Peroxidation Processes in Rats during the Delayed Period after Chronic Administration of Dihydroquercetin

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Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 147, No. 5, pp. 532-535, May, 2009
Original article submitted November 11, 2008

Peroxidation processes in male outbred albino rats were studied 1, 2, and 3 months after 90-fold administration of dihydroquercetin in doses of 86, 860, and 3000 mg/kg and flavonoid rutin (reference preparation) in a dose of 86 mg/kg. Dihydroquercetin in a dose of 86 mg/kg was as potent as rutin in modulating the process of peroxidation. Changes in the nonenzymatic antioxidant system and activation of lipid peroxidation were not observed in the delayed period after administration of dihydroquercetin.

Key Words: *dihydroquercetin; rutin; antioxidant properties; peroxidation processes*

Much recent attention is paid to antioxidant activity of flavonoids, including dihydroquercetin (DHQ, taxifolin). Increasing interest in DHQ is related to the use of this compound as a biologically active food additive (the efficacy of DHQ is associated with its antioxidant properties), experience of course administration of DHQ to patients, and unique resources for DHQ. In the area of DHQ manufacturing in the Siberian region [4,6,10]. Recent studies showed that DHQ has no genotoxic activity [1]. Most studies of biological activity (*e.g.*, antioxidant properties) were performed *in vitro* on model systems [3,4,8,9]. Little is known about *in vivo* properties [3,10] and long-term effect of DHQ [2]. Some studies have focused on chemiluminescence assay of the antioxidant properties in biological samples or antioxidant enzyme activity. The influence of DHQ on the nonenzymatic antioxidant system (particularly, in the delayed period after drug treatment) is poorly understood.

Here we studied the process of peroxidation in experimental animals during the delayed period after chronic administration of DHQ in a wide range of doses.

MATERIALS AND METHODS

Experiments were performed on male outbred albino rats weighing 180-220 g (n=90). The animals were housed in a vivarium and had free access to water and food. The rats were divided into 5 groups of 6 specimens each. Group 2-4 animals daily received a dietary supplement of DHQ for 3 months (daily dose 86, 860, and 3000 mg/kg, respectively). Reference preparation rutin in a dose of 86 mg/kg was given to group 5 animals. Intact animals of control group 1 received a standard diet and were maintained under similar experimental conditions. The rats were killed by decapitation 1, 2, and 3 months after the last treatment with test compounds.

The degree of peroxidation was evaluated from the content of water-soluble nonenzymatic antioxidants and intermediate and end products of lipid peroxidation (LPO). The concentration of reduced glutathione (SH-glutathione) in liver homogenates was estimated from the amount of nonprotein SH groups using 5,5'-dithio-bis-(2-nitrobenzoic acid). Ascorbic acid (AA) concentration in blood plasma was measured in the reaction with 2,4-dinitrophe-

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0007-4888/09/1475-0603

nylhydrazine [7]. The content of LPO products (lipid hydroperoxides; and 2-TBA-reactive products, TBA-RP) was estimated in liver homogenates [7].

Experiments were performed with DHQ from Siberian larch wood (LLC Sibirskii Kedr). The purity of DHQ (97%) was evaluated by high-performance liquid chromatography with an external standard (LLC Flavir) on a Milikhrom A-02 chromatograph (CJSC EkoNova).

Intergroup differences were evaluated by one-way analysis of variance (median test, Kruskal—Wallis ANOVA, paired Mann—Whitney test). The data are expressed as the median value (Me) and interquartile range (upper limit of the lower quartile, LQ; and lower limit of the upper quartile, UQ). The differences were significant at $p<0.05$.

RESULTS

The changes were most pronounced 1 month after 90-fold administration of the test compounds. One-way analysis of variance revealed the existence of intergroup differences in the concentration of AA (plasma) and amount of SH-glutathione and TBA-RP (liver, Table 1). AA concentration in rats of all groups was much higher compared to the control. However, AA concentration in group 3 and 4 animals was lower than in group 2 specimens. Pairwise comparison revealed a decrease in the amount of SH groups in group 2 rats (compared to the control). The content of TBA-RP in group 2 animals did not differ from the control. However, the content of TBA-RP in group 3 and 4 rats was higher

TABLE 1. Effect of DHQ in Various Doses on the Nonenzymatic Antioxidant System and TBA-RP Content in Rat Liver Tissue (Me, LQ — UQ, n=6)

