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# The Effect of Cpd 861 on Chronic Hepatitis B Related Fibrosis and Early Cirrhosis: A Randomized, Double Blind, Placebo Controlled Clinical Study



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**Objectives**

To further assess the clinical antifibrotic efficacy of Cpd 861 on chronic hepatitis B related fibrosis and early cirrhosis using a randomized, double-blind, and placebo controlled clinical trial.

**Methods**

Total of 136 patients with HBV-related fibrosis and early cirrhosis were allocated randomly into Cpd 861 treatment group and placebo group for 24 weeks treatment. Serum fibrosis markers including hyaluronic acid (HA), IV collagen (IV-C), amino terminal propeptide of type III procollagen (PIIIP), laminin (LN), and serum MMP1, 2, 9, TIMP1, 2 level were determined before and after 24 weeks treatment. Liver biopsies before and after 24 weeks of treatment were assessed according to modified Scheuer and Chevallier's scoring system.

**Results**

Total of 52 patients in Cpd 861 treatment group and 50 patients in placebo-controlled group completed the 6 months. ALT level decreased from 68.2 U/L ± 68.6 U/L to 45.9 U/L ± 26.1 U/L, AST level decreased from 60.4 U/L ± 62.6 U/L to 46.7 U/L ± 39.0 U/L ($P < 0.05$) after 24 weeks treatment, whereas there was no significant change in placebo group (ALT: 65.3 U/L ± 48.3 U/L to 85.4 U/L ± 115.5 U/L; AST: 60.4 U/L ± 44.6 U/L to 77.6 ± 89.6 U/L, $P > 0.05$). Serum fibrosis markers, including HA, IV-C, PIIIP, and LN were decreased after treatment, but there is no statistically significant compared with placebo group. Compared with placebo group, serum TIMP1 and MMP9 level decreased significantly (TIMP1 172.0 ng/ml ± 79.6 ng/ml vs. 133.5 ng/ml ± 66.8 ng/ml; MMP9 116.1 ng/ml ± 88.2 ng/ml vs. 80.4 ng/ml ± 79.0 ng/ml), and the ratio of TIMP1/MMP1 (48.3 ± 96.3 vs. 19.9 ± 28.0) were also decreased after 861 treatment. In patients treated with Cpd 861, hepatic inflammatory score (from 14.0 ± 6.0 to 10.2 ± 6.1), fibrosis score (from 11.9 ± 6.5 to 8.2 ± 4.5), and relative content of collagen (from 18.9% ± 9.5% to 14.9% ± 8.4%) decreased significantly. In contrast, there was no significant change in placebo group. The reversal (fibrosis score decrease ≥ 2) rate of fibrosis in Cpd 861 group was 38.9% in S2, 53.3% in S3 (pre-cirrhotic) and 78.6% in S4 (cirrhosis), significantly higher than those in placebo group (14.3%, 25.0%, 41.7%, respectively). The overall reversal rate was 52.0% in Cpd 861 group, and 20.0% in placebo group ($P < 0.05$). No serious adverse effects were observed during Cpd 861 treatment.

**Conclusion**

Liver fibrosis and early cirrhosis due to HBV infection in man could be definitely reversed by herbal remedy Cpd 861.

The original publication is in Chinese. This study is recorded on NCBI Pubmed and available from https://www.ncbi.nlm.nih.gov/pubmed/15329205. The study is translated by Zou CL from Beijing Friendship Hospital and revised by Jia JD in 2019

## Abbreviation

| | |
|---|---|
| AST | Aspartate aminotransferase |
| ALT | Alanine aminotransferase |
| Cpd 861 | Compound 861 |
| HBV | Hepatitis B virus |
| MMP | Matrix metalloproteinase |
| TIMP | Tissue inhibitor of metalloproteinase |
| P III P | Type III procollagen |
| LN | Laminin |
| HA | Hyaluronic acid |

Cpd 861 has the effect of blocking and reversing liver fibrosis and early cirrhosis in rats and humans, and the mechanism is to restore the balance of synthesis and degradation of extracellular matrix[1]. To further validate the clinical efficacy of Cpd 861, a randomized, double-blind, placebo-controlled clinical study was performed in patients with chronic hepatitis B. Serum levels of matrix metalloproteinases (MMPs) and, tissue inhibitor of metalloproteinase (TIMPs) were measured to understand the therapeutic mechanism of Cpd 861.

## Methods

**Study population.** Patients with chronic hepatitis B who were hospitalized to the Changzhou Infectious Disease Hospital of Hebei Province, Shandong Provincial Hospital, Shandong Province, Weifang People's Hospital, Shandong Province, the 89th Hospital of the People's Liberation Army, the Dongfeng Company General Hospital of Shiyan City, Hubei Province, and the Traditional Chinese Medicine Hospital of Shiyan City, Hubei Province during the period between September 1999 and September 2001 were enrolled in the study according to the inclusion and exclusion criteria. The diagnosis met the criteria of the 2000 Viral Hepatitis Prevention Protocol[2].

