EFFECTS OF KOJIC ACID ON OXIDATIVE DAMAGE AND ON IRON AND TRACE ELEMENT LEVEL IN IRON-OVERLOADED MICE AND RATS

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SUMMARY

Since members of hydroxypyrone series posses iron chelating properties, kojic acid (KA), 5-hydroxy-2- (hydroxymethyl)-4H-pyran-one, a fungal metabolite of natural origin, has been suggested to might play a role in iron-overload diseases and in oxidative stress conditions involving transition metal. In our experiments in vivo models of iron-overload were used to study iron-chelating properties of KA and its effect on oxidative damage in mice and rats. The treatment of iron-preloaded rats (25 mg Fe.kg^{-1} b.w., i.p., daily for five days) with 0.5% KA in drinking water for four weeks did not lower the iron concentration accumulated in the liver, neither diminished the induced hepatic lipid peroxidation in iron-loaded rats. The GSH level decreased in KA-treated group. Similarly, in iron-loaded mice model experiment, the following oral treatment with KA (100 mg.kg^{-1}) daily for 7 days did not decrease the level of Fe accumulated in the liver and the lipid peroxidation even enhanced after KA treatment. Though in our experiments in vivo the ability of kojic acid to affect iron kinetics in the organism could not be proved, kojic acid as a molecule of natural origin may serve as a template for the preparation of new biologically active derivatives possessing capability of chelating iron.

Key words: kojic acid, chelator, iron accumulation, oxidative damage, trace elements

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INTRODUCTION

Kojic acid (KA), 5-hydroxy-2-(hydroxymethyl)-4H-pyran-one, is a fungal metabolite commonly produced from carbohydrate sources (glucose, saccharose, starch etc.) in aerobic processes by a variety of microorganisms (species of *Aspergillus*, *Acetobacter* and *Penicillium*). It is a component of number of fermented foods and beverages (soybeen paste, soy sauce, sake) and widely used as a food additive for preventing enzymatic browning, and in cosmetic preparations as a skin lightening or bleaching agent (1, 2). It has been shown to act as a competitive and reversible inhibitor of animal and plant polyphenol oxidases and xanthine oxidase. Acute and subchronic toxicity resulting from oral doses has not been reported; chronic feeding studies in mice and rats indicated the development of thyroid follicular cell adenomas primarily due to inhibition of iodine uptake and metabolism leading to decreases in T3 and T4 and increase in TSH (3, 4). The proliferative effects of kojic acid on thyroid is not related to genotoxic pathway, and within 48h of withdrawal of KA-treatment serum T3, T4 and TSH returned to normal (5). Since members of the hydroxypyrone series posses iron chelating properties (6), kojic acid has been suggested to might play a role in iron-overload diseases and in oxidative stress conditions involving transition metal (7, 8, 9). In our experiments the effect of kojic acid treatment on parameters of oxidative damage, iron accumulation and essential elements content has been studied in the target tissues of iron toxicity in mice and rats.

METHODS

In experiment A, male CD mice (Anlab, Prague, CZ; 35 ± 3g body mass) were loaded with iron polyisomaltosate [Ferrum Lek, Slovenia] in daily dose 25 mg Fe.kg^{-1} i.p. for 10 days. After 5 days break the chelator deferoxamine or kojic acid were applied to mice for 7 days. Single daily doses: deferoxamine hydrochloride [Desferal – Novartis] - 354 mg.kg^{-1} i.p., kojic acid (synthesized Dept. of organic chemistry, STU, Bratislava, SR) 100 mg.kg^{-1} per os in 0.25% methylcellulose. The animals were sacrificed 24h after the last injection of chelators.

Peroxidation of lipids (LP) expressed as malondialdehyde (MDA) production was estimated in liver homogenates by thiobarbituric acid test (10). The level of the reduced form of glutathione (GSH) was determined in liver homogenates with Ellman's reagent (11). The liver, kidneys and brain tissues were analyzed for iron and essential elements using atomic absorption spectrometry.

In experiment B, male Wistar rats (Anlab, Prague, CZ; 300 ± 30g body mass) were divided into two major groups of 16 animals. First group has been loaded with iron polyisomaltosate in daily dose of 25 mg Fe.kg^{-1} i.p. for 5 days. Second group served as control receiving saline. After 3 days break one half of rats from each group was treated with kojic acid (0.5% KA in drinking water, *ad libitum*) for 4 weeks, the second half of animals received drinking water without KA. The experiment was finished on the

36th day. Peroxidation of lipids and GSH level were determined in liver homogenates as stated above. The activity of glutathione peroxidase (GSH-Px) and catalase (CAT) were measured in liver homogenates (12), (13). The liver and kidneys were analyzed for iron and essential elements.

The animals were kept under standard laboratory conditions with free access to standard pellet diet in a controlled-temperature room (21-23 °C) and 12 h light/dark cycle. At the end of the experiment the rats were sacrificed under ether anesthesia. The liver and kidney were quickly excised, washed in ice-cold saline, weighed and stored frozen at –70 °C until analysis. Data are presented as means ± SD; the statistical significance of differences was evaluated using unpaired Student's t-test. Numbers of analyzed samples (n) per group are stated in the tables.

