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Original Article

Zinc sulfate inhibits the enterohepatic cycling of unconjugated bilirubin in subjects with Gilbert's syndrome

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Abstract

We have previously observed that UCB binds to $ZnSO_4$ *in vitro,* and suppresed the biliary bilirubin secretion in the hamster. The aim of this study was designed to investigate whether Zn salts might inhibit the enterohepatic cycling of UCB in subjects with Gilbert´s syndrome. Fifteen patients with Gilbert´s syndrome and 5 normal healthy volunteers were included in this study according to the following criteria: fasting hyperbilirubinemia, no hemolysis, and free of any medication. Patients were randomly assigned to receive acute o chronic treatment. Subjects treated in acute form and normal healthy volunteers were treated with 40 mg of $ZnSO_4$ in a single dose, where as patients treated in chronic form received 100 mg $ZnSO_4$ in a single dose daily for 7 days. The serum UCB levels (mg/dL) decreased from 2.64 ± 1.04 to 2.02 ± 0.87 ($p < 0.001$) and 1.8 ± 0.36 to 1.48 ± 0.32 ($p < 0.005$) in subjects treated in acute an chronic form respectively, but not in the control group. Whereas, the serum Zn levels (μg/dL) increased from 96.3 ± 16.8 to 118.8 ± 19. 5, ($p < 0.01$) and from 117.6 ± 8.5 to 130.7 ± 6.6 ($p < 0.03$) in subjects treated in acute an chronic form and also in subjects in the control group (98.0 ± 7.3 to 128.0 ± 21.9) $p < 0.03$. This study showed that acute and chronic oral administration of $ZnSO_4$ decreased serum UCB levels significantly in subjects with Gilbert´s syndrome. Most likely by the inhibition of the "normal" enterohepatic cycling of UCB.

Key words: Bilirubin, Zinc, Bile salts, Cholestasis, Gilbert´s syndrome, Pigmented gallstones.

Introduction

The Gilbert's syndrome is a chronic disease in which unconjugated bilirubin (UCB) is not efficiently conjugated in the liver and it is accumulated in the blood.[1-3] Recently, it has been associated with an extra TA in the promoter region of both alleles for bilirubin UDP-glucuronosyltransferase 1 (UGT1A1).[4-8] The resultant 65% decrease in transcription of the (TA)7TAA mutant alleles explains the impaired conjugation of bilirubin found in all Gilbert´s syndrome subjects.[9,10]

Under normal conditions conjugation of bilirubin takes place in the hepatic cell, and two or one molecules of glucuronic acid leads to form bilirubin diglucuronides (BDG) and bilirubin monoglucuronides (BMG) respectively.[11] In humans, BDG constitutes the major conjugate in bile (about 80%). But in subjects with Gilbert's syndrome the proportion of BDG/BMG is inverted.[12] Interestingly, the BMG are the major conjugate species in mice, sheep, pigs, rabbits, guinea pigs, and hamsters.[13] According to this information, we believed that both subjects with Gilbert's syndrome and hamsters have and an EHC of UCB.

On the other hand, we recently obtained evidence for EHC of UCB in rats following distal, but not proximal, small bowel resection.[14] We proposed that EHC of UCB was secondary to an interruption in the EHC of bile salts. These findings are consistent with results in patients with Crohn's disease involving the ileum,[15,16] who present with total bilirubin and UCB levels significantly elevated in gallbladder bile.

Furthermore, we recently observed that at physiological pH $ZnSO_4$ adsorb UCB almost entirely from unsaturated micellar bile salt solution *in vitro*.[17] In addition, in hamsters which have principally BMG in bile,[13] when we fed them with 1% $ZnSO_4$ for one week, the biliary bilirubin secretion was suppressed suggesting an inhibition of the EHC of UCB.[7] The aim of this study was designed to investigate whether Zn salts might inhibit the EHC of UCB in subjects with Gilbert´s syndrome.

