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SHORT COMMUNICATION

Effect of Quercetin on the Intestinal Carbohydrases Activity in the Offspring of the Lead Intoxicated Mother

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Abstract

Aim: This work aims to investigate the effect of quercetin on the development of small intestine disaccharidase activity in the offspring intoxicated with lead acetate. **Material and Methods:** The experiments were carried out on white outbred rats. Rats were divided into control and three experimental groups. In the control and 3rd experimental group, rats were nursed by intact mothers. In the 1st and 2nd experimental groups, rats were nursed by mothers who replaced drinking water with a 0.2% lead acetate solution. All experimental groups of rats were orally treated with quercetin (20 mg/kg/24 h) from the 3rd to 20th days of postnatal life. Body weight, small intestine weight as well as the activities of intestinal maltase and lactase were determined on the 7th, 14th, and 21st days after birth. **Results:** In rats nursed by mothers who used a solution of lead acetate instead of drinking water a body weight and the small intestine mucosa weight decreased, but intestinal maltase and lactase activity increased. Treatment of lead-intoxicated growing rats with quercetin restored the body weight, small intestine mucosa weight, and development rate of maltase and lactase activity. **Conclusion:** The effect of quercetin on the activity of intestinal maltase and sucrase in growing rats nursed by lead acetate consumed mother is mediated through the restoration of intoxication damage since treatment of intact growing rats with quercetin in the same way and at the same time did not affect on the intestinal disaccharidases activity.

Keywords: Growing Rats, Lactase, Lead Acetate, Maltase, Maternal Intoxication, Quercetin, Small Intestine

1. Introduction

Quercetin is one of the important bioflavonoids which acts as an agent to lower inflammation, hypertension, overweighting and cholesterol level. It has been proven that the multiple effects of quercetin are mediated by the enhancement of antioxidant defense systems in tissues due to the removal of free radicals[1-4]. Excess free radicals in the body generate oxidative stress. This process plays an important role in the development of various diseases and damages, including lead-induced oxidative stress[5]. Lead ions with water and food, bypassing the intestinal barrier, can enter the hemocirculation[6]. Lead ions even could enter the fetus by passing the transplacental barrier[7,8], and to the infant with the mother's milk[9,10]. The transfer of the toxicant from the mother's milk to the offspring is also facilitated by high intestinal permeability during breastfeeding[11]. Therefore, the search for harmless biologically active substances that neutralize the negative effect of lead on the growing organism is in demand.

Since lead ions are pro-oxidants[6,7], we suggested that quercetin, which has pronounced antioxidant

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properties[1-4], could be used to correct the development of intestinal enzyme systems in case of lead poisoning.

The purpose of the work is to investigate the effect of quercetin on the small intestine disaccharidase activity in the offspring intoxicated with lead acetate.

2. Materials and Methods

2.1 Animals

The experiments were carried out on outbred white rats. The rats were kept in well-ventilated rooms with natural light and room temperature. The rats were fed a standard vivarium diet with unlimited access to food and tap water once a day at the same time (9-10 am). All animal procedures were carried out in accordance with the 1975 Declaration of Helsinki on Ethical Principles for Medical Research, as revised in 2000.

To obtain offspring, 4 females weighing 200-220 g were planted in plastic cages 50×30×28 cm in size. After the female adaptation to the new environment, one male of the same weight was placed in a cage for 2 days. On the 14th-15th days after mating, females with signs of pregnancy were separated into individual cages 35×28×28 cm in size, and the appearance of offspring was monitored. After birth, rats from different litters were mixed and 8 pups were left for each female. Growing rats stayed with the nursing mother until the end of the experiments.

2.2 Treatment of Animals

Rats were divided into one control and three experimental groups. In the 1st experimental group, rats were nursed by mothers whose drinking water was replaced with a 0.2% solution of lead acetate. In the 2nd experimental group, lactating rats also consumed a 2% solution of lead acetate, but their sucklings were taken orally quercetin (20 mg/kg/24 h) from the 3rd to the 21st day of postnatal life. The 3rd experimental group was nursed by females consuming ordinary drinking water, but growing rats, as well as in the second experimental group, were orally administered quercetin from the 7th to the 21st day of postnatal life in the same dose. Preliminary experiments showed that oral administration of saline did not affect the activity of intestinal enzymes, so intact growing rats served as control. Rats were sacrificed on the 7th, 14th, and 21st days of postnatal life in the morning between 9.00-10.00 h.