RESULTS

Experiment A

Data in Table 1 demonstrate iron accumulation in the liver and kidneys of iron polyisomaltosate treated mice. The 17-fold increase of iron hepatic concentration compared to control mice was diminished by deferoxamine treatment to 12-fold increase, kojic acid treatment exerted no effect on iron hepatic accumulation. In the kidneys of iron-loaded mice a 1.4-fold increase of iron was found and was not significanly changed by both chelator treatment.

As shown in Fig. 1, iron treatment induced lipid peroxidation expressed in terms of MDA formation in liver homogenates (440% of control level). There was a further increase in lipid peroxidation after kojic acid treatment (525% of control level), DFO treatment exerted no effect. Fig. 2 shows the level of GSH in liver homogenates, no significant difference was found when compared to control group. The administration of iron caused a decrease of zinc concentration in the liver as well as copper concentration in the kidneys and both remained unaffected by further treatment with the chelators (data not shown).

Experiment B

The data in Table 2 show the accumulation of iron in the liver and kidneys of iron-loaded rats. Four weeks supplementation of kojic acid had no effect on iron accumulation in the liver or kidneys. Kojic acid treatment alone moderately increased iron level in the liver compared to control animals. Fig. 3 demonstrate the inductive effect of iron loading on lipid peroxidation (220% of control level). No effect of kojic acid treatment was proved in both iron-loaded and control rats. Fig. 4 shows a significant decrease of GSH level in the liver of iron-loaded rats treated with kojic acid. The activity of GSH-Px measured in liver homogenates was equal in all experimental group. The catalase activity was not influenced by iron exposure, in KA-only treated group a decrease of CAT activity (89% of control level) was found (data not shown).

Table 3 shows the concentration of essential elements in the liver and kidney tissues. The 4-week-supplementation with kojic

Table 1. *The effect of kojic acid and deferoxamine on iron level in the tissues of iron-loaded male mice*

Treatment	n	Liver	Kidneys
Control	8	78 ± 12	48 ± 4
Fe	8	1392 ±242**	68 ± 6**
Fe + kojic acid	7	1430 ±227**	71 ± 8**
Fe + deferoxamine	7	1012 ±208**#	63 ± 7**

mean±SD; μg/g of wet tissue weight
** p<0.01 versus control group; # p<0.05 versus Fe group

Table 2. *The effect of kojic acid on iron level in the tissues of iron-loaded and control Wistar rats.*

Treatment	n	Liver	Kidneys
Control	8	110 ± 23	55 ± 5
Fe	7	1135 ±126**	80 ± 7**
Fe + kojic acid	7	1059 ±123**	84 ± 8**
Kojic acid	8	139 ± 20*	55 ± 3

mean±SD; μg/g of wet tissue weight
** p<0.01 versus control group; * p<0.05 versus control group

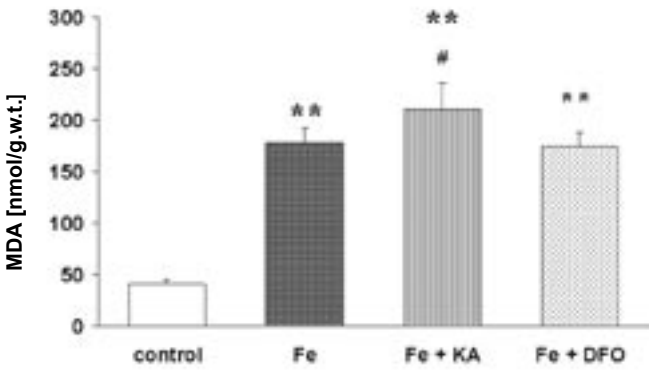

Fig. 1. *The influence of kojic acid on lipid peroxidation in the liver of iron-loaded mice [** p<0.01 versus control, * p<0.05 versus control, # p<0.05 versus Fe-group].*

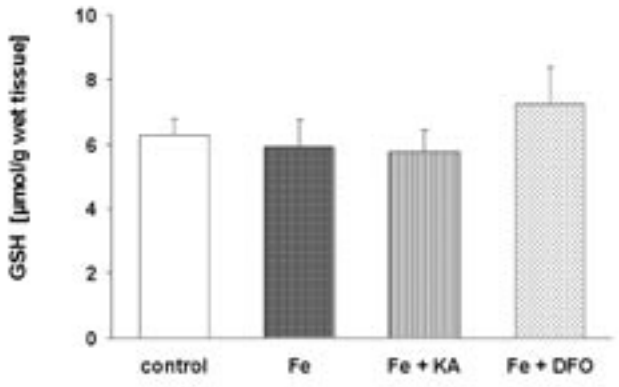

Fig. 2. *The influence of kojic acid on glutathione level in the liver of iron-loaded mice.*

