JPP Journal of Pharmacy and Pharmacology
ROYAL PHARMACEUTICAL SOCIETY

JPP Journal of Pharmacy and Pharmacology

Research Paper

JPP 2011, 63: 627–635

Received June 30, 2010
Accepted January 18, 2011
DOI
10.1111/j.2042-7158.2011.01259.x
ISSN 0022-3573

Oltipraz therapy in patients with liver fibrosis or cirrhosis: a randomized, double-blind, placebo-controlled phase II trial

Sang Geon Kim[a,b], Young Mi Kim[b], Jong Young Choi[c], Joon-Yeol Han[c], Jeong Won Jang[c], Se-Hyun Cho[c], Soon Ho Um[d], Chae Yoon Chon[e], Dong Hoo Lee[f], Ja-June Jang[g], Eunsil Yu[h] and Young Sok Lee[c]

[a]College of Pharmacy and Research Institute of Pharmaceutical Sciences, Seoul National University, [b]Interdisciplinary Program of Clinical Pharmacology, Seoul National University, [c]Department of Internal Medicine, The Catholic University of Korea, [d]Department of Internal Medicine, Korea University Hospital, [e]Department of Internal Medicine, Yonsei University Hospital, [f]Department of Internal Medicine, Hanyang University Hospital, [g]Department of Pathology, Seoul National University Hospital and [h]Department of Pathology, Asan Medical Center, Seoul, Korea

Abstract

Objectives Oltipraz, a cancer chemopreventive agent, has an anticirrhotic effect in animals. A phase II trial was designed to investigate the preliminary efficacy of oltipraz therapy in liver fibrosis or cirrhosis.

Methods Of 83 patients who were randomized to receive placebo, oltipraz 60 mg bid or oltipraz 90 mg qd for 24 weeks, 68 completed the study without any major protocol violation. Pre- and post-treatment liver biopsies, and blood fibrosis markers were assessed.

Key findings Twenty-four weeks of oltipraz treatment showed no significant differences in the proportions of patients showing an improvement in histological outcomes, including Ishak fibrosis score. In the oltipraz 60 mg bid group, there was a trend of decreases in hepatic collagen area and plasma transforming growth factor-β1 (TGF-β1, a blood fibrosis marker) levels from baseline to week 24. In the per-protocol population ($n = 68$), decreases in plasma TGF-β1 correlated with those in the Ishak fibrosis score, suggesting that circulating TGF-β1 serves a possible indicator for fibrosis treatment.

Conclusions No significant differences in liver histological outcomes were seen among the three treatment groups in this 24-week pilot study. Our finding indicates an association between TGF-β1 repression and improvement in the histological index of fibrosis.

Keywords clinical trial; liver fibrosis and cirrhosis; oltipraz; TGF-β1

Introduction

Liver fibrosis (LF) is defined as the injury of hepatocytes and accumulation of fibers, and is a prepathologic state of liver cirrhosis (LC); LC involves the formation of septae and nodules, alteration of blood flow and nodular regeneration as well as parenchymal cell destruction and connective tissue formation. Worldwide, chronic hepatitis types B and C are the major causes of LF and LC, which may develop in one-quarter of infected patients so that it is one of the major leading causes of death.[1–3] A few hepatotonic agents (e.g. ursodeoxycholic acid and silymarin) have been recommended for these patients, while other medications symptomatically manage fluid retention, peritoneal ascites, encephalopathy and variceal hemorrhage. An antiviral agent (e.g. lamivudine) may improve hepatic function in decompensated LC patients with replicating HBV, but these patients require continuous treatment. Unfortunately, a limitation of this therapy is its viral resistance.[4,5]

Transforming growth factor β1 (TGF-β1) regulates cell growth and differentiation. In a variety of liver diseases it causes synthesis of extracellular matrix (ECM) proteins and their receptors, ECM deposition and inhibition of collagenase activity in the liver after injuries,[6,7] thus playing a key role in LF and LC. In response to TGF-β1, hepatic stellate cells produce large quantities of ECM.[6,7] TGF-β1 receptor blockade experiments in animals have verified the importance of TGF-β1 in LF.[8] However, no therapeutic agent that inhibits TGF-β1 expression is currently available. It should be pointed out that insulin resistance is observed in the peripheral organs of most cirrhotic patients, and diabetic patients are at increased risk

Correspondence: Sang Geon Kim, College of Pharmacy, Seoul National University, San 56-1, Sillim-dong, Kwanak-gu, Seoul 151-742, South Korea.
E-mail: sgk@snu.ac.kr
Young Sok Lee, Division of Gastroenterology, Department of Internal Medicine, The Catholic University of Korea, 505 Banpo dong, Seocho-gu, Seoul, 137-701, South Korea.
E-mail: ysleecumc@hanmail.net

NIH clinical trial registration number: NCT00956098.
Both SGK and YMK equally contributed to the data analyses, interpretation, and manuscript writing.

of developing hepatic diseases.[9–11] Hence therapeutic interventions by new tailor-made drugs that not only repress TGF-β1, but also prevent insulin resistance and alterations in energy metabolism would be beneficial to patients with LC.

One of the potential drug candidates for the treatment of LF and LC is 4-methyl-5-(2-pyrazinyl)-1,2-dithiol-3-thione (oltipraz), which has been extensively studied as a cancer chemopreventive agent.[12–16] Comprehensive mechanistic studies have revealed the chemopreventive efficacy of oltipraz, a finding further supported by the outcome of phase II studies.[17,18] In our previous studies, oltipraz was identified as the agent effective in resolving accumulated fibers and regenerating cirrhotic liver in animal models; oltipraz reduced the intensities of cirrhotic nodules and eliminated accumulated ECM.[19,20] Consequently, oltipraz treatment improved the viability of animals with LC. Moreover, our subsequent investigation led to the identification of oltipraz as the candidate that inhibits insulin resistance and hyperglycemia by a mechanism involving AMP-activated protein kinase-mediated p70 ribosomal S6 kinase-1 inhibition.[21] Hence oltipraz treatment may have an additional benefit in sensitizing the insulin response in the liver. Recently, we also reported that this agent exerts an inhibitory effect on liver X receptor-α activity and liver X receptor-α-dependent hepatic lipogenesis.[22]

In view of the anticirrhotic effectiveness and other beneficial effects of oltipraz in animal models, a randomized, double-blind, placebo-controlled phase II multicenter trial was designed and conducted to evaluate the preliminary efficacy of 24-week oltipraz treatment in patients with LF or LC induced by chronic hepatitis type B or C. This is the first study to evaluate the efficacy of oltipraz, a potential cancer chemopreventive agent, in fibrotic or cirrhotic patients with the aim of developing it as a new class of therapeutics for LF and LC.