A total of 136 patients were enrolled, and 102 patients completed the 24-week medication and paired liver biopsies before and after the treatment, including 52 in the treatment group and 50 in the placebo group. The mean age, sex ratio, and mean disease duration in the two groups had no statistically significant differences. 34 patients (15 in the treatment group and 19 in the placebo group) were lost to follow-up during the course of medication, with 20 of them due to declining the second liver biopsy.

**Eligibility criteria.** The inclusion criteria were as follows: (1) 16-70 years old, (2) history of HBV infection for more than 6 months, (3) elevation in at least 2 of the 4 serum markers of liver fibrosis: aminoterminal propeptide of type III procollagen (PIIIP), hyaluronic acid (HA), type IV collagen, and laminin (LN), (4) histologically-proved fibrosis of S2-S4. All Patients agreed to participate in the clinical research and signed informed consent.

The exclusion criteria were as follows: (1) decompensated cirrhosis at enrolment, (2) presence of severe comorbidities, (3) pregnant or lactating women, (4) use of glucocorticoids, immunomodulators, or anti-fibrotic drugs within 6 months of enrolment.

**Study design and intervention.** A random table generated by the NDST software (created by Professor Sun Ruiyuan and the New Drug Evaluation Center of the Ministry of Health, China) were used in the study. The therapeutic drug was compound capsule (Cpd 861) consisting of several traditional Chinese medicines which was provided by Tianjiang Pharmaceutical Co., Ltd. Patients were allocated to receive Cpd 861 capsules or placebo capsules according to the random code, and two capsules were same in appearance and packaging. The capsules were given orally at a dose of 7 capsules, three times a day. After 24 weeks of treatment, the patients in the placebo group were given Cpd 861 for 6 months.

**Laboratory tests.** Symptoms, signs, liver function test, serum fibrosis markers, levels of MMP1, 2, 9 and levels of TIMP1, 2 were evaluated before and after treatment.

**Liver biopsy.** Liver biopsies before and after 24 weeks of treatment were assessed according to modified Scheuer scoring[3] (Proposed by Liver Fibrosis Study Group, Chinese Society of Hepatology and modified by professor Tailing Wang) and Chevallier's[4] scoring system, by three physicians[5,6]. Liver histology was analysed with HPIAS-1000 High-Resolution Pathology Report Analysis System after reticulin and Masson's staining, and immunohistochemical staining for α-smooth muscle actin (α-SMA).

**Statistical analysis.** Statistical analyses were performed using SPSS 10.0 software and Medcalc software. Pre-and post-treatment comparison

was analysed by paired t-test. Differences among continuous and categorical variables were examined for significance by Student's t-test and chi-squared test, respectively. Semi-quantitative scores were examined with non-parametric statistical methods. The effect of drugs on histopathology was examined

## Results

**Demographic characteristics.** There were no differences in improvement rate of symptoms and signs between the two groups: 55. 5% in the treatment group improved, and 51.0% in the placebo group.

**Change in liver function tests and liver fibrosis markers.** In the treatment group, the normalization rates of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were 54.5% (12/22) and 44.4% (8/18), whereas in the placebo group, the normalization rates were 34.5% (10/29) and 20% (6/30), respectively, with no significant difference between the two groups. Serum fibrosis markers decreased after treatment with Cpd 861, but except serum PIIIP, there was no significant difference in changes when compared with placebo group (Table 1).

**Change in MMPs and TIMP1.** MMP2 were measured before and after treatment in 43 patients (25 in the treatment group and 18 in the placebo group) and other MMPs were measured in 88 patients (46 in the treatment group and 42 in the placebo group). Compared with the control group, Cpd 861 significantly reduced the serum levels of TIMP1, MMP9 and ratio of TIMP1 to MMP1 (Table 2).

