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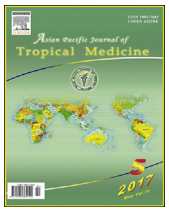

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Pyrrolidine dithiocarbamate alleviates the anti-tuberculosis drug-induced liver injury through JAK2/STAT3 signaling pathway: An experimental study

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ABSTRACT

Objective: To study the effect of pyrrolidine dithiocarbamate (PDTC) on the anti-tuberculosis drug-induced liver injury and the molecular mechanism.

Methods: Clean male SD rats were selected as experimental animals and randomly divided into normal group, model group, PDTC group and AG490 group. Animal model of anti-tuberculosis drug-induced liver injury was established by intragastric administration isoniazid + rifampicin. PDTC group received intraperitoneal injection of PDTC, and AG490 group received intraperitoneal injection of AG490. Twenty-eight days after intervention, the rats were executed, and the liver injury indexes, inflammation indexes and oxidative stress indexes in serum as well as JAK2/STAT3 expression, liver injury indexes, inflammation indexes and oxidative stress indexes in liver tissue were determined.

Results: p-JAK2, p-STAT3, TNF-α, IL-1β, IL-6, ROS, 8-OHdG and MDA expression in liver tissue as well as TBIL, ALT, AST, γ-GT, TNF-α, IL-1β, IL-6, 8-OHdG and MDA levels in serum of model group were significantly higher than those of normal group while p-JAK2, p-STAT3, TNF-α, IL-1β, IL-6, ROS, 8-OHdG and MDA expression in liver tissue as well as TBIL, ALT, AST, γ-GT, TNF-α, IL-1β, IL-6, 8-OHdG and MDA levels in serum of PDTC group and AG490 group were significantly lower than those of model group.

Conclusions: PDTC can inhibit the inflammation and oxidative stress mediated by JAK2/STAT3 signaling pathway to alleviate the anti-tuberculosis drug-induced liver injury.

1. Introduction

Drug-induced liver injury is a common clinical liver injury, which specifically refers to the liver injury caused by the drugs themselves or drug metabolites in the process of drug treatment, and is associated the increased sensitivity or decreased tolerance of the body's special constitution to the drugs. Combined use of isoniazid, rifampicin, pyrazinamide and other anti-tuberculosis drugs is a common cause of drug-induced liver injury [1,2], but the specific molecular pathway of anti-tuberculosis drugs to cause liver injury is not yet clear, and the targeted drugs are also short for clinical treatment of anti-tuberculosis drug-induced liver injury. The studies about anti-tuberculosis drug-induced liver injury in recent years have shown that the activation of inflammation and the activation oxidative stress reaction in liver tissue are closely related to the occurrence of liver injury, and janus kinase 2 (JAK2)/Signal Transducer and Activator of Transcription 3 (STAT3) are the key signaling pathways in the body that regulate inflammation and oxidative stress reaction

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[3,4]. So it was speculated that anti-tuberculosis drugs can cause liver injury through the inflammation and oxidative stress response mediated by JAK2/STAT3. Pyrrolidine dithiocarbamate (PDTC) is the drug with extensive anti-inflammatory and antioxidant effect, and exerts protective effect on the inflammatory injury and oxidative damage of myocardium [5], kidney [6], liver [7], pancreas [8] and other tissues. In the following study, the protective effects of PDTC on anti-tuberculosis drug-induced liver injury and the role that JAK2/STAT3 signaling pathway played in liver protection by PDTC were analyzed.

2. Materials and methods

2.1. Experimental materials

Clean male SD rats with body mass of 200–250 g were purchased from the laboratory animal center of Shandong University. Animal experiments were upon the approval of the Hospital Animal Ethics Committee, and all animal experiments and the processing after execution were conducted according to the regulations. PDTC was purchased from Sigma company, and AG490 was purchased in Alexis company; isoniazid and rifampicin were purchased from Guangdong Huanan Pharmaceutical Group Co., Ltd., and the specifications were 0.1 g/tablet and 0.15 g/pill; RIPA protein lysis buffer and BCA protein assay kits were purchased from Shanghai Beyotime Company; enzyme-linked immunosorbent assay kits were purchased from Shanghai Westang Biotechnology Company; monoclonal antibodies of JAK2 and STAT3 were bought in Abcam company.