Materials and Methods

Patients and study design

Patients were recruited from seven different hospitals in South Korea. Patients (ages 25–65) were eligible for enrollment if they had been positive for hepatitis B surface antigen, or for hepatitis C virus (HCV) RNA or anti-HCV, and had been diagnosed with LF or LC by histological examination and ultrasonography. The participants individually provided written informed consent.

Patients were excluded if they had any of the following criteria: (i) treatment with antiviral agents, immunosuppressants or glucocorticoids within the previous 6 months, or with biphenyl dimethyl dicarboxylate within the previous 4 weeks, (ii) treatment with any other investigational drug within 30 days before the initiation of study treatment, (iii) Child–Pugh class C, (iv) mean weekly consumption of ≥80 g alcohol within the previous 30 days, use of enzyme inducers or inhibitors, or drug abuse that might affect this study, (v) a known hypersensitivity to oltipraz or its structurally related compounds, (vi) decompensated LC with the history of ascites, hemorrhage from varicoses or hepatic encephalopathy within the previous 6 months, (vii) evidence of hepatocellular carcinoma (a rising serum level of α-fetoprotein or suspicious foci on abdominal sonography), (viii) a history of liver transplantation, (ix) pregnancy, lactation or unwillingness of contraception during the study period, (x) other serious concurrent illness, e.g. severe hemorrhagic gastrointestinal, renal, pulmonary, neurological, cardiovascular diseases (congestive heart failure of class III or above or a history of myocardial infarction within the previous 6 months), cancer, autoimmune or severe psychological diseases, (xi) bilirubin concentration >2.0 mg/dl, serum albumin concentration <2.5 g/dl, or prolongation of prothrombin time by more than 4 s, or (xii) any patient who was judged inappropriate for clinical study by the investigators.

This was a multicenter, randomized, double-blind, placebo-controlled study conducted between February 1, 2006 and April 13, 2007. A total of 100 patients with LF or LC were evaluated, and 83 eligible patients were randomly assigned in a 1 : 1 : 1 ratio to receive placebo, oltipraz 60 mg bid or oltipraz 90 mg qd for 24 weeks. All study personnel and participants were blinded to treatment assignment throughout the study. Patients were instructed to take the study drug twice a day 1 h after the morning and evening meals (placebo, three placebo tablets at each time; 60 mg bid group, two 30 mg oltipraz tablets plus one placebo tablet at each time; and 90 mg qd group, three 30 mg oltipraz tablets in the morning and three placebo tablets in the evening). The patients who completed 24 weeks of treatment were followed for an additional 12 weeks. Block-randomization with a block size of three or six was centrally performed to obtain balanced groups, and the randomization code was developed using a computer random number generator. The study protocol and informed consent were approved by the Institutional Review Board at each study site. This clinical trial was conducted according to the GCP guidelines and the Declaration of Helsinki.

Objectives

The primary objective of the study was to evaluate the preliminary efficacy of 24-week administrations of oltipraz (60 mg bid or 90 mg qd) compared with placebo in patients with LF or LC induced by chronic hepatitis type B or C.

Outcomes

The primary outcome was the proportion of patients showing a reduction of one or more points in the Ishak fibrosis score (range, 0–6)[23] on liver biopsy after treatment with oltipraz for 24 weeks (improved, a decrease of at least one point; unchanged, no change or an increase of one point; and worsened, an increase of greater than one point). Secondarily, effectiveness was assessed by evaluating changes in the following parameters from baseline to the end of treatment: (i) the Ishak fibrosis score, (ii) modified Knodell's histological activity index (HAI) score (necro-inflammatory score; range, 0–18) (improved, 2-point or more decrease; unchanged, less than 2-point decrease or increase; and worsened, 2-point or more increase),[23] (iii) Child–Pugh score and (iv) hepatic collagen area. The pre-specified exploratory analyses included a comparison of paired blood levels of TGF-β1, hyaluronic acid (HA), and alanine aminotransferase (ALT) from baseline to week 24.

Assessments

Patients were assessed at weeks 0, 1, 2, 4, 8, 12, 16, 20 and 24 after the initiation of study treatment. During the post-treatment follow-up period, patients were monitored every 6 weeks (weeks 30 and 36). At every visit, all clinical adverse events were recorded and general physical examination, laboratory tests (blood chemistry, hematology and urinalysis) and the measurement of vital signs (weight, blood pressure and pulse) were performed.

Liver biopsy samples were collected at baseline and within 1 month after the end of treatment. If liver biopsy samples collected within 3 months before the initiation of study treatment were available for histological and morphometrical evaluation, additional pre-treatment biopsy was not conducted. Liver biopsy specimens were reviewed by two independent histopathologists who were blinded to the treatment assignments. In addition, blood samples obtained at weeks 0, 12 and 24 were analysed for TGF-β1 and HA using a human TGF-β1 immunoassay kit (R&D Systems, Minneapolis, MN, USA) and HA test kit (Corgenix Inc., Broomfield, CO, USA), respectively.