**Change in liver histology.** 102 patients completed the paired liver biopsy before and after treatment (52 in the treatment group, 50 in the placebo group). 50 in the treatment group were included in histopathological analysis, and 2 was excluded because the liver tissue sizes were too small to conduct adequate assessment and scoring. Compared with the placebo group, after treatment with Cpd 861 for 6 months, the three key lesions including piecemeal necrosis (PN), bridging necrosis

**Table 1** Changes of serum biochemical and liver fibrosis markers before and after treatment ($\bar{x}\pm s$)

| Group | Number | ALT (U/L) | AST (U/L) | PIIIP (ng/ml) | C IV (ng/ml) | HA (ng/ml) | LN (ng/ml) |
|---|---|---|---|---|---|---|---|
| **Treatment** | | | | | | | |
| before | 52 | 68.2±68.6* | 60.4±62.6* | 1.3±0.8* | 199.9±139.6 | 157.7±87.9 | 215.7±36.2 |
| after | | 45.9±26.1 | 46.7±39.0 | 1.1±0.5 | 168.4±120.5 | 139.3±86.2 | 205.5±41.7 |
| **Placebo** | | | | | | | |
| before | 50 | 65.3±48.3 | 60.4±44.6 | 1.0±0.6 | 173.6±141.9 | 135.0±85.6 | 205.2±36.1 |
| after | | 85.4±115.5 | 77.6±89.6 | 1.2±0.8 | 165.3±101.6 | 145.4±107.5 | 198.6±44.7 |

*ALT, alanine aminotransferase; AST, aspartate aminotransferase; PIIIP, type III procollagen; C IV, type IV collagen; HA, hyaluronic acid; LN, laminin. Compared with placebo group, * t values was 2.315, 2.168 and 3.173, respectively, P<0.05.*

**Table 2** Changes of serum collagenases and TIMP1 before and after treatment in two groups (ng/ml,$\bar{x}\pm s$)

| Group | Number | MMP1 | MMP2 | MMP9 | TIMP1 | TIMP2 | TIMP1/MMP1 |
|---|---|---|---|---|---|---|---|
| **Treatment** | | | | | | | |
| before | 25 | 9.3±4.7 | 621.1±165.8 | 116.1±88.2 | 172.0±79.6 | 15.0±17.9 | 48.3±96.3 |
| after | | 10.1±4.5 | 641.8±224.6 | 80.4±79.0* | 133.5±66.8# | 30.5±78.9 | 19.9±28.0** |
| **Placebo** | | | | | | | |
| before | 18 | 9.4±5.2 | 640.1±199.0 | 87.9±52.6 | 157.5±80.5 | 12.2±7.1 | 32.9±41.7 |
| after | | 8.9±4.7 | 606.8±186.1 | 126.3±98.7 | 165.5±78.2 | 19.4±33.9 | 48.1±112.3 |

*MMP, matrix metalloproteinase; TIMP, tissue inhibitor of metalloproteinase. Compared with placebo group, * t=2.723, #t=2.433, **t=2.248, P<0.05.*

**Table 3** Changes of pathological semi-quantitative scores and PCA before and after treatment before and after treatment in the two groups ($\bar{x} \pm s$)

| Group | Number | Inflammation score | Fibrosis score | PCA (%) |
|---|---|---|---|---|
| **Treatment** | | | | |
| before | 50 | 14.0±6.0 | 11.9±6.5 | 18.9±9.5 |
| after | | 10.2±6.1* | 8.2±4.5# | 14.9±8.4** |
| **Placebo** | | | | |
| before | 50 | 11.4±6.8 | 9.1±5.4 | 15.6±10.1 |
| after | | 14.5±6.6 | 10.7±5.5 | 17.5±9.3 |

*Compared with placebo group, * t=3.223, #t=3.354, **t=2.202, P<0.05, PCA: proportional collagen area*

(BN), and fibrous septa were significantly improved. PN was dramatically alleviated or disappeared after treatment. BN was alleviated, and large loose wide fibrous septa vanished or only became delicate. At the same time, hepatocyte regeneration was remarkable, and the pseudo-lobular structure became atypical or even disappeared.

With HPIAS-1000 High-Resolution Pathology Report Analysis System the liver sections with reticulin and Masson's staining were analysed, and the proportional collagen area (PCA, the relative percentage of collage to total area of the liver tissue) was calculated. The pathological semi-quantitative scores and PCA of the two groups before and after treatment were shown in Table 3.

The histopathology was considered as progressed if the semi-quantitative scores of fibrosis increased by ≥ 2 points; the histopathology was considered as reversed (or regressed) if the scores declined by ≥ 2 points; the histopathology was considered as stable if the change was less than 2 points. The reversal rates of each stage of fibrosis in the two groups were shown in Table 4.

By ITT analysis, the data of patients who were lost to follow-up were also included in the statistical analysis. The histopathology which remained stable or worsened was considered as ineffective, whereas histopathological reverse was considered as effective. The effective rate was 26/67 (38.8%) for treatment group and 10/69 (14.5%) for the placebo group. Compared with placebo group, $\chi^2 = 9.705$, $P<0.05$.