2.2. Experimental methods

2.2.1. Animal grouping and intervention methods

Experimental animals were randomly divided into normal group, model group, PDTC group and AG490 group, with eight in each group. Model group, PDTC group and AG490 group were established into anti-tuberculosis drug-induced liver injury animal model according to the following method: isoniazid and rifampicin solution with mass volume concentration of 30 g/L was configured for intragastric administration in accordance with the drug dose of 50 mg/kg/d, once a day; normal group received intragastric administration of same dose of solvent, once a day. During lavage process, the intervention methods for four groups of rats were as follows: normal group and model group were given intraperitoneal injection of same dose of saline, PDTC group was given intraperitoneal injection of 50 mg/kg/d PDTC, and AG490 group was given intraperitoneal injection of 8 mg/kg/d AG490. Four groups of rats received continuous lavage and intraperitoneal injection for 28 d.

2.2.2. Serum sample collection and index detection methods

Twenty-eight days after intervention, the rats were put to death immediately, about 8–10 mL peripheral blood was collected, let stand at room temperature for 20–30 min for natural blood coagulation and then centrifuged in centrifuge for 20 min at a speed of 3000 r/min to separate serum, then automatic biochemical analyzer was used to detect the levels of total bilirubin (TBIL), alanine aminotransferase (ALT), aspertate aminotransferase (AST) and γ-glutamyltransferase (γ-GT), and the enzyme-linked immunosorbent assay kits were used to detect the levels of tumor necrosis factor-α (TNF-α), interleukin-1β (IL-1β), IL-6, 8-hydroxy-2-deoxyguanosine (8-OHdG) and malondialdehyde (MDA).

2.2.3. Liver tissue collection and index detection methods

Twenty-eight days after intervention, the rats were put to death to collect blood specimens and then anatomized to get the liver tissue and freeze it with liquid nitrogen, then proper amount of liver tissue was collected, added in RIPA protein lysis buffer and grinded, the obtained tissue suspension was centrifuged in a centrifuge at 4 °C and 12 000 r/min for 20 min to separate supernatant, enzyme-linked immunosorbent assay kits were used to determine TNF-α, IL-1β, IL-6, ROS, 8-OHdG and MDA content, the BCA kits were used to determine total protein content, and the TNF-α, IL-1β, IL-6, ROS, 8-OHdG and MDA content per mg total protein were calculated; another part of the supernatant was taken, Western-blot method was used to detect the p-JAK2, p-STAT3 and β-actin protein bands, the grey value of protein bands were scanned, and then the p-JAK2 and p-STAT3 protein expression were calculated.

2.3. Statistical methods

SPSS19.0 software was used to input and statistically process the experimental data, measurement data analysis among groups was by variance analysis, pair-wise comparison was by LSD-t test and $P < 0.05$ indicated statistical significance in differences.

3. Results

3.1. p-JAK2 and p-STAT3 expression in liver tissue

Analysis of p-JAK2 and p-STAT3 expression in liver tissue among four groups of rats was as follows. p-JAK2 and p-STAT3 expression in liver tissue of model group was significantly higher than those of normal group, and p-JAK2 and p-STAT3 expression in liver tissue of PDTC group and AG490 group were significantly lower than those of model group (Table 1).

3.2. Serum liver injury index levels

Analysis of serum liver injury indexes TBIL, ALT, AST and γ-GT levels among four groups of rats was as follows: serum TBIL, ALT, AST and γ-GT levels of model group were significantly higher than those of normal group, and serum TBIL, ALT, AST and γ-GT levels of PDTC group and AG490 group were significantly lower than those of model group (Table 1).

3.3. Inflammatory factor levels in serum and liver tissue

Analysis of inflammatory factors TNF-α, IL-1β and IL-6 levels in serum and liver tissue among four groups of rats was as follows. TNF-α, IL-1β and IL-6 levels in serum and liver tissue of model group were significantly higher than those of normal group, and TNF-α, IL-1β and IL-6 levels in serum and liver tissue of PDTC group and AG490 group were significantly lower than those of model group (Table 2).

Table 1

p-JAK2 and p-STAT3 expression in liver tissue as well as serum liver injury indexes of four groups of rats (n = 8; mean ± SD).

