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Effects of curcumin on antioxidative activities and cytokine production in Jian carp (*Cyprinus carpio var.* Jian) with CCl_4-induced liver damage

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ABSTRACT

We investigated the protective effects of curcumin on liver-damaged *Cyprinus carpio var.* Jian (Jian carp). The carp were fed 0.1%, 0.5%, or 1.0% curcumin for 60 days, then injected intraperitoneally with 30% carbon tetrachloride solution. Liver and blood samples were collected to measure the liver index, serum- and liver-associated enzymes, liver histology, nuclear factor-κB (NF-κB)/c-Rel, interleukin-1β (IL-1β), tumor necrosis factor-α (TNF-α), and IL-12 mRNA expression, and the level of NF-κB/c-Rel protein in the liver, and for a comet assay. We found that 0.5% and 1.0% curcumin significantly reduced the CCl_4-induced increase in the liver index. The comet assay showed that the tail moment, olive tail moment, tail length, and tail DNA% improved in fish pretreated with 0.5 or 1.0% curcumin. CCl_4-induced histological changes, including extensive hepatocyte degeneration, indistinct cell borders, nuclear condensation, and karyolysis were clearly reduced after treatment with 0.5% and 1.0% curcumin. Moreover, 0.5% and 1.0% curcumin significantly inhibited the CCl_4-induced increase in serum glutamic oxaloacetic transaminase and promoted the restoration of superoxide dismutase in the liver; 1.0% curcumin significantly reduced serum glutamic pyruvic transaminase and lactate dehydrogenase and hepatic malondialdehyde, but significantly increased the total antioxidant capacity and glutathione levels in the liver. The CCl_4-induced upregulation of NF-κB/c-Rel, IL-1β, and TNF-α mRNAs and NF-κB/c-Rel protein levels was inhibited by 0.5% and 1.0% curcumin, and IL-12 mRNA was reduced by all three doses of curcumin. The effects of curcumin on the liver index, enzymes, histological changes, and cytokines were dose-dependent. Our results indicate that curcumin reduces CCl_4-induced liver damage in Jian carp by upregulating antioxidative activities and inhibiting NF-κB, IL-1β, TNF-α, and IL-12 expression.

1. Introduction

In the rapidly developing aquaculture industry, the fish liver is influenced by many risk factors, including environmental pollution, the use of high-protein, high-fat, and moldy feed, and the abuse of antibiotics and pesticides [1]. These risk factors clearly affect normal hepatic cell functions in the fish, causing liver damage such as liver lesions and liver cell disruption, ultimately resulting in death. Currently, the occurrence of fish liver diseases cannot be effectively controlled by antibiotics, insecticides, or antiviral medications [2]. Various enzymes and free radicals are dramatically affected in liver cells in many liver diseases, together with lipid peroxidation [3]. Many cytokines are activated in the progression of liver damage, directly inducing damage to liver cells and indirectly exerting toxic effects by the induction of inflammatory mediators [4,5].

Carbon tetrachloride (CCl_4), a classic hepatotoxicant, is widely used to generate liver damage models in which to screen liver-

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directed drugs [6]. CCl_4 is degraded into $CCl_3\cdot$ by cytochrome P450, and $CCl_3\cdot$ selectively damages liver cells in the center of the liver lobules [7], where it is transformed into CCl_3O_2. These free radicals not only cause lipid peroxidation, which damages liver cells, but also induce the overproduction of inflammatory mediators (interleukin-1β [IL-1β], tumor necrosis factor-α [TNF-α], IL-4, and IL-12) by activating nuclear factor-κB (NF-κB). These mediators then activate inflammatory cells, such as neutrophils, leading to liver cell death [8,9].

Traditional Chinese medicine (TCM) has been widely used to enhance immunity. Previous studies have demonstrated that various TCMs play important roles in regulating various cytokines, enhancing the antioxidant system in the body, and promoting antiviral, antitumor, and anti-aging effects [10,11]. With lower toxicity and fewer adverse effects than other drugs and no bioaccumulation, TCMs have been widely used in the control of aquatic animal disease in recent decades. In particular, TCMs have been effective in treating enteritis and improving the immunity of *Ctenopharyngodon idellus*, *Anguilla anguilla*, and *Acipenser baeri* [12,13]. Recent studies have demonstrated that TCMs are significantly effective in preventing liver damage in fish [14,15].

Curcumin, or 1,7-bis(4-hydroxy-3-methoxyphenyl) −1,6-heptadiene-3,5-dione, is the major active component in *Curcuma longa* L. and the only natural medicine containing phenolic and quinone groups [16]. Curcumin has strong anti-inflammatory, antioxidative, antitumor, and antidepressant activities [17,18], so is widely used in clinical therapy. In recent years, it has been reported that curcumin exerts a protective effect against liver damage. In a CCl_4-induced rat model of liver damage, curcumin significantly reduced the levels of serum alanine aminotransferase and aspartate aminotransferase [19]. Rukkumani, Aruna [20] demonstrated that curcumin and a synthetic analogue exerted significant protective effects against the oxidative stress induced by alcohol and thermally oxidized sunflower oil, confirming that curcumin can be used as an effective antioxidant. Nanji, Jokelainen [21] reported that curcumin prevented alcohol-induced liver disease in rats via the NF-κB signaling pathway. However, the effects of curcumin on liver damage in fish and the associated mechanisms have rarely been studied.

