Effect of an Excessive Intake of Quercetin on the Vitamin E Level and Antioxidative Enzyme Activities of Mouse Liver under Paraquat-Induced Oxidative Stress

Noriko B ANDO, Saori WAKAMATSU, and Junji TERAO

Department of Food Science, Graduate School of Nutrition and Bioscience, The University of Tokushima, Tokushima 770-8503, Japan

Received May 9, 2007; Accepted June 20, 2007; Online Publication, October 7, 2007 [doi:10.1271/bbb.70282]

The liver α-tocopherol level of the paraquat fed mice group was lower than that of the control diet-fed group. An excessive intake of quercetin lowered the liver α-tocopherol level of the control diet-fed mice group, but did not affect it in the paraquat-fed mice group. The same quercetin intake significantly increased the superoxide dismutase and glutathione peroxidase activities in the liver of both groups, indicating that excessive quercetin intake can either promote or attenuate oxidative stress in the liver.

Key words: quercetin; paraquat; glutathione peroxidase; superoxide dismutase; prooxidant

The antioxidative defense system involves antioxidative enzymes and small molecular antioxidants, both of which are responsible for attenuating oxidative stresses in the body. In particular, superoxide dismutase (SOD) and glutathione peroxidase (GPX) are key enzymes for protecting biological tissues and fluids from oxidative stress. On the other hand, dietary flavonoids from plant foods have attracted considerable attention as essential dietary antioxidants together with vitamin E and vitamin C.1) Quercetin (3,3′,4,5,7-pentahydroxyflavone), a typical catechol type of flavonoid, is distributed ubiquitously in fruits and vegetables and is known to act as an effective antioxidant by chelating transition metal ions and/or scavenging free radicals.2) Interestingly, several studies have demonstrated that dietary quercetin enhanced the antioxidative defense system by upregulating antioxidative enzymes.3–5) Although the ability of dietary quercetin to protect the body from oxidative stress has been strongly implied, its excessive intake is suggested to have an adverse effect on the body.6–8) For example, catechol-type compounds including quercetin are able to act as prooxidants by generating reactive oxygen species (ROS) and semiquinone radicals during the autocatalytic oxidation process.9–11) Thus, an adequate intake amount should be assessed for providing to its antioxidative effect without any disorders.

The aim of this study is to clarify the effect of an excessive quercetin intake on the antioxidative defense system under chronic oxidative stress. We have previously found that the liver weight was increased by the intake of quercetin, in spite of there being no change in total body weight, when glycosylated quercetin (100 mg/100 g of diet) was fed to rabbits for one month.12) Paraquat (PQ; 1,1′-dimethyl-4,4′-bipyridilium dichloride) is known to cause oxidative damage to the liver by generating superoxide anion and has been used for estimating the antioxidative effect of dietary flavonoids on experimental animals under oxidative stress.13,14) We therefore focused on the liver as a target organ, and PQ was loaded as an inducer of chronic oxidative stress for evaluating the antioxidative defense system.

Six-week-old female mice (SLC: BALB/c strain) were obtained from Japan SLC Co. (Hamamatsu, Japan). Female mice were used in this study because a preliminary experiment had indicated that the females were more susceptible to paraquat-induced stress than the males. The mice were housed in a room with a 25 °C temperature and 12 h/12 h light–dark cycle, and had free access to water and the experimental diet. The experiments were performed in accordance with the guidelines for the care and use of laboratory animals of The University of Tokushima. In the first experiment, the mice were given a diet containing 0, 1, 2, 5, or 10 mg of PQ (Sigma, St. Louis, MO, USA) in 100 g of the control diet, which consisted of 20% casein, 20% sucrose, 40% corn starch, 5% corn oil, 5% lard, 1.0% vitamin mixture (5 mg of α-tocopherol acetate/g, Oriental mixture, Oriental Yeast Co., Tokyo, Japan), 3.5% mineral mixture (Oriental mixture, Oriental Yeast Co., Tokyo, Japan), 0.3% D,L-methionine, 0.2% choline chloride and 5% cellulose powder, for 40 days. The amount and the origin of fat in the diet were identical to those reported by Umegaki et al.,15) in which the antioxidative and prooxidative activity of dietary β-carotene was meas-

Note

Effect of an Excessive Intake of Quercetin on the Vitamin E Level and Antioxidative Enzyme Activities of Mouse Liver under Paraquat-Induced Oxidative Stress

Noriko B ANDO, Saori WAKAMATSU, and Junji TERAO

Department of Food Science, Graduate School of Nutrition and Bioscience, The University of Tokushima, Tokushima 770-8503, Japan

Received May 9, 2007; Accepted June 20, 2007; Online Publication, October 7, 2007 [doi:10.1271/bbb.70282]
ured. In the second experiment, the control diet and 10 mg of the PQ-supplemented diet with or without 100 mg of quercetin aglycon (Sigma, St. Louis, MO, USA) in 100 g of diet was supplemented to the mice for 40 days. Mice were then sacrificed and the liver was removed. A liver homogenate (10%, w/v) was prepared with a Polytron homogenizer in a cold 100 mM phosphate buffer (pH 7.4). The homogenate was centrifuged at 7,200 × g for 10 min at 4°C, the resulting supernatant was used to measure the activity of SOD\(^{16}\) and GPX.\(^{17}\)

The liver \(\alpha\)-tocopherol content was determined by reversed phase HPLC equipped with fluorescence detector as described previously.\(^{18}\) The mice individually were maintained in stainless steel metabolic cages to collect urine for 3 days just before the end of the feeding periods. Urine was stored at \(-28\) °C until its 8-hydroxy deoxyguanosine (8-OHdG) content was determined by using an 8-OHdG determination ELISA kit (Japan Aging Research, Hamamatsu, Japan). All data were analyzed by one–way ANOVA followed by Bonferroni/Dunn’s multiple comparison test, using Statview software.