Statistical analysis

The sample size was calculated by considering the proportion of patients showing a reduction in the Ishak fibrosis score; the proportions for placebo and oltipraz-treated groups were assumed to be 5 and 35%, respectively, on the basis of previous reports.[24,25] At least 21 subjects were required for each group to have a power of 80% at the 5% level of significance. Assuming a dropout rate of 20%, the estimated sample size was 27 per group. The primary efficacy outcome was analysed in both the modified intention-to-treat (MITT) population, who took at least one dose of the study drug, and per-protocol (PP) population, who completed the study without any major protocol violation. In the MITT population-based analysis, patients without post-treatment liver biopsy were considered to have no change. The assessments of secondary efficacy outcomes, including exploratory analyses, were primarily based on the PP population.

Differences between treatment groups were compared using ANOVA or *t*-test for continuous variables and chi-square or Fisher's exact tests for categorical variables. Stratified analyses were performed using the Cochran–Mantel–Haenzsel test. The Kruskal–Wallis test was used to compare the changes in the Ishak fibrosis score, Child–Pugh score or collagen area among the three treatment groups. Pre- and post-treatment levels were compared using paired *t*-test and Wilcoxon signed rank test for blood fibrosis markers and collagen area, respectively. Correlation between variables was analysed by Pearson's correlation test. All statistical analyses were performed using SPSS for Windows (version 15.0; SPSS, Chicago, IL, USA). A two-sided *P*-value of less than 0.05 was considered statistically significant.

Results

Patient disposition and baseline characteristics

Of 83 randomized patients, 2 withdrew their consent (Figure 1). The MITT population consisted of 81 patients; 28 in placebo, 28 in oltipraz 60 mg bid and 25 in the oltipraz 90 mg qd groups. Of the 81 patients, 5 withdrew consent during the study and 7 discontinued treatment because of adverse events. One patient in the 60 mg bid group and 4 in the 90 mg qd group permanently stopped the study drug due to mild-to-moderate drug-related adverse events.[26] Of the 69 patients who completed the 24-week treatment, all were evaluable at week 24, except for one with major protocol violation (use of prohibited concomitant medication) in the 90 mg qd group (PP population, $n = 68$). The demographic and baseline characteristics of the patients are summarized in Table 1, and were not significantly different among the three treatment groups. Of the 81 patients, 55 (68%) had advanced fibrosis or cirrhosis (Ishak fibrosis score of 4 to 6) at baseline. All patients were in Child–Pugh class A.

Efficacy

Paired liver biopsies from 68 patients (PP population) were compared. The proportions of patients showing an improvement in the Ishak fibrosis score, the primary end point of this study, were not significantly different among groups (Table 2). In the MITT population-based analysis, the results were similar: 21% (6/28), 18% (5/28) and 12% (3/25) for the placebo, 60 mg bid and 90 mg qd groups, respectively ($P = 0.709$). Additional analyses using the Cochran–Mantel–Haenszel test stratified by duration of LF or LC, or concomitant administration of other hepatotonic agents (e.g. ursodeoxycholic and silymarin) showed no significant differences. The proportions of patients showing an improvement in modified Knodell's HAI score were also similar in the three treatment groups (Table 2).

The mean changes in the Ishak fibrosis, modified Knodell's HAI or Child–Pugh score before and after therapy were not different in the three treatment groups (data not shown). No differences between pre- and post-therapy scores in the liver histological outcomes were observed either (Supporting Table S1 online). In patients treated with 60 mg bid, however, hepatic collagen area tended to decrease (from $16.9 \pm 15.7\%$ to $12.6 \pm 11.2\%$, $P = 0.093$) (Figure 2 and Supporting Table S1 online).

Plasma TGF-β1, HA and ALT levels pre- and post-therapy were compared, which was a pre-specified exploratory analysis. Interestingly, there was a trend of decrease in plasma TGF-β1 content from baseline to week 24 in the 60 mg bid group (9.7 ± 4.5 vs 8.3 ± 2.8 ng/ml), but it did not reach statistical significance ($P = 0.149$) (Figure 3, upper). Mean percentage change in plasma TGF-β1 also tended to be lower in the 60 mg bid group than that in placebo (–6.9 vs 12.4%, $P = 0.068$) (Figure 3, lower). The proportions of patients who showed the decrease in TGF-β1 level were 42% (11/26), 64% (16/25) and 53% (9/17) for the placebo, 60 mg bid and 90 mg qd groups, respectively. There were no differences in plasma HA content pre- and post-therapy with oltipraz in each group or among the three treatment groups (Supporting Figure S1 online). Unlike the case of TGF-β1, plasma HA content in patients treated with 60 mg bid tended to increase by week 24 compared with baseline (from 69.7 ± 52.7 to 88.6 ± 68.5 ng/ml, $P = 0.052$). In patients treated with placebo or oltipraz 60 mg bid, mean ALT was elevated by 18.5 and 23.1 U/l, respectively. Treatment with 90 mg qd resulted in a decrease

