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Analysis of the Role of Satiety of the Parents during Irradiation in the Assimilation of Substrates of the Protein Origin in two Generations of their Posterity

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Abstract

Thanks to the use of nuclear energy a society lives under the conditions of increased radiation background. Malformations and other inherited diseases caused by damage to the genetic apparatus by low doses of irradiation appear even in the remote generations. It determines the need for research of the remote effects of irradiation of parents on their posterity. One of the most radiosensitive tissues is small intestine which is responsible for the last stages of digestion and absorption of nutrients. Therefore the aim of our work was the analysis of the role of satiety of the parents during irradiation in the assimilation of substrates of the protein origin in two generations of their posterity.

Materials and methods: *In vitro* were studied parameters of transport of free glycine and "peptide" glycine formed from glycyl-glycine in the small intestine of the 2nd generation of posterity of male rats, that were irradiated by a dose 0,5 Gy after taking meal, and intact females.

Results: Parameters of the transport of the free glycine and "peptide" glycine in the small intestine of the 2nd generation of posterity of irradiated satiety male rats are lower than corresponding parameters in the 1st generation and in the intact groups but are in the borders of active component of transport. Stability of transport of the free glycine and "peptide" glycine are higher than in the corresponding intact groups.

Conclusions: In comparison with the corresponding data for 2nd generation of irradiated fasting male rats, satiety of the male-predecessors lead to the stabilization of the activity of transport system as for free glycine, so for the "peptide" glycine both in the small intestine of their posterity. Apparently, the protective effect of the presence of food in the gastrointestinal tract of precursor at exposure is realized by stabilizing of the activity of transport system for free glycine and "peptide" glycine both in the small intestine of two generations of posterity, and maintaining its work at the level necessary and sufficient for normal body functioning.

Keywords: Irradiation; Generations of posterity; Satiety; Glycine; Glycyl-glycine; Transport

Introduction

An important role in the energy supply of society still plays a nuclear energy. A side effect of this is to increase of the background radiation. It determines the need for research not only an immediate effects of ionizing radiation on the human body but also its effect on the distant future generations. Irradiation leads to breaks in the DNA molecule and disorders of interphase chromatin, to the inhibition of the synthesis of the DNA-membrane complex. As a result, it can increase the membrane permeability and disturb of the processes of the regulation of energy metabolism in the cells, resulting in their death because of irradiation. One of the most important aspects of ionizing radiation is to reduce the functional capacity of the descendants of the irradiated organism, which further leads to the manifestation of long-term radiation damage - tumours, genetic changes and disorders of embryonic development [1,2].

The bases of radiation pathology at the cellular level are 3 types of disorders that result from the direct effects of radiation: cell death, conservation of non-lethal hereditary disorders and hereditary changes that consistently reproduced during reproduction of somatic cells [2-4]. The most radiosensitive are tissues with high proliferative activity - red bone marrow, intestines, and gonads cells [5]. The small intestine, where the final stages of digestion and formation of monomer nutrients occurs, is the most radiosensitive part of the gastrointestinal tract. Distant radiation pathology of the digestive tract may develop as a result of the incorporation of radio nuclides for which the gastrointestinal tract is one of the major routes of getting and excretion from the body, and the influence of external sources of radiation both.

The actions of small doses of radiation leads to violations of ultra-structures of the mucous membrane of the small intestine, changes the lipid composition of the apical membrane of enterocytes and the viscosity of the lipid bilayer and increase its permeability for inorganic ions, disruption of oxidative-antioxidant status membranes [6]. Thus, the existence of the whole organism to a certain extent depends on the functional activity of small intestine. Functional disorders due to the

action of high doses of radiation usually appear within a few hours or days, but malformations and other inherited diseases caused by damage to the genetic apparatus - only in the next generations. Complete elimination of the negative effects of exposure of predecessors in the population happens till 7-10-th generation, and about 56% of genetic diseases of the 1st generation appear in the 2nd [7].

