Phenidone, a dual inhibitor of cyclooxygenase and lipooxygenase, inhibits carbon tetrachloride-induced acute liver injury in rats

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Abstract: This study was carried out to find whether phenidone (1-phenyl-3-pyrazolidinone), a cyclooxygenase as well as a lipooxygenase inhibitor, exhibits the preventive effect on carbon tetrachloride (CCl₄)-induced acute liver injury in rats. Rats were pretreated with phenidone at a dose of 50 or 200 mg/kg (p.o.) once daily for 3 consecutive days before CCl₄ administration. Serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were measured. Malondialdehyde (MDA) production was determined as an index of lipid peroxidation in the liver and serum. The histopathological changes in the liver were also examined in each group. The reduction in body weights was significantly inhibited in the phenidone-treated group than in the CCl₄ control group. Significant increase in the relative liver weights of the phenidone-treated groups was observed compared with either the vehicle or CCl₄ groups. Elevation of serum AST and ALT activities occurred after CCl₄ treatment was significantly attenuated by the pretreatment with phenidone. The elevation of MDA levels in liver and serum were completely inhibited in phenidone-treated groups. The protective effects on phenidone-treated groups were confirmed histopathologically. These results suggest that phenidone may be a useful protector through modulation of hepatic inflammation in CCl₄-induced acute liver injury.

Keywords: ALT, AST, carbon tetrachloride (CCl₄), malondialdehyde, phenidone

Introduction

Carbon tetrachloride (CCl₄) is a well-known hepatotoxicant inducing liver injury in experimental animals. When the metabolism of CCl₄ is initiated by NADPH-dependent cytochrome P-450 enzyme, the trichloromethyl radicals (•CCl₃) are produced in liver microsomes and react with O₂ to form trichloromethyl peroxy radicals (Cl₃COO•) that cause membrane lipid peroxidation [5].

The cleavage products of the lipid peroxides, namely malondialdehyde, 4-hydroxy-2-pentenal, 2,4-hexadienal, and 4-hydroxy-2-nonenal have the toxic mechanisms of causing the breakdown of the smooth endoplasmic reticulum (sER) structure, decreased activity of the sER enzyme, and the inhibition of the protein synthesis which leads to fatty liver connected with hepato cellular necrosis [1]. The CCl₄ also causes direct and acute toxicity in the liver and results in the central lobular necrosis of the liver histopathologically.

Cyclooxygenase (COX) and/or lipooxygenase (LOX), two major pro-inflammatory enzymes that mediate the arachidonate cascade pathway, are believed to be involved in the progression of liver injury by CCl₄ [3]. When CCl₄-induced liver damage occurs, the levels of leukotrienes mediated by LOX are known to increase at an early stage while prostaglandins mediated cyclooxygenase-2 (COX-2), the isoform of inducible type, increases at a later stage during hepatic inflammation [3]. Recently, a strategy of combination therapy with inhibitors of COX and LOX has been found to improve hepatic function [2].

Phenidone (1-phenyl-3-pyrazolidinone) is known to exhibit a dual inhibitory action of COX and LOX in various inflammatory diseases. Previously, we had reported that phenidone has a protective action on
various neurotoxic or neuroinflammatory disease models in \textit{in vitro} and \textit{in vivo} studies \cite{4, 6, 10}. Therefore, in this study, we examined whether pretreatment of phenidone exhibited protective effects on CCl\textsubscript{4}-induced hepatotoxicity through alleviation of lipid peroxidation in rats.

\textbf{Materials and Methods}

\textbf{Experimental animals}
As many as 12 female and male Sprague-Dawley rats which were ten weeks old were purchased from Orient Bio (Korea). Rats were housed in plastic cages and maintained at 23 ± 2°C for 12 h / 12 h light-dark cycle. The feed was 5L79, rat formula, manufactured by PMI nutrition in USA. Feed and water were given \textit{ad libitum} before fasting. The rats were fasted for 20 h before CCl\textsubscript{4} injection, but water was supplied \textit{ad libitum} during the whole experimental period. The rats were sacrificed 24 h after CCl\textsubscript{4} injection. All animal studies were performed in accordance with the National Institute of Health (USA) Guide for the Humane care and Use of Laboratory Animals.

\textbf{Preparation of materials}
Phenidone, CCl\textsubscript{4}, thiobarbituric acid (TBA), and sodium dodecyl sulfate (SDS) were purchased from Sigma (USA). Acetic acid and KCl were purchased from Showa Chemical (Japan) and Yakuri Pure Chemicals (Japan), respectively. Olive oil was purchased from Public Market in Chuncheon (CJ, Korea).

\textbf{Instruments}
Spotchem EZ SP-4430 (Arkray, Japan), T10 basic homogenizer (IKA Ultra-Turrax, Germany), and Spectra Max Plus Spectrophotometer (Molecular Devices, USA) were used for every biochemical assay.

\textbf{Grouping of the experimental rats}
The rats were divided into four groups. Each group was classified as follows: control group (vehicle), CCl\textsubscript{4} group, CCl\textsubscript{4} + phenidone (50 mg/kg) group, and CCl\textsubscript{4} + phenidone (200 mg/kg) group. Each group consisted of six rats, three females and three males. A 1 : 1 (v/v) mixture of CCl\textsubscript{4} and olive oil was injected (2 mL/kg) intraperitoneally to the rats of the experimental groups. Phenidone was administered orally once a day for 3 days before CCl\textsubscript{4} injection. Rats were fasted and sacrificed 24 h after CCl\textsubscript{4} injection.

\textbf{Preparation of serum and liver homogenate}
The rats were anesthetized using urethane (ethyl carbamate, 1.5 g/kg, \textit{i.p.}) for the sampling of blood and liver. Blood samples were collected from the abdominal aorta. The samples were kept in the refrigerator for 1 h and centrifuged at 3,000 rpm for 15 min. The supernatant was used as serum.

The blood in the liver was eliminated by washing immediately with refrigerated saline. The saline on the surface of the liver was removed using gauze and the relative liver weights per 10 g of body weight measured. Similar portion of tissues removed from livers were analyzed for malondialdehyde (MDA). Liver tissues were placed in ten-time refrigerated 1.15% KCl-10 mM phosphate buffer (pH 7.4) in order to be homogenized.

\textbf{Measurement of MDA in the liver tissue}
The MDA production induced by the injection of carbon tetrachloride was measured by the procedure of Ohkawa \textit{et al.} \cite{7}. Briefly, 200 µL of the liver homogenates was put into each test tube and 8.1% SDS solution was added and mixed. After 1.5 µL of the 20% acetic acid was added and mixed, 1 µL of the 1.2% TBA solution was added and heated in the water bath for 1 h. The tubes were cooled to room temperature and centrifuged for 10 min at 2,000 rpm. The absorbance was measured at 532 nm. The concentration of the lipid peroxides was expressed as MDA nmol per gram liver weight.

\textbf{Measurement of the serum aspartate aminotransferase (AST), alanine aminotransferase (ALT) and MDA}
Serum AST and ALT were evaluated by Spotchem EZ SP-4430. The serum was diluted if the value of the measurement was out of range. Serum MDA was quantified using thiobarbituric acid reactive substances Assay kit (ZeptoMetrix, USA). The 100 µL serum supernatant was put into the test tubes and 100 µL SDS solution was added and mixed. Simultaneously, a 2.5 mL TBA/ Buffer Reagent was mixed and heated at 9.5°C in a water bath for 60 min and cooled for 10 min in an ice box. It was centrifuged for 15 min at 3,000 rpm and the absorbance value of the supernatant was measured at 532 nm.