In Vivo Study of Dihydroquercetin Genotoxicity

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Genotoxic properties of dihydroquercetin were *in vivo* studied by the method of chromosome aberrations counting and DNA-comet assay. Dihydroquercetin administered repeatedly (5 times, 0.15 and 1.5 mg/kg) or once in doses of 15, 150, and 2000 mg/kg induced no DNA damages in mouse bone marrow, blood, liver, and rectal cells. Single administration of this preparation in doses of 1.5 and 150 mg/kg and 5-fold administration in a dose of 1.5 mg/kg had no effect on the level of chromosome aberrations in mouse bone marrow cells.

Key Words: *dihydroquercetin; genotoxicity; DNA-comets; chromosome aberrations; mice*

Flavonoid dihydroquercetin (DHQ) belongs to a group of natural polyphenols widely presented in natural products [4]. All these compounds are characterized by pronounced antioxidant activity. It is currently accepted that diets enriched with natural antioxidants reduce the risk of cardiovascular diseases, malignant neoplasms, and decelerate aging processes [2,8], therefore polyphenols are often used as drugs, bioactive supplements, and as component of functional foodstuffs.

However, the safety of polyphenols is not yet proven [8]. There are data on genotoxicity of polyphenols, which are often determined by dose-dependent inversion of antioxidant effects into prooxidant ones [1,8]. For instance, *in vitro* and *in vivo* genotoxic effects of quercetin were demonstrated [7]. At the same time, DHG the closest structural analog of quercetin, is widely used in humans without reliable information on its genetic safety [4]. Genotoxicity of DHQ was studied only on microorganisms [6]. The results of prokaryotic tests by some reasons mentioned above [1], cannot serve as a proof in evaluation of the genotoxic risk of chemical compounds for humans.

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Here we evaluated genotoxicity of DHQ *in vivo* by counting chromosome aberration in bone marrow cells and by the analysis of DNA damage (comet assay) in bone marrow, blood, liver, and rectal cells of mice.

MATERIALS AND METHODS

The study was carried on 8-12-week-old male and female C57Bl/6 mice weighing 18-20 g. The animals were maintained in a vivarium of Central Laboratory of Pharmacological Genetics, V. V. Zakusov Institute of Pharmacology, (10-12 animals per cage) at 12-h light-dark regimen with free access to standard briquette chow and water.

The test for induction of chromosome aberrations was performed in accordance with methodical recommendations [3]. DHQ (CAS Number 98006-93-0; Flavit Company) was administered to male mice in doses of 1.5 and 150 mg/kg (cell material was fixed after 24 h) and to males and females in a dose of 1.5 mg/kg once a day for 5 days (cell material was fixed 6 h after the last dose). Control mice (negative control) received an equivalent amount of the solvent (1% ethanol). Cyclophosphamide (Sigma) was used as the positive control and was injected intraperitoneally in a dose of 20 mg/kg.

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The animals were sacrificed 24 h after treatment. DHQ dissolved in 1% ethanol was administered *per os*.

Cytogenetic preparations of femoral bone marrow were prepared using standard air-drying technique [3]. Cytogenetic analysis was performed on a Standart-20 microscope (Carl Zeiss) at 10×100 magnification (oil immersion). Cells with achromatic gaps, single and paired chromosome fragments, and exchanges of different types were counted. Cells with multiple damages were analyzed separately, metaphases with more than 5 chromosome aberrations were counted. A total of 100 metaphases from each animal were analyzed. Statistical processing of the experimental data (f test) was performed by comparing the percent of damaged metaphases in the control and experimental groups.

In the test for induction of DNA damage, DHQ was administered once and repeatedly. In series I, DHQ was administered in doses of 0.15 and 1.5 mg/kg once a day for 5 days. The animals were sacrificed 3 h after the last dose. In series II, DHQ was administered once in doses of 15, 150, and 2000 mg/kg; after 3 h the animals were sacrificed and the preparations were made. Control mice (negative control) received an equivalent amount of the solvent (1% ethanol). Methylmethane sulfonate (Sigma) was used as the positive control and was injected intraperitoneally in a dose of 40 mg/kg over 3 h.

Gel electrophoresis of isolated cells was performed in alkaline version [9] as described previously [5]. The preparations were stained with SYBR Green I (1:10,000 in TE-buffer) for 30 min in darkness. Analysis was performed under a Mikmed-2 12T epiluminescent microscope (LOMO) equipped with VEC-335 high definition digital camera (EVS) at ×200. The images of DNA comets were analyzed using CASP 1.2.2 software [10]. DNA content in the tail of DNA comets (in %) was used as the index of DNA damage. At least 100 cells in each micropreparation was analyzed. The data were processed statistically using ANOVA post-hoc Dunnet-test.

RESULTS

In animals receiving methylmethane sulfonate (positive control), the level of DAN damage significantly increased in all studied organs and tissues (Table 1).

Spontaneous level of DNA damage in bone marrow cells of control animals was 4.5±1.1%. In animals repeatedly treated with DHQ in doses of 0.15 and 1.5 ng/kg, this parameter was 4.9±1.1 and 3.6±0.5%, respectively, *i.e.* did not significantly differ from the control. In control animals, the level of DNA damage in blood cells was 3.4±0.4% DNA. In animals treated with DHQ in doses of 0.15 and 1.5 mg/kg, this parameter did not significantly differ from this value (3.4±0.8 and 3.0±0.5%). The level of DNA damage in liver cells of control animals was 4.1±0.6%, while after 5-fold treatment with DHQ in doses of 0.15 and 1.5 mg/kg this parameter was 4.8±0.6 and 3.2±0.9%, respectively. No significant differences between these values were revealed. Similar results were also obtained for rectal cells. No significant differences between the level of DNA damage in mice of the control (8.0±1.5%) and experimental groups (7.9±1.3 and 6.4±0.4% for 0.15 and 1.5 mg/kg, respectively) were revealed.