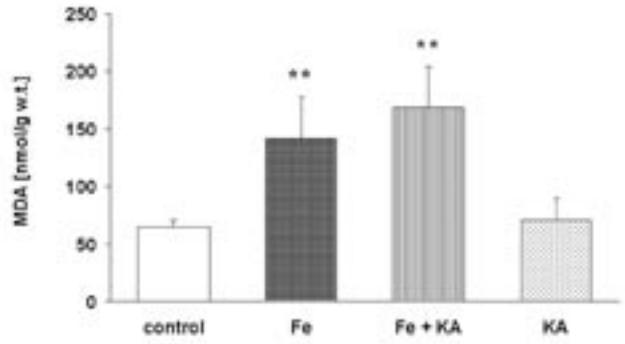

*Fig. 3. The influence of kojic acid on lipid peroxidation in the liver of iron-loaded Wistar rats [** p<0.01 versus control].*

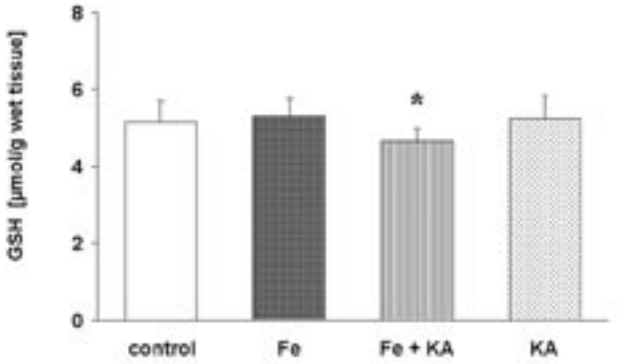

Fig. 4. The influence of kojic acid on glutathione level in the liver of iron-loaded Wistar rats [p<0.05 versus control].*

Table 3. *The effect of kojic acid on essential elements level in the liver and kidneys of iron-loaded and control Wistar rats*

Liver	**n**	**Ca**	**Mg**	**Zn**	**Cu**
Control	8	31.3 ±2.4	195 ± 5	30.4 ±2.0	4.09 ±0.24
Fe	8	28.7 ±4.3	196 ± 6	29.7 ±2.5	4.14 ±0.12
Fe + kojic acid	7	33.2 ±4.1	199 ± 6	31.9 ±2.3	4.52 ±0.32*
Kojic acid	8	35.0 ±6.5	203 ± 5*	32.8 ±1.7*	4.45 ±0.23*
Kidneys	**n**	**Ca**	**Mg**	**Zn**	**Cu**
Control	8	55.4 ±4.6	181 ± 6	23.7 ±1.6	10.4 ±2.2
Fe	8	54.6 ±3.3	174 ± 8	22.3 ±0.6	10.6 ±1.5
Fe + kojic acid	7	51.3 ±3.5	173 ± 8	23.6 ±1.2	9.5 ±.2.1
Kojic acid	8	49.7 ±2.1*	164 ± 4*	22.8 ±1.0	9.0 ±2.7

mean±SD; µg/g of wet tissue weight; *p<0.05 versus control group

acid resulted in enhanced copper concentration in the liver of both KA-treated groups and increased the level of magensium and zinc in KA-only treated group. In kidney tissue a decrease in level of calcium and magnesium was estimated in KA-only treated group.

DISCUSSION

The aim of our study was to evaluate the effect of kojic acid on iron accumulation and oxidative damage in the in vivo models of iron-overload in mice and rat liver tissue. Also the influence of long-term administration of kojic acid on other essential elements - zinc, copper, calcium and magnesium - concentration in the liver and kidney tissue was followed.

Since members of hydroxypyrone series have iron chelating properties, kojic acid has been suggested to might positively influence disturbances in iron metabolism and to be able to interrupt or ameliorate some of the effect of free radical generation, leading to tissue damage.

In our experiment A model of iron overload in mice the daily oral treatment with 100 mg.kg^{-1} of kojic acid for 7 days did not affected the level of iron accumulated in the liver in contrast to comparative iron chelator deferoxamine. Increased lipid peroxidation in the liver of iron-loaded mice was further enhanced by kojic acid treatment. In the experiment B model of iron overload in rats the 4-week treatment with 0.5% kojic acid in drinking water exerted no effect on iron accumulation or induced lipid peroxidation in iron-loaded mice. The results received in our study confirm conclusions from in vitro experiment in which kojic acid treatment did not show any protective effect against reperfusion injury contrary to catechol, mimosine and deferoxamine (14). In similar experiment on isolated rat hearts no protective effect of KA on contractile function during reperfusion was found compared to significant protection by iron-chelator deferiprone (L1) (15). In both our experiments the influence of kojic acid treatment on trace element concentration in the liver and kidneys was minor.

Though in our experiments in vivo the ability of kojic acid to affect iron kinetics in the organism could not be proved, kojic acid as a molecule of natural origin may serve as a template for the preparation of new biologically active derivatives possessing capability of chelating iron.

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