Methods

Subjects

We recruited subjects from one medical center in Mexico City (Medica Sur Clinic & Foundation). Mexi-

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can subjects male and female patients 20-46 years of age with Gilbert´s syndrome were included in this study according to the following criteria: a) positive test of fasting hyperbilirubinemia, b) no hemolysis, and c) free of any medication. Normal healthy volunteers acting as controls. Written informed consent was obtained from each subject. The study was approved by the Human Subjects Committee at The Medica Sur Clinic & Foundation as conforming to the ethical guidelines of the 1975 Declaration of Helsinki.

Study design

This was a clinical trial carried out in fifteen patients with Gilbert´s syndrome and 5 normal healthy volunteers acting as controls. Subjects who met the entrance criteria were randomly assigned to acute o chronic treatments, subjects treated in acute form and normal healthy volunteers were treated with 40 mg of $ZnSO_4$ in a single dose. Whereas patients treated in chronic form received 100 mg of $ZnSO_4$ in a single dose daily for 7 days.

Clinical monitoring

Subjects treated in acute form (n = 10) and normal healthy volunteers B (n = 5) were hospitalized one day before treatment in order to avoid physical activities. After a period of 14 h overnight (baseline) they received a carbohydrate (CHD)-rich breakfast (600 kcal) and two h later a single 40 mg oral dose of $ZnSO_4$. Subjects treated in chronic form (n = 5) were treated as outpatients with a single 100 mg oral dose of $ZnSO_4$ daily for 7 days.

Analytical procedures

Serum UCB and Zn levels were monitored by HPLC[18] and atomic absorption spectroscopy at baseline and after oral administration of $ZnSO_4$, and fecal UCB concentrations were determined before and 24 h after oral administration of $ZnSO_4$ by HPLC.[18]

Statistical analysis

All values are expressed as means ± SD, the paired "t" Student and Wilcoxon tests were used to compare the serum UCB and Zn levels. The differences were considered as statistically significant when p was < 0.05.[19]

Results

Subjects with Gilbert´s syndrome treated in acute form and normal healthy volunteers were directly observed by the investigators, indicated that they had good tolerance of the $ZnSO_4$. All other subjects treated as out-patients do not referred side effects attributed to $ZnSO_4$. Clinical characteristics of all groups are given in *Table I*.

Figure 1 shows the effect of the $ZnSO_4$ on serum UCB levels (mg/dL) in subjects with Gilbert's syndrome in acute and chronic forms. The UCB levels range from 2.66 ± 0.91 at the baseline to 2.84 ± 0.89 and after administration of $ZnSO_4$ there was an decrease to 2.34 ± 0.90 ($p < 0.0003$). Whereas serum UCB levels in subjects with Gilbert's syndrome. treated in a chronic form decreased from 1.8 ± 0.36 to 1.48 ± 0.32 ($p < 0.005$). The serum Zn levels (μg/dL) in acute an chronic form. The levels increased from 101.51 ± 12.38 to 122.08 ± 20.0, ($p < 0.04$) and from 117.64 ± to 130.7 ± 6.6, ($p < 0.03$) in subjects with Gilbert's syndrome.

Figure 2 shows the effect of $ZnSO_4$ on serum UCB and Zn levels in the control group. There was no change in UCB levels after administration of $ZnSO_4$. Whereas Zn levels increased from 98.0 ± 7.3 to 128.0 ± 21.9) $p < 0.03$. Fecal UCB concentration (μg/g dry) was increased after oral administration of $ZnSO_4$ from 27.25 ± 21.4 to 37.2 ± 23.08, $p < 0.03$ in subjects treated in acute form, but not in the control group *(Table II)*.

Discussion

The results of this study showed that acute and chronic oral administration of $ZnSO_4$ decreases serum UCB levels significantly in subjects with Gilbert´s syndrome. Most likely by inhibition of the EHC of UCB.