The mice that received 10 mg of paraquat per 100 g of the control diet (PQ10) showed a lower \(\alpha\)-tocopherol level than those that received the control diet (Fig. 1A). In contrast, both the SOD and GPX activities in the liver of mice fed with 5 mg of PQ per 100 g of diet (PQ5) were significantly higher than those of the other groups. No difference was apparent between the control diet group and PQ10 diet group in liver GPX and SOD activities (Fig. 1B and C). The body weight gain of the mice that received PQ10 was significantly lower than that of the control diet group, and the PQ10 plus quercetin-fed group showed no decrease in the body weight gain (data are not shown here), indicating that dietary quercetin suppressed the toxic effect of paraquat in the body. In contrast, the \(\alpha\)-tocopherol content in the control diet plus quercetin-fed group was significantly lower than that in the control diet without quercetin-fed group, although the quercetin intake did not affect the decrease in \(\alpha\)-tocopherol content in the PQ10-fed group (Fig. 2A). It is unlikely that the \(\alpha\)-tocopherol intake affected its content in the liver of each group because there was no significant difference in the intake of the diet during the \textit{ad libitum} feeding period. This phenomenon can be explained instead by the idea that quercetin accelerated the consumption of \(\alpha\)-tocopherol by acting as a prooxidant in the control-diet group. The GPX activity of the PQ10 plus quercetin-fed group was higher than that of the PQ10 without quercetin-fed-group (Fig. 2B). Furthermore, the SOD activity of the quercetin-containing-diet fed group was higher than that of the quercetin free-diet fed-group in the cases of both the control diet and PQ10 (Fig. 2C). These results indicate that the excessive quercetin intake enhanced the activity of antioxidant enzymes, irrespective of the paraquat-induced chronic oxidative stress. The concentration of 8-OHdG in the urine from the mice that received PQ10 was significantly higher than that from the mice fed with the control diet. This elevation tended to be suppressed by the addition of quercetin to the PQ10 diet (Fig. 3). The addition of quercetin tended to increase the 8-OHdG content in the control diet-fed group, indicating that dietary quercetin acted as a prooxidant.

Previous studies using rodent animals have demonstrated that a quercetin intake prevented and protected the liver from the oxidative damage induced by the ingestion of ethanol,\(^{19}\) and streptozocin\(^{20}\) and by biliary obstruction.\(^{21}\) However, Choi \textit{et al.}\(^{22}\) have found that orally administered quercetin acted as both an antioxidant and prooxidant in the rat liver. Our study here also indicated that dietary quercetin exerted a prooxidative effect in the case of the control diet, as the liver vitamin E content was decreased and urine 8-OHdG was increased by its excessive intake (Fig. 2A and Fig. 3). Nevertheless, this excessive intake of quercetin appa-
rently protected the body from paraquat-induced oxidative stress, as the simultaneous intake of quercetin with PQ 10 suppressed the paraquat-induced loss of body weight gain and increase of urine 8-OHdG content (Fig. 3).

The mechanism for the antioxidative action of dietary antioxidants may involve not only ROS scavenging but also upregulation of the antioxidant enzymes through the modulation of cellular redox thiols and its related cell signaling pathway.23) Our results confirm that the intake of quercetin induced upregulation of the liver GPX and SOD activities (Fig. 2) which may have affected the antioxidative defense system of the liver exposed to chronic oxidative stress. On the other hand, it is likely that oxidative stress at a moderate level enhanced the activities of the two antioxidant enzymes by modulating the cellular redox thiols, as indicated by the fact that these enzyme activities were significantly increased at the dosage of 5 mg of PQ to the control diet (Fig. 1). Therefore, enhancement of the liver GPX and SOD activities by the intake of quercetin may reflect its prooxidative activity of generating ROS after incorporation into the body. Although dietary quercetin is known to be completely metabolized to its glucuronide/sulfate conjugates during intestinal absorption,24) a rat study has clarified that such metabolites are widely distributed in various tissues including liver.25) Thus, quercetin seems to accumulate in the liver as its conjugated metabolites and modulates the liver antioxidative defense system.

Taken together, an effect of dietary quercetin as either an antioxidant or prooxidant in the liver is likely to depend on the balance of the antioxidative defense and oxidative stress induced by external factors. This result warrants a further study on the adverse effect of an excessive quercetin intake on health.

References
5) Alia, M., Mateous, R., Ramos, S., Lecumberri, E.,