2.3 Preparation of Small Intestine Enzyme-active Samples

To determine the disaccharidase activity after the rats' decapitation the abdominal cavity was quickly opened. The small intestine was removed, separated from adjacent tissues, washed with 10 ml of cold saline, and dried with filter paper. Then the small intestine was placed on a glass plate and the mucosa was carefully separated with a plastic spatula. The mucosa was weighed and placed in a glass homogenizer. Cold Ringer's solution (pH 7.4) was added to the mucosa at the rate of 0.9 ml per 100 mg of tissue and homogenized at a speed of 300 g for a minute. Then the homogenate was centrifuged at 3000 rpm for 15 min, and the activity of enteral maltase (α-D-glucoside-glucohydrolase; EC 3.2.1.20) and lactase (3-D-galactoside galactohydrolase, EC 3.2.1.23), as well as the content of protein, was determined. All operations for the preparation of enzymatically active preparations of the small intestine were carried out in cold conditions.

2.4 Biochemical Assay

Maltase and lactase activity was determined by conventional methods[12]. To determine the protein content, sets of special reagents (Human, Germany) were used. The specific activity of intestinal enzymes was expressed in μmol of reduced glucose per 1 g of protein. The protein content in the intestinal mucose supernatant was determined by using protein assay kit reagents (Hunam, Germagy).

2.5 Statistics

The obtained results were processed using Student's t-test. The arithmetic mean (M), the mean error of the mean (m), and the significant coefficient (P) were calculated. If the P value was less than 0.05, the difference between the control and experimental rat groups was considered statistically significant.

3. Results

3.1 Body Weight and Small Intestine Mucosa Weight

Body weight and small intestine mucosa weight were increased both in the control and experimental groups of growing rats (Table 1). However, the rate of body

Table 1. Effect of quercetin on body weight and intestinal mucosa weight of rats nursed by lead-intoxicated mothers (M ± m, n = 6)

Age (days)	Control	Experiment 1	Experiment 2	Experiment 3
	Body weight (g)			
7P	10,1 ± 1,1 -	10,7 ± 0,3>0,5	9,9 ± 1,2>0,5	10,9 ± 0,6>0,5
14P	22,5 ± 1,4 -	20,4 ± 1,2>0,3	20,1 ± 2,2>0,3	24,9 ± 2,1>0,2
21P	32,1 ± 1,2 -	27,9 ± 0,9<0,01	29,3 ± 1,6>0,2	29,6 ± 0,9>0,1
	Small intestine mucosa weight (mg)			
7P	143,2 ± 10, 2 -	151,1 ± 17,1>0,5	154,1 ± 13,1>0,5	150,7 ± 13,9>0,5
14P	497,1 ± 25,8 -	421,1 ± 23,3<0,05	486,2 ± 30,5>0,5	459,9 ± 23,4>0,3
21P	731,9 ± 34,2 -	604,1 ± 21,2<0,01	681,4 ± 32,1>0,3	728,3 ± 45,2>0,4

Control – intact rats, experiment 1 - rats nursed by lead-intoxicated female mother; experiment 2 - rats nursed by lead-intoxicated mothers and treated with quercetin; experiment 3 - rats nursed by intact females mothers and treated with quercetin, P - statistical significance between control and experimental groups.

weight gain in rats nursed by lead-intoxicated mothers lagged behind that in other groups. It was 13.0% less than in the control group on the 21st day of postnatal life.

The weight of the small intestine mucosa in rats nursed by intoxicated mothers (experiment 1) was 15.3% and 17.5% less than in the rats nursed by intact mothers on the 14th and 21st days of postnatal life respectively. Oral administration of quercetin to intoxicated nursing rats (experiment 2) led to an approximation of the body weight and small intestine mucosa weight to control values by the end of observations. However, oral administration of quercetin to growing rats nursed by intact females (experiment 3) did not have any effect on the body and small intestinal mucosa weight (Table 1).

3.2 Intestinal Maltase and Lactase Activity

The results on the effect of quercetin on the small intestine maltase and lactase activity of rats nursed by intact and lead-intoxicated mothers are represented in Table 2.

