The protective effect of diammonium glycyrrhizinate and polyene phosphatidyl choline on liver damage caused by anti-tuberculosis drugs

Li Wang

Department of Pharmacy, Chongqing Infectious Disease Medical Center, Chongqing, 400036, China

ARTICLE INFO
Article history:
Received
Received in revised form
Accepted
Available online

Keywords:
Tuberculosis
Liver damage
Diammonium glycyrrhizinate
Polyene phosphatidyl choline

ABSTRACT
Objective: To study the protective effect of diammonium glycyrrhizinate and polyene phosphatidyl choline on liver damage caused by anti-tuberculosis drugs. Methods: Patients who received initial 2HRS(E)Z/4HR short-range anti-tuberculosis treatment in our hospital from October 2013 to October 2015 were selected and randomly divided into diammonium glycyrrhizinate group (group A) and polyene phosphatidyl choline group (group B), and after 4 weeks of liver protection treatment, serum levels of liver damage marker molecules, stress marker molecules and NF-κB-mediated inflammatory molecules as well as protein expression levels of bile acid metabolism genes were determined. Results: Serum ALT, AST, GGT, ALP, GDH, TBIL, NF-κB, IL-1β, TNF-α and MCP-1 levels of group B were significantly lower than those of group A while HO-1, GSH-Px, SOD, ERK, MEK and SIRT1 levels were significantly higher than those of group A; serum CYP7A1, FXR and SHP protein expression levels of group B were significantly lower than those of group A while BESP protein expression level was significantly higher than that of group A. Conclusion: Polyene phosphatidyl choline has better protective effect on liver damage caused by anti-tuberculosis drugs than diammonium glycyrrhizinate, and the molecular mechanisms of polyene phosphatidyl choline to protect the liver are enhancing the antioxidant effect mediated by HO-1 and SIRT1, inhibiting the inflammatory response mediated by NF-κB and regulating the expression of bile acid metabolism genes.

1. Introduction

Tuberculosis is a contagious disease caused by mycobacterium tuberculosis infection, China is a great power in TB and the incidence of tuberculosis shows rising trend in recent years. 2HRS(E)Z/4HR short-range chemotherapy is the first clinical choice for initial treatment of positive sputum pulmonary tuberculosis, and the overall regular use of chemotherapy drugs is the key to insure the treatment effect. In 2HRS(E)Z/4HR anti-tuberculosis scheme, isoniazid and rifampicin have strong liver toxicity and can cause liver damage during anti-tuberculosis treatment, and part of the patients with severe liver injury have to stop using anti-tuberculosis drugs, which affects the anti-tuberculosis treatment effect[1,2]. Therefore, anti-tuberculosis treatment requires liver-protecting drugs to alleviate the liver injury caused by anti-tuberculosis drugs[3]. Diammonium glycyrrhizinate and polyene phosphatidyl choline are two common clinical liver-protecting drugs[4,5], but the effect of the two drugs on liver injury caused by anti-tuberculosis drugs is unclear. In the following study, the protective effect of diammonium glycyrrhizinate and polyene phosphatidyl choline on liver damage caused by anti-tuberculosis drugs was analyzed.

2. Subjects and Methods

2.1 Research subjects

A total of 78 patients who received initial treatment of tuberculosis in our hospital from October 2013 to October 2015 were selected as
the research subjects, were all diagnosed with tuberculosis by clinical symptoms, imaging examination and etiology examination, and received 2HRS(E)Z/4HR short-range anti-tuberculosis treatment after admission. Patients with viral hepatitis, diabetes, cardiac and renal insufficiency as well as pregnant women and breast-feeding women were excluded. Random number table was used to divide the included patients into diammonium glycyrrhizinate group (group A) and polyene phosphatidyl choline group (group B) \((n=39)\) who received diammonium glycyrrhizinate liver protection treatment and polyene phosphatidyl choline liver protection treatment respectively. Group A included 22 male cases and 17 female cases that were \((39.5\pm5.2)\) years old; group B included 24 male cases and 15 female cases that were \((38.7\pm4.9)\) years old. The two groups of patients were not significantly different in general information \((P>0.05)\).