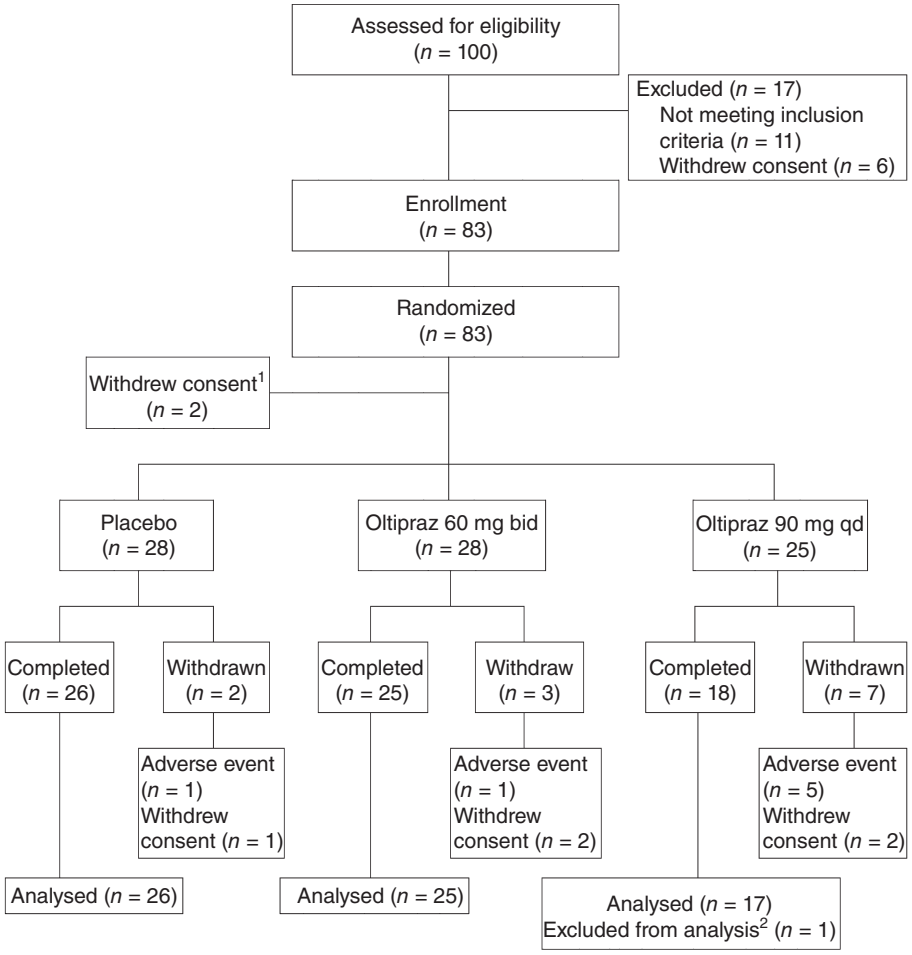

Figure 1 Patient flow diagram.

in mean ALT by 15.7 U/l. Nonetheless, no statistically significant differences were noted in the changes of ALT ($P = 0.301$) (Supporting Figure S1 online).

Association of antifibrotic efficacy and circulating TGF-β1 repression

In this trial, a significant correlation was observed between changes in the Ishak fibrosis score and percentage changes in circulating TGF-β1 content in all patients of the three treatment groups (PP population, $n = 68$; $P = 0.046$, $r = 0.243$) (Figure 4a). By contrast, percentage changes in serum HA content did not correlate with changes in the Ishak fibrosis score (PP population, $n = 68$; $P = 0.505$, $r = -0.082$) (Supporting Figure S2 online). These results were also supported by univariate logistic regression analyses (Supporting Table S2 online). Percentage changes in plasma TGF-β1 levels between pre- and post-therapy were associated with the response in Ishak fibrosis score ($P = 0.014$), but no such association was found with other markers such as blood HA or ALT levels. Our finding, showing an association of antifibrotic efficacy and TGF-β1 repression, supports the concept that plasma TGF-β1 serves as a possible indicator for fibrosis treatment.

Consistently, hepatic collagen area was significantly decreased from baseline to week 24 in patients with decreases in plasma TGF-β1 content (from 19.21 ± 22.57 to 11.65 ± 9.93; $P = 0.047$) (Figure 4b). In the same population ($n = 36$), the proportion of patients showing a reduction in the Ishak fibrosis score was higher than that in patients without a decrease in TGF-β1 (10/36, 27.8% vs 4/32, 12.5%), but this difference did not reach statistical significance ($P = 0.144$, Fisher's exact test).

Discussion

In previous animal studies, oltipraz treatment reduced the number of cirrhotic nodules and the staining intensities of nodular capsules; both fibrosis index score and collagen accumulation were decreased after treatment of cirrhotic rats with oltipraz for 4 weeks.[19,20] In patients with LC, hepatic disturbances are functionally monitored by peritoneal fluid

Table 1 Baseline characteristics (MITT population, $n = 81$)

Characteristic	Treatment arm			*P*-value
	Placebo ($n = 28$)	Oltipraz 60 mg bid ($n = 28$)	Oltipraz 90 mg qd ($n = 25$)	
Gender				0.502[a]
Male	22 (78.6)	20 (71.4)	16 (64.0)	
Female	6 (21.4)	8 (28.6)	9 (36.0)	
Age (years)	47.5 ± 7.8	46.3 ± 6.6	47.4 ± 6.8	0.782[b]
Weight (kg)	65.1 ± 8.4	68.1 ± 10.6	64.9 ± 9.1	0.379[b]
Route of transmission				0.917[c]
Transfusion	0 (0.0)	1 (3.6)	0 (0.0)	
Contact with the infected	3 (10.7)	5 (17.9)	3 (12.0)	
Vertical	6 (21.4)	7 (25.0)	6 (24.0)	
Unknown	19 (67.9)	15 (53.6)	16 (64.0)	
Duration of hepatitis (months)	131.6 ± 88.7	122.0 ± 88.7	117.0 ± 90.7	0.831[b]
<120 months	15 (53.6)	16 (57.1)	12 (48.0)	0.800[a]
≥120 months	13 (46.4)	12 (42.9)	13 (52.0)	
Duration of liver fibrosis/cirrhosis (months)	52.2 ± 41.6	40.0 ± 52.0	43.5 ± 41.7	0.587[b]
<40 months	11 (39.3)	17 (60.7)	14 (56.0)	0.236[a]
≥40 months	17 (60.7)	11 (39.3)	11 (44.0)	
Child–Pugh class				
A	28 (100)	28 (100)	25 (100)	
Child–Pugh score				0.235[b]
Mean ± SD	5.04 ± 0.19	5.14 ± 0.36	5.04 ± 0.20	
Median (range)	5 (5–6)	5 (5–6)	5 (5–6)	
Ishak fibrosis score				0.325[b]
Mean ± SD	4.11 ± 1.26	4.57 ± 1.50	4.60 ± 1.29	
Median (range)	4 (2–6)	5 (1–6)	5 (2–6)	
Modified Knodell's HAI score				0.682[b]
Mean ± SD	4.14 ± 2.35	4.36 ± 2.38	4.80 ± 3.50	
Median (range)	3 (0–10)	4 (2–11)	4 (1–12)	
Collagen area (%)				0.531[b]
Mean ± SD	15.8 ± 23.1	16.6 ± 15.7	11.7 ± 7.2	
Median (range)	10.2 (1.3–123.3)	11.3 (0.3–60.6)	12.9 (1.4–26.4)	

HAI, histological activity index; MITT, modified intention-to-treat; SD, standard deviation. [a]Chi-square test. [b]ANOVA. [c]Fisher's exact test. Data are number of patients (%) or mean ± SD unless specified otherwise.