As expected, the specific maltase activity of the small intestine mucosa in all group rats was age-dependent and increased because of the transition from milk to definitive nutrition. However, in 14-day-old and 21-day-old rats nursed by an intoxicated mother, the specific maltase activity was higher compared control rats by 27.9% and 47.6%, respectively. Treatment of nursed by lead intoxicated mother growing rats with quercetin resulted in a decrease in the level of maltase activity to control levels. Whereas the administration of quercetin to intact growing rats did not have any effect on the maltase activity.

Table 2. Effect of quercetin on the small intestine maltase and lactase activity (μ/min/g protein) of rats nursed by lead-intoxicated mothers (M ± m, n = 6)

Age (days)	Control	Experiment 1	Experiment 2	Experiment 3
	Maltase			
7P	82,19 ± 6,10 -	96,22 ± 8,11>0,2	89,42 ± 8,22>0,5	86,41 ± 5,29>0,5
14P	155,12 ± 10,29 -	198,41 ± 12,11<0,02	180,22 ± 9,19>0,1	158,37 ± 13,37>0,5
21P	254,55 ± 20,61 -	375,91 ± 21,74<0,002	271,89 ± 21,56>0,5	290,12 ± 21,56>0,3
	Lactase			
7P	59,42 ± 2,11 -	62,22 ± 2,35>0,4	63,91 ± 1,12>0,2	64,34 ± 3,04>0,2
14P	38,82 ± 3,41 -	48,22 ± 3,11<0,05	41,71 ± 2,19>0,5	46,93 ± 3,11>0,1
21P	32,12 ± 2,22 -	38,93 ± 2,44<0,05	29,61 ± 0,99>0,5	37,37 ± 2,06>0,1

Control – intact rats, experiment 1 - rats nursed by lead-intoxicated females mother; experiment 2 - rats nursed by lead-intoxicated mothers and treated with quercetin; experiment 3 - rats nursed by intact females mothers and treated with quercetin, P - statistical significance between control and experimental groups.

The activity of lactase decreased in all animal groups by the time of weaning, which corresponds to the third postnatal week of life in rats (Table 2). However, in rats nursed by lead acetate-intoxicated females, the natural age-dependent decrease in lactase activity was delayed. Lactase activity in rats nursed with intoxicated females on the 14th and 21st day of postnatal life was higher compared to control by 24.2% and 21.2%, respectively. Oral administration of quercitin to rats nursed by intoxicated mothers led to the normalization of lactase activity in intoxicated growing rats. However, administration of quercetin to rats nursed by intact females did not affect the specific lactase activity. The lactase activity in the experiment 3 group of rats was at the control level.

4. Discussion

The results show that nursing of growing rats with lead acetate-intoxicated mothers leads to a decrease in the body and small intestine mucosa weight and shifts in the development of intestinal maltase and lactase activity. The oral administration of quercetin to rats nursed by an intoxicated mother leads to the restoration of the body and small intestine mucosa weight, as well as the normalization of intestinal disaccharidase activity. Oral administration of quercetin to intact growing rats does not cause any changes in the weight parameters and small intestine disaccharidase activity.

These data primarily suggest that maternal lead poisoning results in an increase in the lead amount in the mother's milk and suckling's blood. Indeed Gulson *et al*., showed the contribution of breast milk to blood lead concentration in infants ranged from 36 to 80% during the first 60–90 days after birth[13].

After treatment of rats with lead acetate, it was noted an increase in blood corticosteroid level[14] and an opposite decrease in serum levels of T3 and T4[15]. It is believed that the steroid and thyroid hormones play a decisive role in the development of intestinal disaccharidase activity[16-18]. Administration of steroid hormones or stress causes an increase in the activity of maltase, sucrase, and other disaccharidases in suckling rats[17,18]. So, it can be assumed that the increase in maltase activity is associated with lead-induced changes blood concentration of corticosteroids in sucklings. Thyroid hormones play a critical role in the natural decay of lactase activity during weaning, which corresponds to the third week of a rat's postnatal life[14,18]. So, the decrease in the blood thyroid hormone level[15] may contribute to delaying the natural decline in lactase activity in poisoned with lead acetate growing rats. Based on this, it can be concluded that the effect of quercetin may be mediated by the normalization of hormonal balance in intoxicated rats. The ability of quercetin to suppress the stress-induced activity of the hypothalamic-pituitary-corticoid axis due to the repression of synthesis of mRNA and corticotropin-raising factor is proved[19].