### 2.2. Treatment methods

Both groups received 2HRS(E)Z/4HR short-range chemotherapy regimen for anti-tuberculosis treatment and received liver protection treatment at the same time, group A received diammonium glycyrrhizinate for liver protection treatment, and the method was as follows: oral administration of 150 mg of diammonium glycyrrhizinate enteric capsules \((50 \text{ mg/capsule})\), 3 times/d; group B received polyene phosphatidyl choline for liver protection treatment, and the method was as follows: oral administration of 456 mg of polyene phosphatidyl choline capsules \((228 \text{ mg/capsule})\), 3 times/d.

### 2.3 Detection methods of liver damage–related molecule levels

After 4 weeks of liver protection treatment, serum samples were collected from two groups of patients, automatic biochemical analyzer was used to determine aspartate aminotransferase \((\text{AST})\), alanine aminotransferase \((\text{ALT})\), \(\gamma\)-glutamyl transpeptidase \((\text{GGT})\), alkaline phosphatase \((\text{ALP})\), glutamate dehydrogenase \((\text{GDH})\) and total bilirubin \((\text{TBIL})\) levels, radioimmunoprecipitation kits were used to detect heme oxygenase \((\text{HO-1})\), glutathione peroxidase \((\text{GSH-Px})\) and superoxide dismutase \((\text{SOD})\) levels, and enzyme-linked immunosorbent assay kits were used to determine extracellular regulated protein kinase \((\text{ERK})\), ERK kinase \((\text{MEK})\), silent information regulator 1 \((\text{SIRT1})\), nuclear transcription factor-\(\kappa\) B \((\text{NF-\(\kappa\) B})\), interleukin-1 \(\beta\) \((\text{IL-1}\beta)\), tumor necrosis factor-\(\alpha\) \((\text{TNF-\(\alpha\)})\), monocyte chemoattractant protein-1 \((\text{MCP-1})\), cholesterol 7-hydroxylase \((\text{CYP7A})\), farnesoid X receptor \((\text{FXR})\), small heterodimer partner \((\text{SHP})\) and bile salt export pump \((\text{BSEP})\) levels.

### 2.4 Statistical methods

SPSS 20.0 software was used to input and analyze data, measurement data analysis was by \(t\) test and \(P<0.05\) indicated statistical significance in differences.

### 3. Results

#### 3.1 Serum liver damage marker molecule levels

After 4 weeks of treatment, analysis of serum liver damage marker molecules \(\text{ALT, AST, GGT, ALP, GDH and TBIL}\) between two groups was as follows: serum \(\text{ALT, AST, GGT, ALP, GDH and TBIL}\) levels of group B were significantly lower than those of group A. Differences in serum \(\text{ALT, AST, GGT, ALP, GDH and TBIL}\) levels were statistically significant between two groups after 4 weeks of treatment \((P<0.05)\).

#### 3.2 Serum stress marker molecule levels

After 4 weeks of treatment, analysis of serum \(\text{HO-1}\) antioxidant pathway between two groups was as follows: serum \(\text{HO-1}\), \(\text{GSH-Px}\) and \(\text{SOD}\) levels of group B were significantly higher than those of group A; analysis of \(\text{ERK/MEK/SIRT1}\) pathway was as follows: serum \(\text{ERK}\), \(\text{MEK}\) and \(\text{SIRT1}\) levels of group B were significantly higher than those of group A. Differences in serum \(\text{HO-1}\), \(\text{GSH-Px}, \text{SOD, ERK, MEK and SIRT1}\) levels were statistically significant

### Table 1.

Comparison of serum liver damage marker molecule levels between two groups.

<table>
<thead>
<tr>
<th>Groups</th>
<th>(n)</th>
<th>ALT (U/L)</th>
<th>AST (U/L)</th>
<th>GGT (U/L)</th>
<th>ALP (U/L)</th>
<th>GDH (U/L)</th>
<th>TBIL ((\mu)mol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group A</td>
<td>39</td>
<td>103.4±14.5</td>
<td>98.3±10.1</td>
<td>79.3±9.5</td>
<td>168.7±22.1</td>
<td>93.2±10.3</td>
<td>24.2±3.5</td>
</tr>
<tr>
<td>Group B</td>
<td>39</td>
<td>76.4±9.4</td>
<td>72.5±8.6</td>
<td>34.2±5.6</td>
<td>104.5±14.6</td>
<td>47.8±6.8</td>
<td>17.1±2.7</td>
</tr>
<tr>
<td>(T)</td>
<td></td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>

### Table 2.

Comparison of serum stress marker molecule levels between two groups.