**Immunohistochemistry results.** In patients with chronic hepatitis B, before Cpd 861 treatment the stellate cell proliferation was activated as demonstrated by increased actin filaments staining in the cytoplasm. The number of α-SMA positive cells (activated proliferating stellate cells) were significantly increased compared with normal liver, mainly around the fibrous septa and inflammatory

**Table 4** Comparison of reversal rates of each fibrosis stage in the two groups

| Group | Number | Worsen | Stable | Reverse | Reversal rate |
|---|---|---|---|---|---|
| **Treatment** | 50 | 6 | 18 | 26# | 52.0 |
| S1 | 3 | 0 | 3 | 0 | |
| S2 | 18 | 5 | 6 | 7 | 38.9 |
| S3 | 15 | 1 | 6 | 8 | 53.3 |
| S4 | 14 | 0 | 3 | 11 | 78.6 |
| S3+S4 | 29 | 1 | 9 | 19* | |
| **Placebo** | 50 | 13 | 27 | 10 | 20.0 |
| S1 | 12 | 2 | 10 | 0 | |
| S2 | 14 | 8 | 4 | 2 | 14.3 |
| S3 | 12 | 1 | 8 | 3 | 25.0 |
| S4 | 12 | 2 | 5 | 5 | 41.7 |
| S3+S4 | 24 | 3 | 13 | 8 | |

*Compared with placebo group, * $\chi^2$=4.478, # $\chi^2$=9.766, P<0.05.*

sinusoids. The number of α-SMA staining positive cells was positively correlated with the degree of fibrosis. After treatment with Cpd 861, the number of α-SMA positive staining cells was significantly reduced, especially around the sinusoids.

**Adverse reactions.** Some patients in both treatment group and placebo group had adverse events such as dry throat, nausea, stomach discomfort and constipation, but there was no statistically significant difference between the two groups.

## Discussion

Previous open label trials showed that both inflammation and fibrosis in 107 patients receiving Cpd 861 with paired liver biopsy were significantly improved, confirming the concept that liver fibrosis and early cirrhosis can be reversed. The reversal rate of S2 (significant fibrosis), S3 (advanced fibrosis) and S4 (cirrhosis) was 78%, 82% and 75%, respectively. The current study further confirms the therapeutic role of Cpd 861 in blocking and reversing liver fibrosis in patients with chronic hepatitis B.

The study demonstrated that after treatment with Cpd 861: (1) the serum PIIIP levels became lower (2) inflammation score, fibrosis score and PCA were improved (3) the reversal rates for S2, S3 and S4 were 38.9%, 53.3% and 78.6%, respectively, with a total reversal rate of 52.0%; whereas in the placebo group the reversal rate was only 14.3%, 25.0%, 41.7%, and 20.0%, respectively. The difference between the two groups was statistically significant. The reversal of S3 (advanced fibrosis) and S4 (cirrhosis) was more remarkable; (4) cell degenerated and necrosis, PN and BN decreased, and hepatocytes regeneration increased. The fibrous septa became thinner, looser, and intermittent. Sirius red staining showed that the main component of the fibrous septa collage type I can be reduced or even dissipated. These data further confirmed from pathologically point of view that Cpd 861 can reverse liver fibrosis and even early cirrhosis in patients with chronic hepatitis B.

The therapeutic mechanism of Cpd 861 for liver fibrosis caused by chronic hepatitis B has the following aspects: (1) attenuation of liver necroinflammation: the level of serum ALT, AST decreased after treatment; lobular inflammation, portal area debris necrosis and bridging necrosis were alleviated or even disappeared after treatment. The interface of portal area became neater, the infiltration of inflammatory cells in the portal area and liver parenchyma was alleviated, and cell degeneration and necrosis were alleviated; (2) inhibit the proliferation of stellate cells thereby suppressing the synthesis of collagen: α-SMA positive cells (activated HSC) in the liver were significantly reduced, suggesting HSC activation was inhibited. In the liver, sirius red staining showed that not only type III collagen was significantly reduced, but more importantly, the main component of fibrous septa collagen type I, was also reduced or even dissipated; pathological image analysis showed that the relative content of total collagen was significantly reduced; (3) increase the activity of collagenase and promoting collagen degradation: the increase of MMP1 level suggested that the effect of reversing liver fibrosis may be achieved by increasing the level of MMP1 to degrade the type I collagen which was difficult to be degraded by other collagenases in the fibrous septa. (4) inhibit TIMP1 levels: serum TIMP1 levels was significantly inhibited by Cpd 861 in patients with chronic hepatitis B, suggesting that Cpd 861 may abolish the inhibitory effect of TIMP1 on collagenase degradation activity by decreasing the ratio of serum TIMP1/MMP1. In contrast, the ratio of TIMP1/MMP1 increased in the placebo group, suggesting that inhibition of TIMP1 levels through inhibition of TIMP1/MMP1 ratio, was important for the reversal of liver fibrosis.

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