Group	p-JAK2 and p-STAT3 expression in liver tissue		Serum liver injury indexes			
	p-JAK2	p-STAT3	TBIL (μmol/L)	ALT (U/L)	AST (U/L)	γ-GT (U/L)
Normal	1.00 ± 0.15	1.00 ± 0.18	22.58 ± 4.61	25.21 ± 4.56	31.58 ± 5.16	15.61 ± 3.26
Model	2.95 ± 0.51*	2.44 ± 0.39*	97.63 ± 10.15*	221.43 ± 37.85*	252.76 ± 31.29*	164.26 ± 22.68*
PDTC	1.48 ± 0.18#	1.55 ± 0.22#	36.58 ± 5.61#	78.76 ± 9.61#	89.65 ± 10.12#	56.53 ± 7.65#
AG490	1.54 ± 0.20#	1.61 ± 0.25#	34.15 ± 6.10#	85.11 ± 10.25#	95.41 ± 11.38#	52.31 ± 7.18#

*P < 0.05, compared with normal group; #P < 0.05, compared with model group.

Table 2

Comparison of inflammatory factor levels in serum and liver tissue among four groups of rats (n = 8; mean ± SD).

Group	Inflammatory factors in serum (ng/mL)			Inflammatory factors in liver tissue (ng/mg total protein)		
	TNF-α	IL-1β	IL-6	TNF-α	IL-1β	IL-6
Normal	6.49 ± 0.92	18.54 ± 2.78	11.35 ± 1.67	3.20 ± 0.55	4.11 ± 0.64	2.52 ± 0.55
Model	31.35 ± 6.62*	65.58 ± 9.24*	52.31 ± 8.72*	12.49 ± 1.85*	14.21 ± 1.86*	9.21 ± 1.26*
PDTC	13.42 ± 1.88#	27.63 ± 4.14#	19.33 ± 2.75#	5.25 ± 0.77#	6.58 ± 0.92#	3.54 ± 0.62#
AG490	14.95 ± 1.77#	30.21 ± 5.22#	18.46 ± 2.38#	5.41 ± 0.81#	6.81 ± 0.98#	4.66 ± 0.61#

*P < 0.05, compared with normal group; #P < 0.05, compared with model group.

Table 3

Comparison of oxidative stress product levels in serum and liver tissue among four groups of rats (n = 8; mean ± SD).

Group	Oxidative stress products in serum (nmol/mL)		Oxidative stress products in liver tissue (nmol/mg total protein)		
	8-OHdG	MDA	ROS	8-OHdG	MDA
Normal	6.21 ± 0.93	8.93 ± 1.09	2.05 ± 0.44	4.59 ± 0.67	5.51 ± 0.79
Model	29.44 ± 4.12*	42.57 ± 6.49*	8.59 ± 1.26*	19.28 ± 2.87*	25.42 ± 3.62*
PDTC	9.89 ± 1.15#	15.49 ± 1.95#	3.18 ± 0.49#	7.68 ± 0.93#	8.68 ± 1.15#
AG490	10.15 ± 1.25#	16.02 ± 2.25#	2.98 ± 0.45#	7.91 ± 0.98#	9.02 ± 1.08#

*P < 0.05, compared with normal group; #P < 0.05, compared with model group.

3.4. Oxidative stress product levels in serum and liver tissue

Analysis of oxidative stress products 8-OHdG and MDA in serum as well as oxidative stress products ROS, 8-OHdG and MDA levels in liver tissue among four groups of rats was as follows. 8-OHdG and MDA levels in serum as well as ROS, 8-OHdG and MDA levels in liver tissue of model group were significantly higher than those of normal group, and 8-OHdG and MDA levels in serum as well as ROS, 8-OHdG and MDA levels in liver tissue of PDTC group and AG490 group were significantly lower than those of model group (Table 3).

4. Discussion

Combined use of anti-tuberculosis drugs is a common cause of drug-induced liver injury, and how to reduce liver injury in the process of anti-tuberculosis drug treatment has been the hot topic of clinical scholars. PDTC is a drug with protective effect on tissues and cells, studies have shown that the drug can reduce the myocardial tissue [5], kidney tissue [6], liver tissue [7], pancreas tissue [8] and other tissue injury caused by inflammation and oxidative stress, but it is unclear about the effect of the drug on anti-tuberculosis drug-induced liver injury. In the process of drug-induced liver injury, the liver cell rupture can directly cause the release of ALT, AST, γ-GT and many other kinds of metabolic enzymes from the cytoplasm into the blood circulation, and if the bilirubin metabolism is affected, it will indirectly cause the accumulation of bilirubin in the blood circulation. In this study, the analysis of serum liver injury indexes among all groups of rats showed that serum TBIL, ALT, AST and γ-GT levels of model group were significantly higher than those of normal group, and serum TBIL, ALT, AST and γ-GT levels of PDTC group were significantly lower than those of model group. This shows that the combination of anti-tuberculosis drugs isoniazid and rifampicin can cause liver injury in rats, and PDTC intervention can reduce the degree of anti-tuberculosis drug-induced liver injury, and protect the liver.