<table>
<thead>
<tr>
<th>Groups</th>
<th>(n)</th>
<th>(\text{HO-1}) antioxidant pathway (U/L)</th>
<th>(\text{ERK/MEK/SIRT1}) pathway (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>(\text{HO-1})</td>
<td>(\text{GSH-Px})</td>
</tr>
<tr>
<td>Group A</td>
<td>39</td>
<td>35.9±5.2</td>
<td>68.6±8.6</td>
</tr>
<tr>
<td>Group B</td>
<td>39</td>
<td>77.1±9.6</td>
<td>134.5±17.8</td>
</tr>
<tr>
<td>(T)</td>
<td></td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>
between two groups after 4 weeks of treatment ($P$<0.05).

3.3 Serum NF-κB-mediated inflammation-related indexes

After 4 weeks of treatment, analysis of NF-κ B-mediated inflammation-related indexes NF-κ B, IL-1β, TNF-α and MCP-1 in serum between two groups was as follows: serum NF-κ B, IL-1β, TNF-α and MCP-1 levels of group B were significantly lower than those of group A. Differences in serum NF-κ B, IL-1β, TNF-α and MCP-1 levels were statistically significant between two groups after 4 weeks of treatment ($P$<0.05).

3.4 Bile acid metabolism gene protein expression levels

After 4 weeks of treatment, analysis of serum bile acid metabolism genes CYP7A1, FXR, SHP and BESP protein expression levels between two groups was as follows: serum CYP7A1, FXR and SHP levels of group B were significantly lower than those of group A while BESP level was significantly higher than that of group A. Differences in serum CYP7A1, FXR, SHP and BESP levels were statistically significant between two groups after 4 weeks of treatment ($P$<0.05).

4. Discussion

Diammonium glycyrrhizinate and polyene phosphatidyl choline are two common clinical liver-protecting drugs, and after entering the body through oral administration, they exert liver-protective effect through different mechanisms of action. Diammonium glycyrrhizinate mainly exerts liver-protective effect through antioxidant pathways, and can reduce the production of oxygen free radicals and the generation of lipid peroxides in the body, thus protecting liver cell ultrastructure and promoting the liver cell regeneration. The active ingredient of polyene phosphatidyl choline is 1, 2-dilinoleoyl phosphatidyl choline, it can replace the endogenous phospholipids the body, enter the biological membrane and then act as the liver cell membrane and organelle membrane repair material, and it is conducive to the self-repair of damaged liver cells; in addition, the phospholipid composition in the drug can regulate lipid metabolism in the body, reduce the generation of lipid peroxides and inhibit inflammation damage to the liver, and meanwhile, it can also alleviate the liver fibrosis process. In order to define the treatment effect of diammonium glycyrrhizinate and polyene phosphatidyl choline on liver injury caused by anti-tuberculosis drugs, serum levels of liver enzymes and bilirubin of two groups of patients were analyzed in the study at first, and the results showed that serum ALT, AST, GGT, ALP, GDH and TBIL levels of group B were significantly lower than those of group A. It means that polyene phosphatidyl choline has better protective effect on liver injury caused by anti-tuberculosis drugs than diammonium glycyrrhizinate.

Oxidative stress damage is considered as a key link in liver damage caused by anti-tuberculosis drugs, and in the process, a variety of molecules are activated and mediate oxidative stress damage and antioxidant reaction. Heme oxygenase-1 (HO-1) is the induced enzyme regulating heme catabolism and bilirubin anabolism, it is induced as a protective protein under stress and it can protect the liver cells from damage. HO-1 is induced and then involved in heme metabolism, it can produce biliverdin, carbon monoxide and ferrous ion, and biliverdin can also be further transformed into bilirubin to exert cytoprotection, scavenge oxygen free radicals and thus reduce the consumption of antioxidants SOD and GSH-Px. In the study, analysis of the antioxidant pathways mediated by HO-1 confirmed that serum HO-1, GSH-Px and SOD levels of group B were significantly higher than those of group A. SIRT1 belongs to long-acting protein-deacetylase family, and is another kind of protective molecule with anti-oxidative damage effect. MEK/ERK is the upstream signal pathway adjusting the SIRT-1 expression, and a variety of antioxidants can exert cytoprotection through the MEK/ERK/SIRT-1 pathways. In the study, analysis of the SIRT-1 pathway confirmed that serum ERK, MEK and SIRT1 levels of group B were significantly higher than those of group A. It means that polyene phosphatidyl choline can enhance the antioxidant mediated by HO-1 and SIRT1, thereby protecting liver cells from damage by anti-tuberculosis drugs.

