficacy

3.1.1. Hepatic steatosis

The effects of the 8-week treatments on the scores of hepatic steatosis evaluated by ultrasound scan (US) are shown in Table 2. Under BG hepatic steatosis decreased significantly ($p<0.01$) by 25 % of the initial value. Under PLAC a non significant decrease of 3 % was found. The difference between the two treatments is significant ($p<0.001$).

3.1.2. Hepatomegaly

The effects of the 8-week treatments on the scores of hepatomegaly evaluated by dipping the liver area are shown in Table 3. BG elicited a significant ~ <0.05) decrease of the liver area, while under PLAC a not significant increase of 1 % was recorded.

3.1.3. Dyspepsia

The efficacy of the two treatments on dyspepsia complained by the patients is reported in Table 4. Both BG and PLAC were significantly effective in improving dyspepsia and reduced its scores by 40 %. No significant difference was found between BG and PLAC pointing out the notable psychological component involved in dyspepsia.

3.1.4. Discomfort and pain in the upper right quadrant

The scores of discomfort and of pain complained by the patients are reported in Table 5. Discomfort was significantly relieved under BG by 45 % already after the first 4 weeks of treatment. Also PLAC relieved discomfort, but to a significantly smaller degree (15 %) than BG. At the 8th week of treatment BG relieved discomfort in the upper right quadrant by 57 %, significantly more ($p < 0.001$) than PLAC that relieved discomfort by *15%*.

Both BG and PLAC significantly relieved spontaneous pain in the upper right quadrant by *56%*-59 % after 8 weeks of treatment. However, as for dyspepsia, the differences of efficacy between the two treatments were not statistically significant.

3.1.5. Global efficacy judged by the investigator or self-rated by the patient

The judgments of global efficacy of the treatments judged by the investigator and that self-rated by the patients are reported in Fig. 1. Patients with judgment of "good" and of "very good" efficacy were classified "responders", and those of "none" and of "fair" efficacy were classified "non responders". BG elicited a definitely and significantly higher responder rate than PLAC, both judged by the investigator (48 % vs. 17 %) as self-rated by the patients *(52* % vs. 34 %). The exact probability of the difference between BG and PLAC calculated by the double-sided Fisher's test is 9 x l0 for the doctor judgments and 0.017 for the patient self-ratings, in both cases therefore significant.

Table 2. **Efficacy on hepatic steatosis. Average US score ±SE.**

Visit	BG(N=91)	PLAC(N=92)	P of diff.
Week 0	l.93±0.06	1.83±0.067	NS
Week S	1.44 ± 0.07	1.78 ± 0.07	p < 0.001
Decrease (%)	25	3	
P of decrease	< 0.001	NS	

Table 3.. Efficacy on hepatomegaly. Average score ± SE

Visit	BG(N=91)	PLAC(N=92)	P of diff.
Week 0	1.41 ± 0.07	1.43 ± 0.06	NS
Week 8	1.33 ± 0.07	1.45 ± 0.06	0.20
Decrease (%)	6	-1	
P of decrease	< 0.05	NS	

Table 4: **Efficacy on dyspepsia. Average score ± SE.**

Visit	BG(N=91)	PLAC(N=92)	P of diff.
week 0	1.67 ± 0.06	1.61 ± 0.06	
Week 4	1.18 ± 0.06a)	1.09 ± 0.07	
% of W_0	70 (p < O.001)a)	68 (p < 0.001)	
week 8	1.00 ± 0.07a)	0.95 ±0.07	
%ofW_0	60(p<000l)a)	59(p<0.00l)	

a) Non significant difference vs PLAC.

Table 5. Efficacy on upper right quadrant (URQ) discomfort and on URQ pain Average score ±SE.

	URQ DISCOMFORT		URQ PAIN	
Visit	BG(N=91)	PLAC (N=92)	BG (N=91)	PLAC (N=92)
Week 0	1.37 ± 0.06a)	1..32 ± 0. 05	0.68 ± 0.07a)	0.74 ± 0. 07
Week 4	0.75 ± $_{0.08}$b)	1.12 ± 0. 07	0.27 ±0.06a)	0.35 ± 0.06
% ofW_0	55 (p< $_{0\text{-}001}$)b)	85 (p <0.02)	40 (p < 0.001)a)	47 (p <0.001)
Week 8	0.59 ± $_{0,08}$b)	1.12 ± 0. 07	0.30 ±$_{0,06}$b)	0.30 ± 0.07
% ofW_0	43 (p < $_{0,001}$)b)	85 (p = 0.01)	44 (p < O.001)a)	41 (p <0.001)

a) Non significant difference vs. PLAC.
b) Difference v& PLAC p < 0.001.