In experiments with single administration of DHQ, the spontaneous level of DNA damage in bone marrow cells was 5.7±1.2%. After treatment with DHQ in doses of 15, 150 and 2000 mg/kg it corresponded to 5.5±1.0, 6.1±1.0 and 7.3±2.3%, respectively. No significant differences between these values were revealed.

TABLE 1. Effect of DHQ on the Level of DNA Damage in Mouse Organs and Tissues

Experimental conditions	Bone marrow		Blood cells		Liver		Rectum	
	cells	% DNA	cells	% DNA	cells	% DNA	cells	% DNA
Control (1% ethanol, 5 times)	616	4.5±1.1	578	3.4±0.4	528	4.1±0.6	535	8.0±1.5
DHQ, 0.15 mg/kg, 5 times	612	4.9±1.1	518	3.4±0.8	550	4.8±0.6	514	7.9±1.3
DHQ, 1.5 mg/kg, 5 times	546	3.6±0.5	554	3.0±0.5	565	3.2±0.9	524	6.4±0.4
Control (1% ethanol, once)	552	5.7±1.2	558	3.2±1.1	527	5.8±1.1	512	9.3±0.9
DHQ, 15 mg/kg, once	553	5.5±1.0	503	3.8±1.2	532	5.1±1.2	531	9.0±1.0
DHQ, 150 mg/kg, once	529	6.1±1.0	588	2.2±0.3	524	4.4±0.5	518	9.3±0.6
DHQ, 2000 mg/kg, once	557	7.3±2.3	541	3.6±0.5	512	4.6±1.3	528	9.1±0.3
Methylmethane sulfonate, 40 mg/kg	516	16.1±0.3*	520	21.1±0.6*	516	20.6±0.2*	518	21.1±0.3*

Note. *$p<0.01$ compared to the control.

TABLE 2. Effect of DHQ on the Level of Chromosome Aberrations in Bone Marrow Cells of Male and Female Mice

Experimental conditions	Number of cells	Number per 100 cells					Number of abnormal metaphases, %	*p*
		gaps	single fragments	paired fragments	exchanges	cells with MA		
Males								
control (1% ethanol)	500	0.2	1.6	0	0	0	1.8±0.6	
cyclophosphamide, 20 mg/kg	500	0	14.0	1.2	0.8	1.4	12.8±1.5	<0.001
DHQ, 1.5 mg/kg, once	500	0.2	1.2	0	0.6	0	2.0±0.4	>0.05
DHQ, 150 mg/kg, once	500	0.4	0.6	0	0	0	1.0±0.4	>0.05
DHQ, 1.5 mg/kg, 5 times	500	0.6	1.4	0	0	0	2.0±0.6	>0.05
Females								
Control (1% ethanol, 5 times)	500	0.2	1.2	0	0	0	2.0±0.6	
DHQ, 1.5 mg/kg, 5 times	500	0.2	0.6	0	0	0	0.8±0.4	>0.05

Note. *p*: compared to negative control. MA: cells with multiple abnormalities (>5 per metaphase).

Spontaneous level of DNA damage in blood cells of control animals was 3.2±1.1%. In experimental animals receiving DHQ in single doses of 15, 150, and 2000 mg/kg, this parameter was 3.8± 1.2, 2.2±0.3 è 3.6±0.5%, respectively, which did not significantly differ from the control. Similar results were obtained for liver cells: 5.8±1.1% in the control, 5.1±1.2, 4.4±0.5, and 4.6±1.3% for DHQ doses of 15, 150, and 2000 mg/kg, respectively. In rectal cells, no significant differences between the control (9.3±0.9%) and experimental groups (9.0± 1.0, 9.3±0.6, and 9.1±0.3%) were revealed for DHQ doses of 15, 150, and 2000 mg/kg, respectively.

Then we studied the effect of DHQ on the level of chromosome aberrations in bone marrow cells of male and female mice (Table 2).

In male mice receiving no DHQ (negative control), the level of chromosome aberrations in bone marrow cells was 1.8±0.6%. Injection of cyclophosphamide in a dose of 20 mg/kg increased this parameter and it significantly differed from negative control, which agrees with published data characterizing the cytogenetic effect of the mutagen.

After single treatment with DHQ in doses of 1.5 and 150 mg/kg, the level of chromosome aberrations was 2.0±0.4 and 1.0±0.4%, which did not significantly differ from the negative control. In male mice receiving 5×1.5 mg/kg DHQ, the level of chromosome aberrations was 2.0±0.6%, which also did not differ from negative control.

In female mice, spontaneous level of chromosome aberrations in bone marrow cells was 2.0±0.6%. After 5-fold treatment with DHQ in a dose of 1.5 mg/kg, the level of chromosome aberrations in female mice was 0.8±0.4%, *i.e*. the difference from the control was insignificant.

Thus, our experiments showed that DHQ in a wide dose range exhibits no clastogenic and DNA-damaging activities in different organs and tissues of mammals. Together with previous reports, our findings attest to the absence of genotoxic activity in DHQ.

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