Table 3.
Comparison of serum NF-κ B-mediated inflammation-related indexes between two groups (ng/mL).

<table>
<thead>
<tr>
<th>Groups</th>
<th>n</th>
<th>NF-κ B</th>
<th>IL-1β</th>
<th>TNF-α</th>
<th>MCP-1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group A</td>
<td>39</td>
<td>16.58±2.13</td>
<td>34.52±5.26</td>
<td>22.45±3.17</td>
<td>9.35±1.03</td>
</tr>
<tr>
<td>Group B</td>
<td>39</td>
<td>9.38±1.05</td>
<td>15.24±2.09</td>
<td>10.32±1.53</td>
<td>4.58±0.61</td>
</tr>
<tr>
<td>T</td>
<td></td>
<td>7.598</td>
<td>12.458</td>
<td>11.509</td>
<td>11.367</td>
</tr>
<tr>
<td>P</td>
<td></td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>

Table 4.
Comparison of serum bile acid metabolism gene protein expression levels between two groups (ng/mL).

<table>
<thead>
<tr>
<th>Groups</th>
<th>n</th>
<th>CYP7A1</th>
<th>FXR</th>
<th>SHP</th>
<th>BESP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group A</td>
<td>39</td>
<td>1.09±0.14</td>
<td>1.05±0.12</td>
<td>0.94±0.17</td>
<td>0.98±0.15</td>
</tr>
<tr>
<td>Group B</td>
<td>39</td>
<td>0.42±0.06</td>
<td>0.65±0.08</td>
<td>0.31±0.04</td>
<td>2.32±0.41</td>
</tr>
<tr>
<td>P</td>
<td></td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>
Nuclear transcription factor-κ B (NF-κ B) is an important transcription factor in the nucleus that regulates immune and inflammatory response, and it has regulatory effect on the expression of inflammatory cytokines, chemokines, adhesion molecules and acute phase reactive proteins[14]. In the pathophysiological process of drug-induced liver injury and ischemic liver damage, NF-κ B-dependent pathway is significantly activated, and NF-κ B is translocated into the nucleus, starts the expression of target genes of TNF-α, IL-1β and MCP-1, then causes leukocyte infiltration in liver tissue and leads to liver damage through the above inflammatory factors[15,16]. In the study, analysis of the NF-κ B-mediated inflammatory response showed that serum NF-κ B, IL-1β and MCP-1 levels of group B were significantly lower than those of group A. This means that polyene phosphatidyl choline can inhibit the NF-κ B-mediated inflammatory response so as to protect the liver cells from damage by anti-tuberculosis drugs. Anti-tuberculosis drugs are not only with direct liver toxicity, but can also affect the bile acid metabolism, lead to cholestasis and cause liver damage[17]. Animal experiment of domestic XU Yong-ji confirms that rifampicin can affect the expression of bile acid metabolism genes in mice, CYP7A1, FXR and SHP are highly expressed while BESP is lowly expressed, thereby affecting the bile acid metabolism and leading to cholestasis and liver damage[18]. In the study, analysis of the expression levels of above bile acid metabolism genes confirmed that serum CYP7A1, FXR and SHP levels of group B were significantly lower than those of group A while BESP level was significantly higher than that of group A. This means that polyene phosphatidyl choline can adjust the expression of bile acid metabolism genes so as to protect liver cells from damage by anti-tuberculosis drugs.

To sum up, polyene phosphatidyl choline has better protective effect on liver damage caused by anti-tuberculosis drugs than those of group A. This means that polyene phosphatidyl choline can adjust the expression of bile acid metabolism genes so as to protect liver cells from damage by anti-tuberculosis drugs. Polyene phosphatidyl choline protects against indomethacin/bile acid-induced hepatotoxicity in rats. Chem Biol Interact 2014; 213(2): 214-221.

References