In addition to lead-dependent changes in the hormonal balance, the structural and functional state of the small intestine is also affected by oxidative stress[20]. So a wide range of negative effects of lead indicates the need to search for multifunctional drugs to correct or prevent the lead poisoning consequences. Quercetin is a versatile flavonoid that has protective properties against tissue damage caused by various toxicants[21]. In current experiments, the positive effect of quercetin on the morphological and functional functions of the small intestine may also be associated with its antioxidant properties. Quercetin increases the cell's antioxidant capacity by regulating glytation peroxidase level. This is because once oxygen free radicals are formed in the body, superoxide dismutase quickly grabs O^{2-} and converts it into H_2O_2. This enzyme further catalyzes the decomposition of H_2O_2 into non-toxic H_2O. This reaction requires glutation peroxidase as a hydrogen donor. Animal and cell studies have shown that quercetin induces glutation peroxidase synthesis[21]. The effect of quercetin can also be mediated by transcription factors Nrf2 and AP-1 and the activation of antioxidant defense enzymes[22].

It should be noted that the prophylactic and/or corrective effect of quercetin on intestinal enzyme activities was manifested in intoxicated with lead acetate, but not in intact growing rats. In fact, in experiments *in vivo,* the antioxidant effects of quercetin were found mainly in models of induced oxidative stress[23,24] in contrast to studies performed on intact animals[25]. Differences in experimental conditions (including animal species, polyphenol dose, and feeding duration) could also modulate the effects of quercetin.

Thus, quercetin has a prophylactic and/or corrective effect on the activity of intestinal disaccharidases in

lead-intoxicated growing rats but has no effect on enzyme activity in intact rats. These data suggest that the positive effect of quercetin on the activity of intestinal maltase and lactase in suckling rats is associated with the blocking of destroys caused by lead acetate intoxication.