How can we explain the effect of $ZnSO_4$ on the serum UCB concentration in subjects with Gilbert´s syndrome? First, we have shown in our *in vitro* studies that Zn salts at physiological pH and above adsorb UCB essentially completely from unsaturated BS micellar solutions. This indicate that there is a physico-chemical interaction between UCB and Zn salts.[17] The interaction probably is via the carboxyl groups of UCB, as occurs with other divalent cations such as Ca^{2+} [20-22] leading to the formation of calcium bilirubinate [Ca(BH)2].

Second, we have also found that $ZnSO_4$ reduced biliary bilirubin secretion in hamsters *in vivo*.[17] One explanation for the reduced secretion of biliary bilirubin observed in the hamsters fed $ZnSO_4$ is that this is probably the result of the interaction between UCB and $ZnSO_4$ at the distal

Table I. Characteristics of the three treated groups at entry.

	Acute	Chronic	Control	p Value
Clinical				
No. patients	10	5	5	-
Age (yr)	33.3 ± 5.8	31.8 ± 7.9	25.4 ± 1.5	NS
Sex (M/F)	(8/2)	(4/1)	(4/1)	0.001
BMI	22.6 ± 2.7[a]	23.9 ± 2.56	27.4 ± 2.0	0.05
Serum				
UCB (mg/dL)	2.64 ± 1.04[b]	2.22 ± 0.8[b]	0.9 ± 0.17	0.05

BMI, body mass index UCB, unconjugated bilirubin [a] *vs* control [b] *vs* control NS, No significant

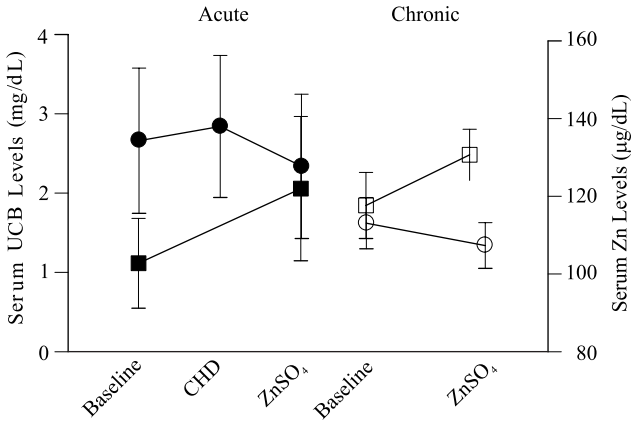

Figure 1. Shows the effect of $ZnSO_4$ on serum UCB levels in subjects with Gilbert's syndrome, in acute and chronic form. In both forms of treatment there were a significant decrease in serum UCB levels, The UCB levels range from 2.66 ± 0.91 at the baseline to 2.84 ± 0.89 and after administration of $ZnSO_4$ there was an decrease to 2.34 ± 0.90 ($p < 0.0003$). Whereas serum UCB levels in subjects treated in a chronic form decreased from 1.8 ± 0.36 to 1.48 ± 0.32 ($p < 0.005$). The serum Zn levels (µg/dL) in acute an chronic form. The levels increased from 101.51 ± 12.38 to 122.08 ± 20.0, ($p < 0.04$) and from 117.64 ± to 130.7 ± 6.6, ($p < 0.03$) . Values are means ± SD.