However, information about the transgenerational phenomenon of radiation-induced genomic instability in offspring of irradiated in small doses parents are scarce, contradictory and require further study [8,9]. Therefore, the problem of the effects of radiation damage does not cease to disturb society, and the search for means to overcome them does not lose relevance. We have previously investigated the activity of the digestive system and absorption of substrates of protein origin in the small intestine of two generations of the descendants of irradiated hungry male rats [10]. It was shown a significant increase in the activity of transport of free glycine in the offspring of the 1st generation and a significant reduction of it in the 2nd generation offspring. But the activity of the enzymatically-transport conveyor, responsible for the hydrolysis of glycyl-glycine and transport of the formed "peptide" glycine were significantly lower than that of intact animals. There was a question about what kind of role in the protection of functional systems bowel of the offspring of irradiated animals may play a presence of food in the gastrointestinal tract of parents at the time of exposure. Previously, we have shown a protective effect of satiety status of parents at the time of irradiation on the activity of the hydrolytic and transport systems of carbohydrate substrates of different degree of polymerization in the small intestine of their posterity of two generations [11]. Therefore, the aim of this work was the study of indicators of digestion and absorption of protein origin substrates varying degrees of polymerization in the posterity of two generations of irradiated in satiety status male rats.

Materials and Methods

Experiments were conducted on two-month male rats of line of Vistar by mass 60-70 gram – posterity of the onetime irradiated male rats and intact female rats. Experimental groups were kept on the standard diet of vivarium and were deprived of meal during 18-24 hours before an experiment. Totally were used 5 groups of posterity: 1-st - intact; 2-nd and 3-rd - descendants of two generations of irradiated hungry by a dose 0,5 Gy males and intact females, 4-th and 5-th - descendants of two generations from males, that were irradiated by a dose 0,5 Gy after taking meal (in satiety status) and intact females. Exposition of male rats performed once by the help of telegamma-setting "AGAT-R1", dose was 120 rad/min, field 20×20, distance from the source of irradiation to the field=75 cm, dose - 0.5 Gy, exposure - 32 sec. Accumulating Mucosa Preparations (APS) produced by the method of Ugolev et al. [12]. APS incubated for 1 hour at t=37°C in the oxygenated medium. As the incubation medium were used solutions 10 mmol / l of glycine and 5 mmol/l of glycyl-glycine that were made on the Ringer solution pH 7.4. In all incubation medium the bile was added. The concentration of free glycine and "peptide" glycine formed due to the hydrolysis of glycyl-glycine was determined using method described in ref. [13] colorimetrically on photoelectrocolorimeter – CFC-2MP, λ=540 nm. The statistical processing of the obtained data was conducted using "Primer Biostatistics" software.