In this study, we established a liver damage model in *Cyprinus carpio* var. Jian (Jian carp), induced with CCl_4, and investigated the effects of curcumin on changes in the liver index, liver-associated enzymes in the liver and serum, liver histology, and the expression of NF-κB/c-Rel, IL-1β, TNF-α, and IL-12 mRNAs in the liver.

2. Materials and methods

2.1. Reagents

Curcumin was purchased from Sigma–Aldrich (St. Louis, MO). CCl_4 (analytical grade) was purchased from Sinopharm Chemical Reagent Co., Ltd (Shanghai, China) and dissolved in olive oil to generate a 30% CCl_4 solution (w/v).

2.2. Fish and treatment

Jian carp were obtained from the Freshwater Fisheries Research Center farm at the Chinese Academy of Fishery Sciences. One week before the experiment, all healthy carp of a similar size and weight were placed in a water circulation system under the following conditions: 27 ± 2 °C, pH 6.8–7.6, >5 mg/L dissolved oxygen, < 0.05 mg/L NH_3, and <0.01 mg/L H_2S. The carp were fed a basal diet (40% crude protein, 10% crude fat, 10% crude fiber, energy 21 kJ/g dry matter) twice daily. This study was performed in accordance with the World Organization for Animal Health (OIE) laws and regulations on animal welfare and was approved by the Ethics Committee of the Chinese Academy of Fishery Sciences.

Healthy carp weighing 30 ± 1.0 g were randomly assigned to six groups, with 20 carp per group. The carp in the normal control group and CCl_4 control group were fed the basal diet for 60 days, and then intraperitoneally injected with olive oil and 30% CCl_4 solution (0.05 mL/10 g bodyweight), respectively. The carp in the curcumin experimental groups were fed a basal diet containing 0.1%, 0.5%, or 1.0% (mass ratio) curcumin for 60 days and injected intraperitoneally with 30% CCl_4 solution. The carp in the curcumin control group were fed the basal diet containing 1.0% curcumin and injected intraperitoneally with olive oil. After injection, all the carp were fasted for 72 h. At the end of the experiment, all the carp were anesthetized with 0.05% tricaine methanesulfonate (MS-222; Sigma–Aldrich) and weighed. Blood was collected from the tail vein. The liver was removed and weighed to determine the liver index according to the following formula: liver index = liver weight (g)/bodyweight (g) × 100%.

2.3. Assessment of DNA damage (comet assay)

Fish were anesthetized with MS-222, and their livers were quickly removed, placed in phosphate-buffered saline (PBS; pH 7.5) on ice, and gently cut with scissors. The isolated hepatocytes were released into suspension by gently stirring the liver with a pipette. The cells were filtered through a 100-mesh sieve, washed, and resuspended in sterile cool Dulbecco's PBS. When the viability of the cells was >90% (as assessed with Trypan Blue exclusion [22]), they were used for a comet assay.

DNA damage was evaluated with a single-cell gel electrophoresis assay, according to the method of Piperakis, Kontogianni [23]. Cells (5×10^3 in 25 μL) were suspended in 75 μL of 0.5% low-melting agarose (LMA). The suspension was then dropped onto a glass slide and immediately covered with a cover slip. Three replicates were performed for each group. The slides were kept on ice for 15 min and the cover slips were then removed. The slides were immersed in cold lysing solution (2.5 M NaCl, 100 mM EDTA, 100 mM Tris, 1% sodium sarcosinate [pH 10.0], with 1% Triton X-100 and 10% DMSO) for 2 h at 4 °C. They were then removed, drained, and placed in horizontal electrophoresis tanks filled with electrophoresis solution (1 mM sodium EDTA, 300 mM NaOH [pH 13.0]) for 20 min at 4 °C. The slides were neutralized by washing them three times with 0.4 M Tris-HCl (pH 7.5) for 5 min each at room temperature. After neutralization, the slides were incubated sequentially in 50%, 70%, 80%, 90%, 100%, and 100% alcohol for 5 min each. The dried slides were stained with DuGreen nucleic acid gel stain (2 μg/ml in distilled water; Yeasen Company, Shanghai, China) for 2 min, covered with a cover glass, and observed and photographed under a fluorescence microscope (Olympus LX70 and Olympus SP6T, Japan) with green light. Randomly chosen nuclei (n = 50) were evaluated per slide. CASP (Comet Assay Software Project, version 1.2.3 b1) was used to determine the extent of DNA damage after the DNA fragments had migrated electrophoretically in the agarose gel. The results are expressed as tail length (μm), tail DNA (%), tail moment (μm), and olive tail moment (μm).

2.4. Liver histology

One portion of liver tissue per fish was weighed, cut, and fixed in 10% formalin, dehydrated, infiltrated with xylene, and embedded in paraffin. Sections (4–5 μm thick) were prepared, deparaffinized, and rehydrated. The slides were stained with the conventional hematoxylin–eosin (HE) protocol, mounted with neutral resin, and examined with optical microscopy.