Table 2 Proportions of patients with improvement in the Ishak fibrosis score and modified Knodell's HAI score on liver biopsy at week 24 compared with baseline (PP population, $n = 68$)

Variable, *n* (%)	Treatment arm			*P*-value
	Placebo ($n = 26$)	Oltipraz 60 mg bid ($n = 25$)	Oltipraz 90 mg qd ($n = 17$)	
Ishak fibrosis score				0.908[a]
Improved	6 (23.1)	5 (20.0)	3 (17.7)	
Unimproved	20 (76.9)	20 (80.0)	14 (82.4)	
Unchanged	18 (69.2)	18 (72.0)	12 (70.6)	
Worsened	2 (7.7)	2 (8.0)	2 (11.8)	
Modified Knodell's HAI[b] fibrosis score				0.560[a]
Improved	5 (19.2)	4 (16.0)	5 (29.4)	
Unimproved	21 (80.8)	21 (84.0)	12 (70.6)	
Unchanged	17 (65.4)	15 (60.0)	7 (41.2)	
Worsened	4 (15.4)	6 (24.0)	5 (29.4)	

HAI, histological activity index; PP, per-protocol. [a]Chi-square test. [b]Necro-inflammatory score (range, 0–18).

accumulation due to a decrease in plasma albumin content and presumed fibrosis-induced portal hypertension.[27] The therapeutic efficacy of oltipraz in decreasing peritoneal fluid accumulation was confirmed by the data in our animal model study. On the basis of these experimental results, the present study investigated the preliminary efficacy of oltipraz in patients with LF or LC induced by chronic hepatitis.

In the 4 weeks of toxicity studies, oltipraz treatment was safe and showed no notable toxicity: no observable adverse effect levels (NOAEL) of oltipraz were 10 and 30 mg/kg/day

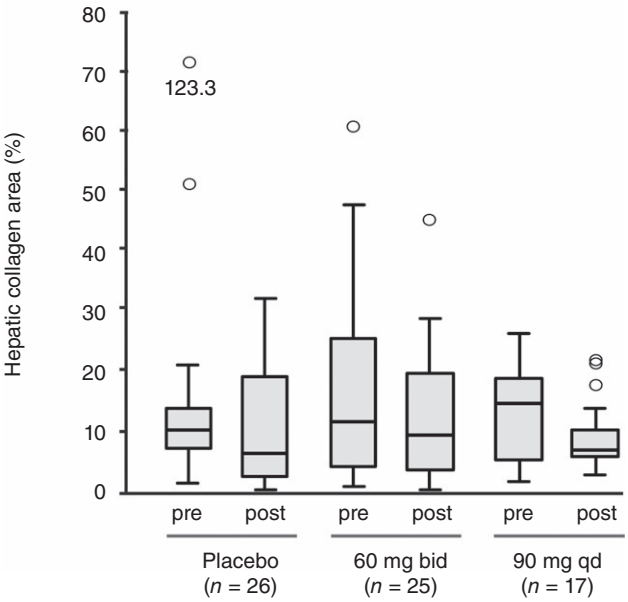

Figure 2 Changes in hepatic collagen area from baseline to week 24 (PP population, $n = 68$). In the box plots, the lower and upper ends of each box represent the 25th and 75th percentiles, the horizontal line inside the box displays the median value and the whiskers indicate 1.5 times the interquartile range below the 25th percentile and above the 75th percentile. Open circles denote outlier values. PP, per-protocol; Pre, pre-treatment; Post, post-treatment.