Results and Discussion

Previously, we have shown that the satiety status of males at the time of exposure provides a level of free glycine transport in the small intestine 1st generation of their descendants on the level of indicators of intact rats - as opposed to increasing of it in the descendants of the 1st generation of the irradiated hungry rats [14]. But in the 2nd-generation of the offspring of male rats exposed in the satiety status, the parameters of the transport of free glycine were 35% lower than in intact rats (33, 37 ± 1, 59 versus 51, 84 ± 3, 62 mmol/l, Table 1, P = 0.002), by 43% lower than in the descendants of the 1st generation (33. 37 ± 1. 59 vs. 58. 92 ± 1. 57 mmol/l, Table 1, P = 0.0001) and lower than that of descendants of the 2nd (and especially the 1st!) generation of hungry exposed rats - by 31% (and respectively 60%!) (33, 37 ± 1, 59 versus 48, 46 ± 3, 97, P = 0, 008 and 33, 37 ± 1, 59 against 83, 84 ± 5, 33 mmol/l, P = 0, 00001; Table 1). However, if we compare absolute levels of free glycine transport in all groups, we can see that the most effective of all the groups have transport in the preparations from the posterity of the 1st generation of the irradiated fasting rats (83, 84 ± 5, 33 mmol/l, Table 1) Previously has already been supposing that this may be the result of hormesis [10]. It is possible that a single exposure of animals-predecessors with relatively small dose by activating of a chain of free radical reactions and by the corresponding changes in the structure of the transport system contributes to the stimulation of its activity in the offspring of the 1st generation. To this favour also evidence a relatively low scatters from the middle data that against the background of a significant increase in its (middle data) do not differ from those in the intact group (6.4% versus 7%, Table 1). Undoubtedly, the violation of the structure of the conveyor should be reflected in the destabilization of his work, so the high stability of the transport of glycine in the offspring of F1 from irradiated fasting male rats can be explained by the positive changes in the structure of the transport system in the small intestine of irradiated male predecessor - obviously, these changes have been secured on the genetic level and implemented in the 1st generation of descendants. It is also likely that in the context of a numerous body damage of the progeny of irradiated animals [1-9] the activation of the transport of amino acid which can be used not only as a material for protein synthesis, but also as a substrate for the gluconeogenesis (it actively occurs in the small intestine) - to meet the energy needs of the body, can be regarded as an adaptive response and is fixed at the level of the genome [14]. But in the 2nd generation this effect disappears and indicators of glycine transport are returning to the indicators of intact group - they are only 6% below those in the intact group (48, 46 ± 3, 97 versus 51, 84 ± 3, 62 mmol/l, Table 1), and even the stability of the transport system is located almost at the same level (8.2% to 7%, Table 1). Apparently, because the irradiation results to genomic instability, it is a manifestation of realization of disorders that have been incorporated into the genome of the male-predecessor during the irradiation, were presented in the recessive form in the offspring F1 and were implemented in the offspring F2 [10]. This assumption is supported by the literature [8,9,15,16]. The trend to increase of the transport activity of glycine in the F1 and the reduction of it in the F2 observed in the offspring of the irradiated in the satiety status rats (Table 1). But unlike the F1 and F2 offspring from irradiated fasting rats in this group differences between generations are smoother - the level of transport in F1 and in the group of intact rats differs by only 12%, but the stability of the transport system in F1 above 60% (2.7% vs. 7%, respectively, Table 1). So, the satiety status of the males - predecessors at the time of irradiation contributes to the stabilization of the transport system for free glycine in their posterity F1. In the F2 on the background of the significant decrease of activity of the

transport system nonetheless the stability of it work is 31% higher than in intact animals (4.8% versus 7%, Table 1) and significantly higher than in all groups of the posterity of the irradiated fasting males - by 25% higher than in F1 and 41% higher than F2 (4, 8% versus 6. 4% and 8. 2% respectively, Table 1). Obviously, reducing the scatter of data has to indicate to the normalization of the activity of transport system. Apparently, the presence of food in the gastrointestinal tract of rats during irradiation yet mitigates effect of irradiation exposure for both generations of posterity. Attention is drawn also to the fact that despite the fact that the absolute levels of glycine transport in F2 are the lowest among all discussed groups, they are still within the active component of the transport (Table 1). Apparently, the protective effect of the presence of food in the gastrointestinal tract of precursor at exposure is realized by stabilizing of the transport system for free glycine in the small intestine of two generations of posterity, and maintaining its work at the level necessary and sufficient for normal body functioning. In favor of this hypothesis is the fact that when comparing the parameters of the activity of the system of hydrolysis of glycyl-glycine and transport produced with "peptide" glycine (P-glycine) in all the analyzed groups, the maximum velocity detected in the F1 posterity from irradiated in satiety status males (63, 25 ± 1, 84 mmol/l; Table 1) on the background of the lowest value scatter - 3% (ibid). Previously, it gave us a reason to conclude about a radio-protective effect of food on the transport system for the dimeric substrate of the protein origin (glycyl-glycine) [14]. Indeed, the absolute values of absorption of P-glycine in the posterity F1 of the irradiated in the satiety status male rats almost coincide those of intact animals (63, 25 ± 1, 84 vs. 63. 29 ± 3.22 mmol/l, Table 1), and they 26% higher than in a similar group F1 from irradiated hungry males (63, 25 ± 1, 84 vs. 46, 97 ± 2, 34 mmol/l, respectively, P = 0, 0001, Table 1). In addition, indicators of stability for this group are even higher than in intact animals group (3% to 5%, Table 1). Absolute parameters of the transport of P-glycine in F2 from irradiated in satiety status males although 25% lower than in a similar group of F2 from the irradiated hungry males, but not significant due to the large scatter of the data in the latter group (4.8% vs. 10.5% respectively: 54%, Table 1). Noteworthy is also the fact of virtually identical parameters of transport of the free and "peptide" glycine in the small intestine of F2 from irradiated in satiety status rats: differences are only in decimals, and scatter indices are generally equal (Table 1). The latest are practically identical with those in intact animals (4.8% vs. 5%, respectively, Table 1). Apparently, these data still support the hypothesis of the radio-protective effect of food on the transport system not only of the free glycine but also P-glycine formed by the hydrolysis of the dimer glycyl-glycine. This is especially interesting in the light of the numerous data that they use different transport systems [17-19]. There is also evidence of a concentration-dependent transport of glycyl-glycine as a peptide from the solution of the concentration not exceeding 40 mmol/l [20]. According to our data, obtained for two generations of posterity of irradiated hungry males, transport of the free glycine and "peptide" glycine indeed are carried out by different systems: start from the parameters of their transport in the intact group (51, 84 ± 3, 62 and 63, 29 ± 3, 22 mmol/l, respectively, Table 1) and ending with multi-directional effects in the offspring of F1 (83, 84 ± 5, 33 and 46, 97 ± 2, 34 mmol/l, respectively, in the same place) transport trends are vary. A common is only an increase of the scatters from the average value in the posterity F2, indicating likely manifestations of disorders in the genome (8.2 and 10.5% respectively, Table 1). This is not observed in two generations of progeny of irradiated in the satiety status males - trends in the transport of the free amino acid and the formed by hydrolysis of the dipeptide in the offspring of different generations almost the same, while the stability of the system of free glycine transport and "peptide" glycine are the same in F2 (Table 1).

