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Effects of dietary baicalin supplementation on iron overload-induced mouse liver oxidative injury

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Abstract

Iron overload is one of the most common metal related toxicity. Under this circumstance, excessive iron deposition in liver will lead to further injury such as hepatocellular necrosis, inflammation, fibrosis, and in some cases even to carcinoma. In this paper, the effect of a nature flavonoid, baicalin, on iron overload-induced mouse liver oxidative injury has been studied. It was found that when iron–dextran-induced iron overload, mice were fed baicalin-containing diet (0.25% and 1%) for 50 days, hepatic iron, liver-to-body weight ratio, and hepatic lipid peroxidation were dose-dependently decreased; while catalase activity, total antioxidant status, and serum iron content were dose dependently increased. The protective effect of baicalin on liver of iron overload mouse may due to both the antioxidant and iron chelation activities of baicalin. These data provide preliminary experimental support for baicalin as medicine for iron overload diseases.

Keywords: Baicalin; Iron overload; Oxidative injury; Liver

1. Introduction

Iron overload is one of the most common metal-related toxicity. Progressive iron-induced liver injury in human is most often encountered in diseases of aberrant iron storage resulting from abnormal increase in the gastrointestinal absorption of dietary iron. Under these circumstances, excessive iron deposition in liver will lead to further injury such as hepatocellular necrosis, inflammation (Deugnier et al., 1992), fibrosis (Arezzini et al., 2003; Gardi et al., 2002), and in some cases even to carcinoma (Niederau et al., 1985). Hepatocellular lipid peroxidation of polyunsaturated fatty acids in membranes has been implicated as a mechanism by which iron causes liver damage (Fletcher et al., 1989). Iron-induced peroxidation of intracellular membranes may lead to cellular dysfunction and eventually sideronecrosis (Arthur, 1996).

At present, two chelating agents, i.e. deferoxamine and 1,2-dimethyl-3-hydroxypyrid-4-one (deferiprone, L1) are commonly used in the treatment of iron overload diseases. Although both drugs are very effective, they exert several side effects and disadvantages (Al-Refaie et al., 1992; Richardson, 1999; Kontoghiorghes et al., 2001). Deferoxamine is not orally absorbed and requires slow subcutaneous administration resulting in poor patient compliance (Olivieri and Brittenham, 1997). A non-toxic and orally active iron chelator is needed.

Flavonoids are phenolic compounds widely distributed in plants, which have been reported to exert multiple biological effects, including antioxidant and free radical scavenging abilities (Bors and Saran, 1987; Negre-Salvayre and Salvayre, 1992). They were also suggested to present a strong affinity to iron ions (Boyer et al., 1988; Havsteen, 1983; Afanas'ev et al., 1989; Morel et al., 1993; Borsari et al., 2001). Baicalin is a flavonoid which is found rich in *Scutellaria baicalensis* Georgi, a commonly used traditional antiinflammatory herb medicine in China, Japan, and Korea. Baicalin is an active antioxidant flavonoid confirmed by some researchers (Yoshino and Murakami, 1998) as well as

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us (Gao et al., 1999), it is also found to be an effective iron chelator (Yoshino and Murakami, 1998). Our previous study found that dietary supplementation of baicalin caused a decrease of rat liver iron content (Gao et al., 2003). These results suggest that baicalin might be used as an iron chelator for iron overload. The aim of the present investigation is testing the effect of baicalin on experimental mouse model of iron overload.

2. Materials and methods

2.1. Materials

Baicalin standard was purchased from National Institute for the Control of Pharmaceutical and Biological Products (China). 2,2′ -azinobis-(3-ethyl-benzothiazoline-6-sulfonic acid) (ABTS) and Trolox were purchased from Sigma. Baicalin was extracted from scutellaria roots according to the method described by Han et al. (1997) and re-crystallized in methanol. The purity of the extraction was tested by polyamine thin-layer chromatography and ultraviolet-visible spectrophotometry; the content of baicalin in the extraction was 98.8%. Other chemicals were purchased from local market. Kunming mice were purchased from Tongji Medical School, Huazhong University of Science and Technology (China).