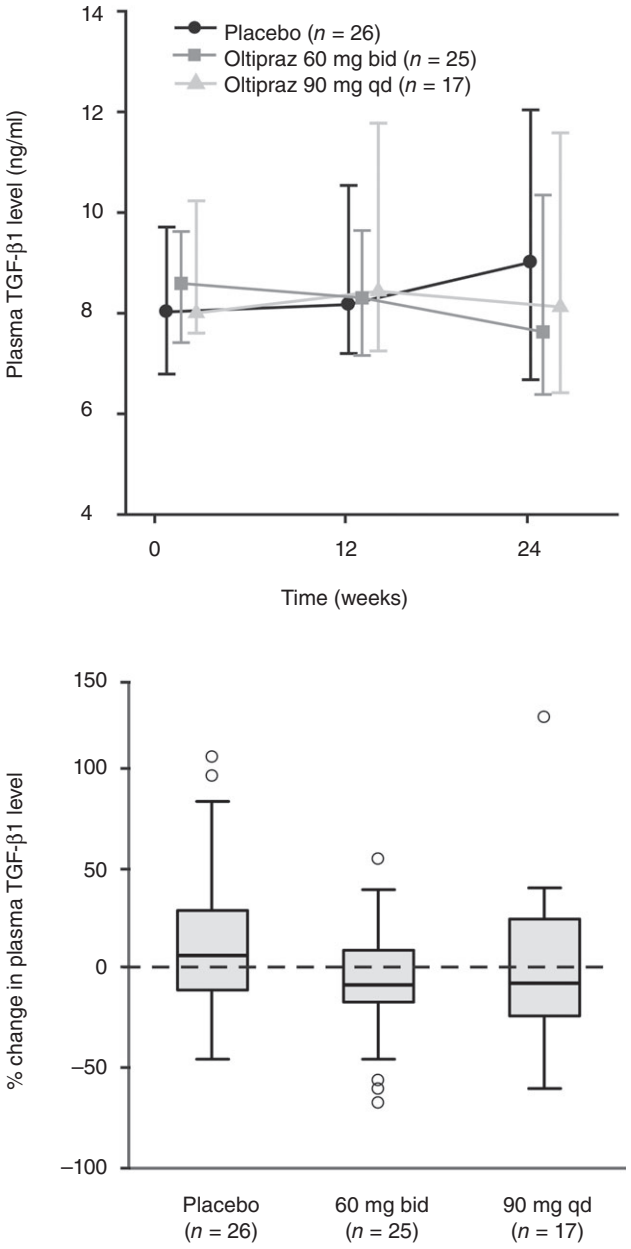

Figure 3 Changes in the blood contents of TGF-β1 from baseline to week 24 (PP population, $n = 68$). Data are plotted as median with error bars indicating 25th and 75th percentiles (upper). In the box plots, the lower and upper ends of each box represent the 25th and 75th percentiles, the horizontal line inside the box displays the median value, and the whiskers indicate 1.5 times the interquartile range below the 25th percentile and above the 75th percentile (lower). Open circles denote outlier values. TGF-β1, transforming growth factor-β1; PP, per-protocol.

in rats and dogs, respectively. Moreover, the NOAEL in chronic toxicity studies conducted in rats and dogs for 26 weeks and 39 weeks, respectively, was 5 mg/kg/day, consistent with a previous report.[28] Oltipraz had been applied to humans at a dose of 30 mg/kg/day as a potential schistosomicidal agent.[29] Moreover, other dose regimens, including 6 months of 125 mg daily dosing and 30 days of intermittent dosing (200 mg × 2 or 500 mg/week), were well tolerated with no serious toxicities in human chemopreventive studies.[30,31] Prior to this multiple-dose study, a single-dose pharmacokinetics and safety study of oltipraz (30–90 mg) in patients with LF and LC had been performed.[26] Five in 31 patients reported 7 cases of adverse events, which were graded as mild and 'unlikely related' or 'not related' to the study drug. Oltipraz was rapidly absorbed, showing dose-dependent pharmacokinetics at the doses of 30–90 mg. For the present study, two dosage regimens (60 mg bid and 90 mg qd) were selected based on the single-dose pharmacokinetic study of oltipraz in LF and LC patients. Oltipraz therapy in patients with LF or LC, at the two doses for 24 weeks, was safe and well tolerated, but the incidences of AEs were marginally higher in the 90 mg qd than those in the placebo group.[26]

TGF-β1 produced from activated hepatic stellate cells plays a key role in promoting liver fibrogenic responses.[7] In animal models, oltipraz's anticirrhotic efficacy is supported by TGF-β1 repression as well as by a decrease in Knodell score.[19,20] Twenty-four weeks of oltipraz treatment led to no significant differences in the improvement in histological outcomes, including Ishak fibrosis score, modified Knodell's HAI and Child–Pugh score. However, there was a trend of decrease in hepatic collagen areas or plasma TGF-β1 levels from baseline to week 24 in the oltipraz 60 mg bid group. Interestingly, most of the patients (71%) who showed an improvement in the Ishak fibrosis score had decreased levels of plasma TGF-β1 at week 24, suggesting that a decrease in circulating TGF-β1 may precede the change in liver histology.