Studies on anti-tuberculosis drug-induced liver injury in recent years have shown that the inflammation and oxidative stress reaction activation in the liver tissue is an important part of the liver injury [9,10]. JAK2/STAT3 is the important signaling pathway regulating inflammation and oxidative stress in the body [11,12]. The JAK2 can be activated in the form of phosphorylation and then cause downstream STAT3 phosphorylation and make it transfer into the nucleus so as to start the expression of inflammation and oxidative stress-related genes, and accentuate the inflammation and oxidative stress reaction in tissue [13,14]. In order to confirm whether anti-tuberculosis drugs caused liver injury through the JAK2/STAT3 signaling pathway, the p-JAK2 and p-STAT3 expression in liver tissue were analyzed in the study, and the results showed that p-JAK2 and p-STAT3 expression in liver tissue of model group were significantly higher than those of normal group. This means that the phosphorylation activation of JAK2/STAT3 signaling pathways is an important pathological link for anti-

tuberculosis drugs to cause liver injury. PDTC is a drug with significant anti-inflammatory and anti-oxidative stress effect, as mentioned previously, PDTC can reduce the degree of anti-tuberculosis drug-induced liver injury, and the effect of PDTC intervention on JAK2/STAT3 signaling pathway activation in the liver tissue was further analyzed on the basis, and it showed that p-JAK2 and p-STAT3 expression in liver tissue of intervention group were significantly lower than those of model group. It means that PDTC has significant inhibitory effect on the phosphorylated activation of JAK2/STAT3 signaling pathways in the process of anti-tuberculosis drug-induced liver injury, and then can inhibit the inflammation and oxidative stress through the JAK2/STAT3 signaling pathway.

In order to define whether the inhibitory effect of PDTC on JAK2/STAT3 signaling pathway in the process of anti-tuberculosis drug-induced liver injury could inhibit the activation of inflammation and oxidative stress, the changes in inflammation and oxidative stress indexes were analyzed in the study. In the activation of the inflammatory response, the secretion of TNF-α, IL-1β, IL-6 and other inflammatory factors increases significantly, and they can mediate cascade amplification of inflammatory response and cause inflammatory liver cell damage [15–17]. In the process of oxidative stress activation, the ROS production in local liver increases significantly and it can have peroxide reaction with the DNA and lipid compositions in liver cells to cause the oxidative liver cell damage; 8-OHdG and MDA are the peroxide reaction products of DNA and lipid composition in the cells, and they can reflect the degree of oxidative stress reaction [18–20]. In the study, analysis of inflammation and oxidative stress indicators in rats after PDTC intervention showed that TNF-α, IL-1β, IL-6, 8-OHdG and MDA levels in serum as well as TNF-α, IL-1β, IL-6, ROS, 8-OHdG and MDA levels in liver tissue of PDTC group were significantly lower than those of model group. It means that PDTC can inhibit the activation of inflammation and oxidative stress in the process of anti-tuberculosis drug-induced liver injury. In order to further clarify the effect of JAK2/STAT3 signaling pathway on the inflammation and oxidative stress in the liver tissue, the JAK2-specific inhibitor AG490 was used in the study for the intervention of anti-tuberculosis drug-induced liver injury model rats, and the results showed that TNF-α, IL-1β, IL-6, 8-OHdG and MDA levels in serum as well as TNF-α, IL-1β, IL-6, ROS, 8-OHdG and MDA levels in liver tissue of AG490 group were significantly lower than those of model group. This means that inhibiting JAK2/STAT3 signaling pathway can reduce the inflammation and oxidative stress activation in the process of anti-tuberculosis drug-induced liver injury.

To sum up, it is believed that the inflammation and oxidative stress mediated by JAK2/STAT3 signaling pathway activation are closely related to the anti-tuberculosis drug-induced liver injury; PDTC can inhibit the inflammation and oxidative stress mediated by JAK2/STAT3 signaling pathway to reduce the degree of anti-tuberculosis drug-induced liver injury.

Conflict of interest statement

We declare that we have no conflict of interest.

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