Table 6: **Enzyme levels. Averages ± SE of lU/1.**

	ALT		AST		γ-GT	
Time	BG	PLAC	BG	PLAC	BG	PLAC
Week 0	42.9 ± 2.2	41.3 ± 2.4	33.5 ± 1.8	30.2 ± 1.2	62.8 ± 6.4	68.1 ± 6.9
Week *8*	38.2 ± 1.6	42.6 ± 2.4	28.8 ± 1.0	32.4 ± 1.0	53.4 ± 3.5	67.3 ± 6.1
%ofW_0	89a)	103	86a)	107	85	99
ANOVAb)	p <0.001		p <0001		p 0.03	

a) Difference vs PLAC p < 0.05.
b) Times x treatments

3.1.6. Enzymatic liver function markers

As shown in Table 6, the 8-week treatment with BG provoked significant decrease of 11 %, 14 % and 15 % of ALT, AST and γ-GT respectively Conversely PLAC was totally ineffective on the liver function markers. The ANOVA proved that the difference of effect of BG vs. PLAC was very significant for all enzymes. Interestingly BG was very effective on ALT and on AST, markers of cellular damage, and less significantly effective on γ-GT, considered a marker of biliary disorders.

3.2. Safety

3.2.1. Adverse events (AES)

Table 7 lists the number of patients reporting AEs with the type and the severity of the AEs. Most of the AEs were mild and affected the gastrointestinal tract. The causal relationship with the treatments is difficult to establish since all reported AEs are common symptoms in patients with liver disorders. The incidence of AEs in the BG group (10 % on the intention to treat patients) is slightly greater than in the PLAC group (7 %) but the difference is not

statistically significant (Fisher's exact probability = 0.43). Severe AEs requiring discontinuation of treatment were not reported and no serious AE occurred.

3.2.2. Vital signs and laboratory tests

Untoward effects on systolic or diastolic blood pressure or on heart rate were not reported for BG or for PLAC. The two treatments did neither provoke untoward effects on hematology, blood chemistry or urinalysis.

Table 7*:* Number of patients with adverse events (AEs).

	AEs under BG
No. of patients	10
Type of AE	2mH, 2mN, 2mD, 2 mod GB, 1 mM, 1 modM
	AEs under PLAC
No. of patients	6
Type of AE	1 mH, 1 mN, 1 mD 1 mGB, 1 modGB, 1mM

m = mild, mod = moderate, H = headache, N = nausea, D = diarrhea, GB gastric burning, M = meteorism.

3.2.3. Conclusions on safety

BG b.i.d. for 8 weeks was well tolerated. The reported AEs during the treatment with BG as well as those with PLAC were mild or moderate, regarded mainly the gastrointestinal tract, were all reversible, did not require discontinuation of treatment and could be likely symptoms of the liver disorder affecting the patients.

4. Discussion

Ultrasound scan and computerized tomography have revealed that NASH is a disorder prevailing in a large proportion of patients in the hepatological practice [12]. Mostly the natural evolution of NASH is benign and symptoms are limited to dyspepsia, bowel irregularities, discomfort or pain in the abdominal upper quadrant that are easily tolerated by the patients. In a substantial number of patients, however, the underlying causes of NASH (toxins, drugs, morbid obesity, non-insulin dependent diabetes, debilitating diseases) may lead to necroinflammatory activity in the liver with outcome of fibrosis, cirrhosis and ultimately liver failure [1, 14]. It is therefore advisable to intervene therapeutically as early as possible to stop the evolution of NASH and possibly to reverse the already existing hepatic lesions. Unfortunately, hitherto effective and reliable treatments for NASH are not known. Besides the intervention on the underlying disorders of secondary NASH (diabetes, morbid obesity, hyperlipidemia, tumors, etc.) there is no single drug able to stop or to reverse the natural evolution of NASH. Possible reason of therapeutic failure are the multifactorial etiological components of NASH, i.e. the endogenous and exogenous toxins, the failure by the liver to manage effectively the metabolism and the clearance of lipids and the deficiency of coenzymes and of substrates required in fat metabolization.

In this therapeutic trial we investigated the efficacy of a drug that combines the liver detoxifying agent glucuronate with the lipotropic effects of betaine. It provides also diethanolamine, a substrate for the synthesis of phospholipids, and coenzymes for the energy-providing redox reactions required for lipid metabolism. The mechanism of action of these agents and the clinical trials conducted on their combination were reviewed in our previous paper [9]. In the present study we report the results of an 8-week course of b.i.d. oral treatment. The therapy was able to significantly reduce liver steatosis and enlargement, discomfort in the abdominal upper quadrant and to partially normalize transaminases. Severe or serious adverse events were not recorded and no discontinuation of therapy was necessary for adverse drug reactions.

In conclusion, the 8-week treatment with betaine glucuronate combined with diethanolamine glucuronate and nicotinamide ascorbate was found effective in non-alcoholic steatohepatitis, a disorder for which the hitherto pharmacological interventions were poorly and inconsistently effective.

5. References

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