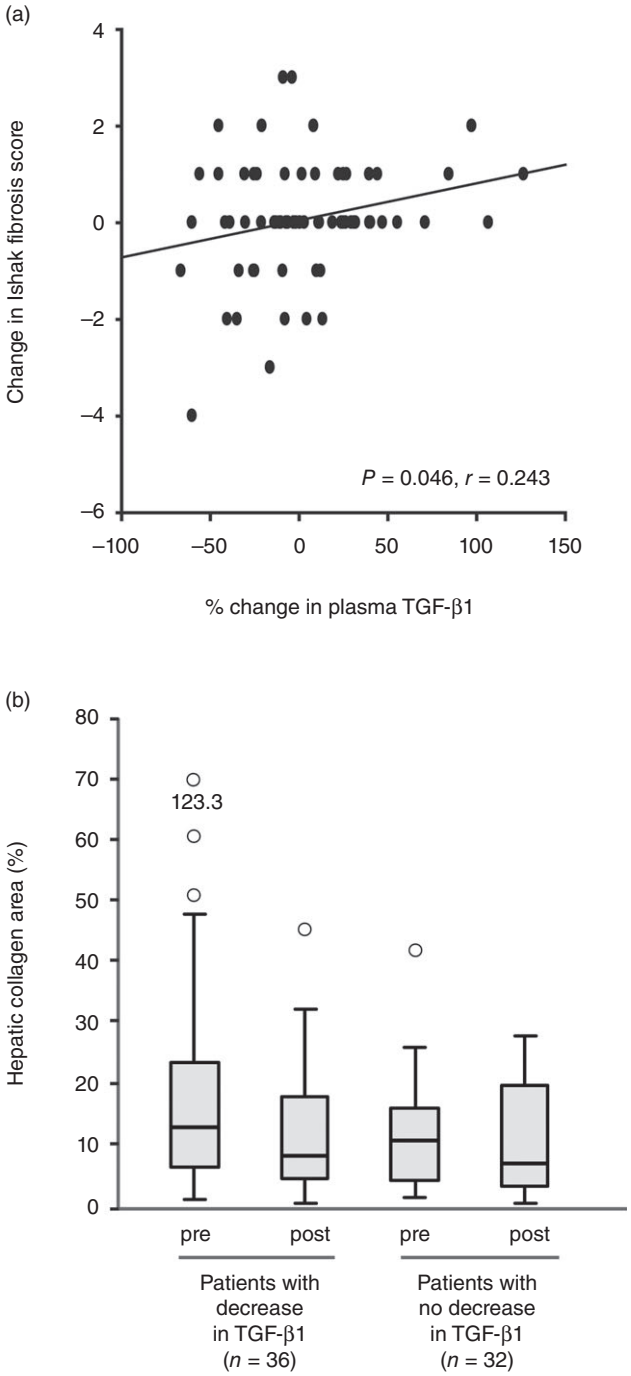

Figure 4 Correlations of histological outcomes with changes in plasma TGF-β1. (a) Correlation between changes in the Ishak fibrosis score and percent changes in plasma TGF-β1 concentration in PP population ($n = 68$). (b) Changes of hepatic collagen area in patients with decreases in plasma TGF-β1 or those with no decreases in it from baseline to week 24 (PP population, $n = 68$). In the box plots, the lower and upper ends of each box represent the 25th and 75th percentiles, the horizontal line inside the box displays the median value and the whiskers indicate 1.5 times the interquartile range below the 25th percentile and above the 75th percentile. Open circles denote outlier values. TGF-β1, transforming growth factor-β1; PP, per-protocol; Pre, pre-treatment; Post, post-treatment.

Taken together, the lack of differences in the liver histological outcomes may be attributed in part to the short period of oltipraz treatment. The anticirrhotic effects of certain antiviral agents also require long periods of treatment.[5,24,25]

Initially, at least 21 patients per group were estimated to be needed for efficacy analysis. However, in the 90 mg qd group, the number of patients who completed the study without major protocol violation was only 17. The statistical power depends on various factors, including statistical significance criterion and magnitude of the effect of interest and sample size in each group. Because of a lack of differences in the primary outcome between groups and the reduced sample size in the 90 mg qd group, this study may have lower statistical power than desired. This was one of the limitations of the present study.

Intriguingly, we found a correlation between change in plasma TGF-β1 and that in the Ishak fibrosis score in all patients from the three treatment groups. Our results parallel previous reports showing that circulating TGF-β1 correlates with hepatic dysfunction in LC patients.[32,33] Our data, illustrating a significant decrease in hepatic collagen area from pre-treatment in patients with TGF-β1 repression, adds support to this contention. The existence of bridging (portal–portal or portal–central) is one important criterion in the Ishak fibrosis scoring system.[23] Hepatic collagen area (%) is obtained by measuring total liver area and blue-stained collagen area in each liver biopsy sample. This difference in measurement or scoring might explain why the proportion of patients showing a reduction in the Ishak fibrosis score was not significantly higher in patients with a decrease in TGF-β1 despite a significant decrease in hepatic collagen area.

According to our previous report, the steady-state plasma concentrations of oltipraz in patients with a decrease in plasma TGF-β1 at week 24 from baseline were significantly higher than in those without a decrease in plasma TGF-β1.[31] Furthermore, the mean plasma concentrations of oltipraz during the 24-week treatment period were significantly higher in the 60 mg bid group than those in the 90 mg qd group.[31] Considering the trend of decreases in hepatic collagen area and plasma TGF-β1, better safety profile and higher steady-state plasma concentrations, the 60 mg bid regimen should be preferred for future expanded studies. Since many patients with LF or LC have comorbid illnesses (e.g. insulin resistance, diabetes and other complications), the effectiveness of oltipraz also needs to be explored in the subgroups of liver fibrotic or cirrhotic patients with those combined illnesses.

Conclusions

No significant differences in liver histological outcomes were seen among the three treatment groups in this 24-week pilot study. Our finding indicates an association between TGF-β1 repression and improvement in histological index of fibrosis.

Declarations

Conflict of interest

The Author(s) declare(s) that they have no conflicts of interest to disclose.

Funding

This work was supported by funds from the Ministry of Health & Welfare (No. A020468) and CJ CheilJedang Co., South Korea, and in part by the National Research Foundation of Korea grant funded by the Korea government (MEST) (No. 2010-0001706), South Korea.