2.2. Animals

Thirty-six male Kunming mice, initially weighing 15.6±0.9 g, were used. Mice were randomly divided into four groups. Three groups received 6 doses (three doses per week) of 100 mg/kg each (i.p. iron–dextran–saline), the other group received the same volume of saline as a blank (B). The blank group and one iron–dextran group (C) were fed commercial animal chow, the other two groups were fed with the same animal chow supplemented with 0.25% (LBa) or 1% of baicalin (HBa) (wt/wt), respectively (about 0.5 g/kg or 2 g/kg b.w. daily, respectively). The amount of other flavonoids in animal food was not considered. Each group of mice were housed in a large plastic cage and given free access to food and tap water. All groups of animals were kept at 23±2 °C under a 12-h dark/light cycle. The body weight of the mice was measured every 5 days. Animal care in this study conformed to the NIH Guide for Care and Use of Laboratory Animals (NIH publication 86-23, revised 1986).

2.3. Tissue preparation

After 50 days of feeding, mice were fasted overnight. They were anesthetized with ethyl ether, and blood was collected by cardiac puncture. The liver was quickly removed and weighted, then perfused with 4 °C saline to exclude the blood cells and then blotted on filter paper; half of them were cut, weighed and homogenized with a glass homogenizer in 9 volume of ice-cold 50 mM phosphate-buffered saline (PBS). Portions of homogenates were immediately pipetted for measuring the levels of thiobarbituric acid reactive substance. The remaining homogenate was centrifuged (10,000×g), then the supernate was used to measure catalase activity and total antioxidant assay. The protein contents in the homogenate and supernate were measured as Peterson (1977) described. The other half liver was weighed and put into a glass flask, then 5 volumes of mixed acid (nitric acid: perchloric acid=4:1) was added, heated until large amount of white vapors could be seen. The volume of the digested sample was adjusted to 10 ml with double distilled water, and then the obtained solution was used to analyze iron contents.

2.4. Lipid peroxidation and antioxidant enzymes analyses

100 μl of liver homogenate was used to measure the lipid peroxidation by the thiobarbituric acid method (Buege and Aust, 1978). Catalase activity in liver homogenate was assayed at 25 °C by a method based on the absorbency disappearance of 10 mM H_2O_2 at 240 nm (Pedraza-Chaverri et al., 1999).

2.5. Total antioxidant status assay

Total antioxidant status was assayed according to the method introduced by Re et al. (1999) and modified by Gao et al. (2003). Briefly, ABTS was dissolved in water to a 7 mM concentration. ABTS radical cation ($ABTS^{\cdot+}$) was produced by first adding MnO_2 powder in the solution and keeping in the dark at room temperature for more than 12 h, then filtrated with syringe filter and kept in dark for another 6 h. Stock solution of $ABTS^{\cdot+}$ was diluted with PBS, pH 7.4, to an absorbance of 0.70 (±0.02) at 734 nm and equilibrated at 30 °C. After adding 1.0 ml of diluted $ABTS^{\cdot+}$ to 10 μl of serum or tissue homogenate supernate, the absorbance reading was taken at 30 °C exactly 2 min after initial mixing. The total antioxidant capacity concentration was compared with equivalent antioxidant capacity of Trolox and was expressed in μmol of Trolox/g of tissue.

2.6. Determination of serum iron and liver iron

Serum iron concentration was determined using the assay based on the generation of an iron–ferrozine complex as described by Galleano and Puntarulo (1992) previously. Iron concentration in the liver digested sample was measured spectrophotometrically at 535 nm, after reaction with 2 mM bathophenanthroline disulfonic acid (Brumby and Massey, 1967).

2.7. Statistical analysis

The results are expressed as means±S.D., (and) statistical significance was determined using a one-way analysis