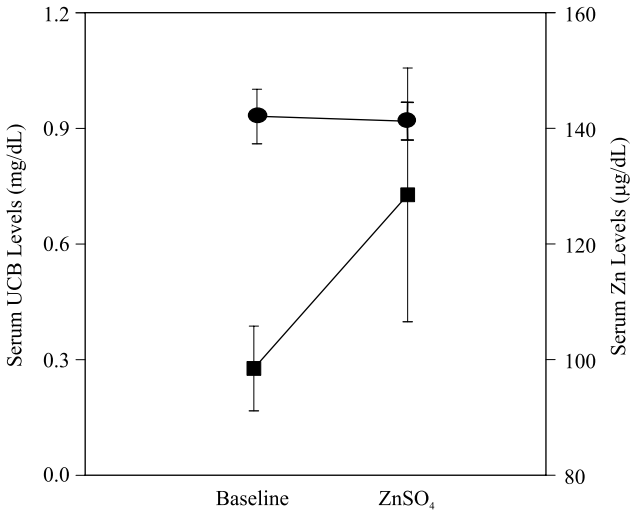

Figure 2. Shows the effect of $ZnSO_4$ on serum UCB levels in healthy volunteers acting as controls. There was no change in serum UCB levels, after administration of $ZnSO_4$. Whereas serum Zn levels (µg/dL) increased from 98.0 ± 7.3 to 128.0 ± 21.9, ($p < 0.03$). Values are means ± SD.

intestinal lumen where the pH is ~ 8, and we know from our *in vitro* experiments that at this pH, UCB might interact with $ZnSO_4$.[17] Then this interaction leads to formation of a flocculated material, which in turn, may be excreted in the fecal content as we shown in this study. As a resulted of this interaction, the final event is an interruption of the EHC of UCB by $ZnSO_4$.

Third, to look the most potent effect of $ZnSO_4$ was seen when we used a carbohydrate-rich diet which is known is able to increase the serum UCB levels.[23,24] In fact, we saw that the effect of $ZnSO_4$ was more evident after a 14-hour fasting period and a carbohydrate-rich diet. The serum

Table II. Fecal unconjugated bilirubin [UCB (µg/g dry)] concentrations before and 24 h after oral administration of $ZnSO_4$.

	Before	After	p Value
Control (n = 4)	5.65 ± 1.7	6.05 ± 0.82	NS
Acute (n = 4)	27.25 ± 21.4	37.2 ± 23.08	0.03

NS, Non significant.

UCB levels decreased significantly after the administration of a 40 mg of $ZnSO_4$ given acutely (see *Figure 1*), and these findings were resembled after oral administration of 100 mg for seven days. Interestingly, the low dose was probably potent enough to decrease the serum UCB concentration.

Four, serum Zn levels increased 20% and 13% in both acute and chronic administration of $ZnSO_4$, suggesting an effect of this salt on serum bilirubin. Whereas fecal UCB concentration was increased after oral administration of $ZnSO_4$ in subjects treated in acute form, but not in the control group.

On the other hand, we believed that $ZnSO_4$ might act as insoluble calcium phosphate which has been proposed as a good cheletor of UCB from bile salts solutions[25] and in fact, oral calcium phosphate/carbonate has been suggested as effective in treating hyperoxaluria in patients with ileal resection.[26-28] In a recent paper, insoluble calcium salts were shown to suppress serum bilirubin levels in patients with Crigler Najjär Type II syndrome.[29] According with the results of the present study Zn which is the least toxic of the trace metals, may be used in the following conditions: a) to inhibit EHC of bilirubin associated with bile salt absorption [distal ileal diseases (Crohn's, AIDS, resection, bypass, and immaturity)], b) to suppress serum bilirubin levels in patients with Crigler Najjär Type II syndrome, c) in patients with chronic cholestasis diseases (primary biliary cirrhosis, primary sclerosing cholangitis), and d) chronic liver diseases (hepatic cirrhosis) interestingly, Dashti et al[30] observed in experimental liver cirrhosis produced by thioacetamide that zinc sulphate- treated animals showed a restoration of normal hepatic and plasma zinc and copper levels plasma. Similarly, plasma levels of aspartate aminotransferase, alanine aminotransferase, gamma-glutamyl aminotransferase, and total bilirubin decreased significantly. Light microscopic studies showed that most of the hepatocytes appeared normal in zinc-treated as compared with untreated cirrhotic animals.

In conlusion, this study showed that acute and chronic administration of oral $ZnSO_4$ decreases serum UCB levels significantly in subjects with Gilbert´s syndrome. This observation suggests inhibition of a "normal" EHC of UCB, and strongly support the potential usefulness of $ZnSO_4$ in the treatment a series of pathological conditions that are characterized by hyperbilirubinemia.

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