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6. References

1. Salehi B, Machin L, Monzote L, Sharifi-Rad J, Ezzat SM, Salem MA, Merghany RM, *et al.* Therapeutic potential of quercetin: new insights and perspectives for human health. ACS omega. 2020; 5(20):11849–72. https://doi.org/10.1021/acsomega.0c01818
2. Sultana B, Anwar F. Flavonols (kaempferol, quercetin, myricetin) contents of selected fruits, vegetables, and medicinal plants. Food Chem. 2008; 108:879–84. https://doi.org/10.1016/j.foodchem.2007.11.053
3. Xu D, Hu MJ, Wang YQ, Cui YL. Antioxidant activities of quercetin and its complexes for medicinal application. Molecules. 2019; 24(6):1123. https://doi.org/10.3390/molecules24061123
4. Anand David AV, Arulmoli R, Parasuraman S. Overviews of the biological importance of quercetin: A bioactive flavonoid. Pharmacogn Rev. 2016; 10(20):84-9. https://doi.org/10.4103/0973-7847.194044
5. Patrick L. Lead toxicity part II: the role of free radical damage and the use of antioxidants in the pathology and treatment of lead toxicity. Altern Med Rev. 2006; 11(2): 114-27.
6. Debnath B, Singh WS, Manna K. Sources and toxicological effects of lead on human health. Indian J Med Spec. 2019; 10(Is.2):66-71. https://doi.org/10.4103/INJMS.INJMS_30_18
7. Bellinger DC. Teratogen update: lead and pregnancy. Birth Defects Res A Clin Mol Teratol. 2005; 73(6):409-20. https://doi.org/10.1002/bdra.20127
8. Rísova V. The pathway of lead through the mother's body to the child. Interdiscip Toxicol. 2019; 12(1):1-6. https://doi.org/10.2478/intox-2019-0001
9. Ettinger AS, Roy A, Amarasiriwardena CJ, Smith D, Lupoli N, Mercado-García A, *et al.* Maternal blood, plasma, and breast milk lead: lactational transfer and contribution to infant exposure. Environ Health Perspect. 2014; 122(1):87-92. https://doi.org/10.1289/ehp.1307187
10. Choi J, Tanaka T, Koren G, Ito S. Lead exposure during breastfeeding. Can Fam Physician. 2008; 54(4):515-6.
11. Kosek MN, Lee GO, Guerrant RL, Haque R, Kang G, Ahmed T, *et al.* Age and sex normalization of intestinal permeability measures for the improved assessment of enteropathy in infancy and early childhood: results from the MAL-ED study. J Pediatr Gastroenterol Nutr. 2017; 65(1):31-9. https://doi.org/10.1097/MPG.0000000000001610
12. Viswanathan L, Rao SS. Intestinal disaccharidase deficiency in adults: evaluation and treatment. Curr Gastroenterol Rep. 2023; 25(6):134-139. https:// doi.org/10.1007/s11894-023-00870-z
13. Gulson BL, Jameson CW, Mahaffey KR, Mizon KJ, Patison N, Law AJ, *et al.* Relationships of lead in breast milk to lead in blood, urine, and diet of the infant and mother. Environ Health Perspect. 1998; 106(10):667-74. https://doi.org/10.1289/ehp.98106667
14. Haider S, Saleem S, Tabassum S, Khaliq S, Shamim S, Batool Z, *et al.* Alteration in plasma corticosterone levels following long-term oral administration of lead produces depression-like symptoms in rats. Metab Brain Dis. 2013; 28(1). https://doi.org/10.1007/s11011-012-9374-y
15. Ibrahim NM, Eweis EA, El-Beltagi HS, Abdel-Mobdy YE. Effect of lead acetate toxicity on experimental male albino rat. Asian Pac J Trop Biomed. 2012; 2(1):41-6. https://doi.org/10.1016/S2221-1691(11)60187-1
16. Drozdowski LA, Clandinin T, Thomson AB. Ontogeny, growth, and development of the small intestine: Understanding pediatric gastroenterology. World J Gastroenterol. 2010; 16(7):787-99. https://doi.org/10.3748/wjg.v16.i7.787.
17. Elnif J, Buddington RK, Hansen NE, Sangild PT. Cortisol increases the activities of intestinal apical membrane hydrolases and nutrient transporters before weaning in mink (Mustela vison). J Comp Physiol B. 2006; 176(3):233-41. https://doi.org/10.1007/s00360-005-0044-9
18. Kuchkarova LS, Kudeshova GT, Dustmatova GA. Hormonal regulation of carbohydrate assimilation in the small intestine of rats during dairy nutrition. Scientific Review. Biological Sciences. 2017; 2:108-16 (in Russia). Available from: https://science-biology.ru/en/article/view?id=1057
19. Kawabata K, Kawai Y, Terao J. Suppressive effect of quercetin on acute stress-induced hypothalamic-pituitary-adrenal axis response in Wistar rats. J Nutr Biochem. 2010; 21(5):374-80. https://doi.org/10.1016/j.jnutbio.2009.01.008
20. Wang Y, Chen Y, Zhang X, Lu Y, Chen H. New insights in intestinal oxidative stress damage and the health intervention effects of nutrients: A review. Journal of Functional Foods. 2020; 75(12):1042-48. https://doi.org/10.1016/j.jff.2020.104248

21. Xu D, Hu MJ, Wang YQ, Cui YL. Antioxidant activities of quercetin and its complexes for medicinal application. Molecules. 2019; 24(6):1123. https://doi.org/10.3390/molecules24061123
22. Lee YJ, Beak SY, Choi I, Sung JS. Quercetin and its metabolites protect hepatocytes against ethanol-induced oxidative stress by activation of Nrf2 and AP-1. Food Sci Biotechnol. 2017; 27(3):809-17. https://doi.org/10.1007/s10068-017-0287-8
23. Badr GM, Elsawy H, Sedky A, Eid R, Ali A, Abdallah BM, *et al*. Protective effects of quercetin supplementation against short-term toxicity of cadmium-induced hematological impairment, hypothyroidism, and testicular disturbances in albino rats. Environ Sci Pollut Res Int. 2019; 26(8):8202-11. https://doi.org/10.1007/s11356-019-04276-1
24. Su JF, Guo CJ, Wei JY, Yang JJ, Jiang YG, Li YF. Protection against hepatic ischemia-reperfusion injury in rats by oral pretreatment with quercetin. Biomed Environ Sci. 2003; 16(1):1-8.
25. Gruse J, Kanitz E, Weitzel JM, Tuchscherer A, Stefaniak T, Jawor P, *et al*. Quercetin feeding in newborn dairy calves cannot compensate colostrum deprivation: study on metabolic, antioxidative and inflammatory traits. PLoS One. 2016; 11(1):e0146932. https://doi.org/10.1371/journal.pone.0146932