Period, months	Parameter	Group					p
		1	2	3	4	5	
1	AA, μmol/liter	29.5 (25-31.8)	41.1 (40.3-43.7)*	35.5 (32.4-40.9)*+	36.3 (35.2-42)*+	33.8 (32.4-39.3)*+	**0.014**/**0.004**
	SH groups, μmol/g tissue	6 (5.4-6.3)	4.8 (4.5-5.2)*	5.3 (4.6-5.8)	5.3 (4.2-6.2)	4.3 (3.4-5.1)*	**0.042**/0.062
	Lipid hydroperoxides, optical density units/g tissue	40.3 (40-41)	40.2 (39.6-48)	42 (39.6-48)	40.3 (35.6-52)	42 (41-44)	0.336/0.914
	TBA-RP, nmol/g tissue	37.7 (34.6-38.5)	40 (38.5-57.7)	48.1 (42.3-57.7)*	51.9 (46.2-61.5)*+	42.3 (36.9-48.5)	**0.017**/0.051
2	AA, μmol/liter	24.4 (23.9-25)	27 (25-28.9)	25.3 (25-26.1)	24.8 (23.9-26.7)	26.7 (26.1-27.8)*	0.124/0.117
	SH groups, μmol/g tissue	6.4 (5.9-6.9)	6.1 (5.1-6.6)	7 (6.5-7.1)	7.2 (6.3-7.7)	5.5 (4.6-6.6)	0.615/0.057
	Lipid hydroperoxides, optical density units/g tissue	55 (36-56)	58 (53-60)	57.5 (52-58)	55 (36-62)	56.5 (55.6-60)	0.523/0.813
	TBA-RP, nmol/g tissue	57.7 (55.4-60)	49.6 (46.2-53.8)*	48.1 (44.6-60)	49.2 (42.3-51.5)*	41.2 (38.5-56.2)*	0.092/**0.045**
3	AA, μmol/liter	28.1 (27.8-28.4)	36.1 (35.8-36.3)*	29.5 (25-30.7)	26.8 (24.4-31.8)	28.4 (28.4-28.4)+	**0.024**/**0.006**
	SH groups, μmol/g tissue	5.1 (4.9-5.4)	4.3 (3.9-4.9)*	4.9 (4.5-4.9)	4.3 (4.2-4.4)*	4.4 (4.3-4.8)*	**0.031**/**0.043**
	Lipid hydroperoxides, optical density units/g tissue	57 (56-57)	60 (56-74)	58.8 (52-64)	58 (56-60)	56.5 (56-57)	0.124/0.788
	TBA-RP, nmol/g tissue	56.5 (53.8-61.5)	57.7 (56.2-69.2)	57.7 (52.3-61.5)	50 (46.2-52.3)*	52.3 (44.6-57.7)	0.155/0.118

Note. Significance level (p) in intergroup comparison: one-way analysis of variance (median test/Kruskal—Wallis ANOVA). Values of $p<0.05$ are shown in bold type. $p<0.05$ (Mann—Whitney U test): *compared to group 1; +compared to group 2.

than in control specimens. These data show that the concentration of AA in group 3 and 4 rats was higher than in control specimens, but lower than in group 2 animals. Moreover, group 3 and 4 rats were characterized by accumulation of end products of LPO. Our findings suggest that DHQ in high doses has the antioxidant properties. The content of reduced glutathione in group 2 animals was lower than in the control. In these rats, the increase in the concentration of AA was not accompanied by changes in the content of TBA-RP. It can be hypothesized that the decrease in tripeptide concentration is related to activation of glutathione S-transferase. Similar changes were revealed in the cytosolic fraction of rat liver homogenates after 2 weeks of treatment with DHQ in a dose of 130 mg/kg [3].

Differences in TBA-RP content in the liver were found after 2 months (analysis of variance). TBA-RP content in group 2, 4, and 5 rats was lower than in group 1 animals. AA concentration in group 5 rats was higher than in control specimens. The content of glutathione tended to increase in rats receiving high doses of DHQ (by 9.4 and 12.5%, respectively).

Intergroup differences in the concentration of AA and amount of SH groups were revealed 3 months after termination of treatment. AA concentration in group 2 rats was higher than in control specimens. However, AA concentration in group 5 animals was lower than in group 2 rats. Pairwise comparison revealed a decrease in the amount of SH groups in group 2, 4, and 5 rats (compared to the control). The content of TBA-RP in group 4 animals was lower than in control specimens. Dose-dependent changes in the test parameters were not revealed during this period (similarly to the previous period). AA concentration in rutin-receiving rats (group 5) did not differ from the control, but was lower than in group 2 animals.

The dose of rutin was equal to the lowest dose of DHQ (86 mg/kg). This flavonoid serves as the reference drug, since rutin and DHQ produce similar antioxidant effect under conditions of LPO induction in liver microsomes [8]. The antioxidant properties of flavonoids (relative inhibitory or protective activity) vary in different oxidative systems [5]. DHQ and rutin produce similar effect on the test parameters (except for AA). After 1 and 3 months, the concentration of AA in group 2 rats was higher than in group 5 animals. The increase in AA concentration was probably related to the ability of flavonoids to interact with other antioxidant (*e.g.*, AA and glutathione) in biological systems. Our results are consistent with published data that rutin oxidizes AA with the formation of the ascorbate radical. By contrast, DHQ can reduce this radical [5].

We conclude that 90-fold treatment of experimental animals with DHQ and rutin (86 mg/kg) has similar antioxidant effects in the delayed period. Changes in the nonenzymatic antioxidant system (SH-glutathione and AA), accumulation of TBA-RP, and variations in the amount of lipid hydroperoxides were not observed in all groups of DHQ-receiving rats during various periods of the study.

We are grateful to Prof. G. G. Yushkov, A. A. Gushchina (Institute of Biophysics, Angarsk State Technical Academy), and A. L. Vereshchagin (Limnological Institute, Siberian Division of the Russian Academy of Sciences, Irkutsk) for their help in performance of the study and discussion of the results.

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