Group of animals	Glycine	Glycyl-glycine
I		
1.Intact n = 5	51,84 ± 3,62* 7%	63,29 ± 3,22* 5%
2.Posterity F1 n = 5	X83,84 ± 5,33* 6,4% P1-2 = 0, 001	X46,97 ± 2,34* 5% P1-2 = 0, 003
3.Posterity F2 n = 10	XX 48,46 ± 3,97* 8,2% P2-3 =0, 0001	44,84 ± 4,69* 10,5% P1-3 = 0, 022
II		
1.Intact n = 5	51,84 ± 3,62* 7%	63,29 ± 3,22* 5%
2.Posterity F1 n = 5	X58,92 ± 1,57** 2,7% P*=0. 002	X63,25 ± 1,84** 3% P*= 0, 0001
3.Posterity F2 n = 5	XX33,37 ± 1,59 4,8% P1-3=0, 002 P2-3 =0, 0001 Pxx =0, 022	33,67 ± 1,63 4,8% P1-3 = 0, 0001 P2-3 = 0, 0001

Note: In all incubation mediums the rabbit bile was added.

Under the parameters of accumulation presents the percentage of the scatters from the middle value

* - Data obtained earlier [10] used to compare

** - Data obtained earlier [14] used to compare

x - the difference between the values in groups of the 1st generation of posterity from irradiated hungry and fed rats;

xx - the difference between the values in groups of the 2nd generation of posterity from irradiated hungry and fed rats

Table 1: Assimilation of substrates (mmol/l*mg) by the accumulation mucosa preparations from the mucosa of the small intestine of the 2-months-old rats – posterity of the 1-st and 2-nd generation of the irradiated by 0, 5 Gy hungry (I) and in satiety status (II) males rats and intact females rats (M ± m).

Conclusions

Detected in the posterity F1 of the irradiated fasting male rats the activation of the transport system for the free glycine and inhibition of the system for the P-glycine, then in the posterity F2 decrease till the level of intact group for the transport of the free glycine, and level of the transport of P-glycine is lower than in intact group - on the level of transport of the free glycine in F1. It has happening on the background of the destabilizing of the activity of both transport systems - as for the free glycine also for P-glycine. In the two generations of posterity of irradiated in satiety status male rats, takes place the stabilization of activity of transport systems as for free glycine and for P-glycine both, despite the decline in absolute parameters in posterity F2 compared to

both the F1 and with the intact group. Apparently, the protective effect of the presence of food in the gastrointestinal tract of precursor at exposure is realized by stabilizing of the activity of transport system for free glycine and "peptide" glycine both in the small intestine of two generations of posterity, and maintaining its work at the level necessary and sufficient for normal body functioning.

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