References

1. DeBakey SF *et al*. Liver cirrhosis mortality in the United States, 1970–1993. National Institute on Alcohol Abuse and Alcoholism; Surveillance Report #41, 1996.
2. Yoon Y *et al*. Liver cirrhosis mortality in the United States, 1970–1998. National Institute on Alcohol Abuse and Alcoholism; Surveillance Report #57, 2001.
3. Kakizoe T. *Cancer Statistics in Japan 1999*. Tokyo: Foundation for Promotion of Cancer Research, 1999.
4. Yao FY *et al*. Lamivudine treatment is beneficial in patients with severely decompensated cirrhosis and actively replicating hepatitis B infection awaiting liver transplantation: a comparative study using a matched, untreated cohort. *Hepatology* 2001; 34: 411–416.
5. Dienstag JL *et al*. Histological outcome during long-term lamivudine therapy. *Gastroenterology* 2003; 124: 105–117.
6. Freidman SL. The cellular basis of hepatic fibrosis. Mechanisms and treatment strategies. *N Engl J Med* 1993; 328: 1828–1835.
7. Gressner AM, Weiskirchen R. Modern pathogenetic concepts of liver fibrosis suggest stellate cells and TGF-beta as major players and therapeutic targets. *J Cell Mol Med* 2006; 10: 76–99.
8. Qi Z *et al*. Blockade of type beta transforming growth factor signaling prevents liver fibrosis and dysfunction in the rat. *Proc Natl Acad Sci USA* 1999; 296: 2345–2349.
9. Petrides AS *et al*. Pathogenesis of glucose intolerance and diabetes mellitus in cirrhosis. *Hepatology* 1994; 19: 616–627.
10. Cavallo-Perin P *et al*. Mechanism of insulin resistance in human liver cirrhosis. Evidence of a combined receptor and postreceptor defect. *J Clin Invest* 1985; 75: 1659–1665.
11. Dixon JB *et al*. Nonalcoholic fatty liver disease: predictors of nonalcoholic steatohepatitis and liver fibrosis in the severely obese. *Gastroenterology* 2001; 121: 91–100.
12. Wattenberg LW, Bueding E. Inhibitory effects of 5-(2-pyrazinyl)-4-methyl-1,2-dithiol-3-thione (oltipraz) on carcinogenesis induced by benzo[a]pyrene, diethylnitrosamine and uracil mustard. *Carcinogenesis* 1986; 7: 1379–1381.
13. Bolton MG *et al*. Transient intervention with oltipraz protects against aflatoxin-induced hepatic tumorigenesis. *Cancer Res* 1993; 53: 3499–3504.
14. Clapper ML *et al*. Chemopreventive activity of oltipraz against N-nitrosobis(2-oxopropyl)amine-induced ductal pancreatic carcinoma development and effects on survival of Syrian golden hamsters. *Carcinogenesis* 1995; 16: 2159–2165.
15. O'Dwyer PJ *et al*. Modulation of gene expression in subjects at risk for colorectal cancer by the chemopreventive dithiolethione oltipraz. *J Clin Invest* 1996; 98: 1210–1217.
16. Benson AB 3rd *et al*. Chronic daily low dose of 4-methyl-5-(2-pyrazinyl)-1,2-dithioiole-3-thione (Oltipraz) in patients with previously resected colon polyps and first-degree female relatives of breast cancer patients. *Clin Cancer Res* 2000; 6: 3870–3877.
17. Wang JS *et al*. Protective alterations in phase 1 and 2 metabolism of aflatoxin B1 by oltipraz in residents of Qidong, People's Republic of China. *J Natl Cancer Inst* 1999; 91: 347–354.
18. Jacobson LP *et al*. Oltipraz chemoprevention trial in Qidong, People's Republic of China: study design and clinical outcomes. *Cancer Epidemiol Biomarkers Prev* 1997; 6: 257–265.
19. Kang KW *et al*. Oltipraz regenerates cirrhotic liver through CCAAT/enhancer binding protein-mediated stellate cell inactivation. *FASEB J* 2002; 16: 1988–1990.
20. Kang KW *et al*. Inhibition of dimethylnitrosamine-induced liver fibrosis by [5-(2-pyrazinyl)-4-methyl-1,2-dithiol-3-thione] (oltipraz) in rats: suppression of transforming growth factor-beta1 and tumor necrosis factor-alpha expression. *Chem Biol Interact* 2002; 139: 61–77.
21. Bae EJ *et al*. Identification of a novel class of dithiolethiones that prevent hepatic insulin resistance via the adenosine monophosphate-activated protein kinase-p70 ribosomal S6 kinase-1 pathway. *Hepatology* 2007; 46: 730–739.
22. Hwahng SH *et al*. Role of adenosine monophosphate-activated protein kinase-p70 ribosomal S6 kinase-1 pathway in repression of liver X receptor-alpha-dependent lipogenic gene induction and hepatic steatosis by a novel class of dithiolethiones. *Hepatology* 2009; 49: 1913–1925.
23. Ishak K *et al*. Histological grading and staging of chronic hepatitis. *J Hepatol* 1995; 22: 696–699.
24. Medical review part I and statistical review part I of the drug approval package for adefovir dipivoxil (NDA 21-449). 2002. Available at http://www.accessdata.fda.gov/drugsatfda_docs/nda/2002/21-449_Hepsera.cfm
25. Suzuki Y *et al*. Histological changes in liver biopsies after one year of lamivudine treatment in patients with chronic hepatitis B infection. *J Hepatol* 1999; 30: 743–748.
26. Kim SG *et al*. Pharmacokinetics of oltipraz and its major metabolite (RM) in patients with liver fibrosis or cirrhosis: relationship with suppression of circulating TGF-β1. *Clin Pharmacol Ther* 2010; 88: 360–368.
27. Ginès P *et al*. Management of cirrhosis and ascites. *N Engl J Med* 2004; 350: 1646–1654.
28. Crowell JA *et al*. Chronic toxicity studies of 5-(2-pyrazinyl)-4-methyl-1,2-dithiole-3-thione, a potential chemopreventive agent. *Fundam Appl Toxicol* 1997; 35: 9–21.
29. el Tayeb M *et al*. Praziquantel and oltipraz: the treatment of school children infected with *Schistosoma mansoni* and/or *Schistosoma haematobium* in Gezira, Sudan. *Ann Trop Med Parasitol* 1988; 82: 53–57.
30. Dimitrov NV *et al*. Oltipraz concentrations in plasma, buccal mucisa cells, and lipids: pharmacological studies. *Cancer Epidemiol Biomarkers Prev* 2001; 10: 201–207.
31. Benson AB 3rd *et al*. Phase I study of 5-(2-pyrazinyl)-4-methyl-1,2-dithoiole-3-thione (Oltipraz, RP35972) in male patients with previously resected colon polyps and first degree relatives of breast cancer patients. *Proc Am Soc Clin Oncol* 1992; 11: 145.
32. Flisiak R *et al*. Circulating transforming growth factor beta1 as an indicator of hepatic function impairment in liver cirrhosis. *Cytokine* 2000; 12: 677–681.
33. Neuman MG *et al*. Serum tumour necrosis factor-alpha and transforming growth factor-beta levels in chronic hepatitis C patients are immunomodulated by therapy. *Cytokine* 2002; 17: 108–117.

Supporting information

Additional Supporting Information may be found in the online version of this article:

Figure S1 Changes in the blood contents of HA and ALT from baseline through week 24 (PP population, $n = 68$). (a) Serum HA levels. (b) Serum ALT levels. Data are plotted as median with error bars indicating 25th and 75th percentiles. HA, hyaluronic acid; ALT, alanine aminotransferase; PP, per-protocol.

Figure S2 No correlation between changes in the Ishak fibrosis score and percentage changes in serum HA concentration in PP population ($n = 68$). HA, hyaluronic acid; PP, per-protocol.

Table S1 Histological outcomes pre- and post-therapy of oltipraz (PP population, $n = 68$).

Table S2 Univariate analyses identifying blood fibrosis markers associated with the response in Ishak fibrosis score (PP population, $n = 68$).