Role of Cyclooxygenases in the Stimulatory Effect of Carcinogen 1,2-Dimethylhydrazine on Stem Cell Survival in the Intestinal Epithelium and Bone Marrow

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In vivo experimental studies showed that 1,2-dimethylhydrazine and product of its metabolism in the body azoximethane improve postradiation survival of two types of stem cells in the adult organism: hemopoietic stem cells and intestinal epithelial stem cells. This effect similar to the well-known radioprotective effect of *E. coli* lipopolysaccharide was observed, when the carcinogen was administered 1 day before γ-irradiation. Treatment with 1,2-dimethylhydrazine prolonged the mean life-span of mice irradiated in supralethal doses inducing death of the majority of intestinal epithelial stem cells. Nonspecific cyclooxygenase inhibitor indometacin weakened this radioprotective effect of 1,2-dimethylhydrazine. We also found that carcinogen 1,2-dimethylhydrazine improved survival of hemopoietic stem cells. However, in contrast to intestinal epithelial stem cells, indometacin did not inhibit the radioprotective effect of the carcinogen. The radioprotective effect of 1,2-dimethylhydrazine and lipopolysaccharide on stem cells in the presence of indometacin was a sum of individual effects of these preparations and indometacin.

Key Words: *1,2 dimethylhydrazine; indometacin; survival of stem cells, intestinal crypts, endogenous splenic colonies*

Dimethylhydrazine regioisomers 1,1-dimethylhydrazine (1,1-DMH) and 1,2-dimethylhydrazine (1,2-DMH) are well-known carcinogens inducing colorectal tumors. Under *in vitro* conditions, DMH induce methylation and breaks of cell DNA and transformation of normal cells [8]. Azoxymethane, the most potent and specific intestinal carcinogen for rodents, is the best studied chemical and metabolic product of 1,2-DMH. Azoxymethane stimulates survival of intestinal stem cells (SC) in irradiated mice. An important role in this protective effect is played by cyclooxygenases (COX) [11]. These data confirm the hypothesis that blockade of the programs of elimination of excess or damaged cells or triggering of programs of cell survival is an important stages of carcinogenesis.

Here we tested the hypothesis that 1,2-DMH can stimulate survival of other types of SC, *e.g.* hemopoietic SC, in the adult organism.

MATERIALS AND METHODS

Experiments were performed on F_1(CBA×C57Bl/6) male mice weighing 20-22 g. The animals were maintained in cages in a soundproof room under conditions of natural illumination at 22-24°C and received standard ration and water *ad libitum.*

The animals were subjected to single γ-irradiation in doses of 6.5 and 12.9 Gy on a Luch device with ^{60}Co as the radiation source (0.3 Gy/min dose power).

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We used 1,2-DMH, indometacin, lipopolysaccharide (LPS; *E.coli*, 0111:B4) purchased from Sigma-Aldrich Rus. Company. 1.1-DMH was kindly provided by Prof. A. A. Mandrugin (Chemical Faculty of M. V. Lomonosov Moscow State University). 1,2-DMH (0.21 mmol/kg, 20 mg/kg intraperitoneally) and LPS (0.5 mg/kg) were was injected 1 day before irradiation, indometacin (10 mg/kg intraperitoneally) was injected 3 times: immediately and 24 and 48 h after irradiation.

The animals were sacrificed 8 days after γ-irradiation, the spleens were removed in fixed for counting colonies with a diameter >0.2 mm formed by survived endogenous hemopoietic stem cells (CFU-S-8) [3]. Each experimental group consisted of 12 animals; the experiments were repeated 3 times. The data were processed statistically; the mean number of splenic colonies formed by survived CFU-S-8 was calculated as described previously [2].

For evaluation of the radiomodifying effects of preparations on survival of intestinal epithelial SC we used a method of microcolonies in our modification [3,4]. Three days after whole-body γ-irradiation in a dose of 12.9 Gy (^{60}Co, 0.3 Gy/min), a fragment of the intestine located 1.5-2.0 cm distally from the duodenum was isolated and fixed in 10% neutral formalin. After standard histological treatment, 7-μ transverse section were prepared; similar preparations were made from the intestine of non-irradiated mice. The number of survived crypts containing ≥10 viable cells, descendants of survived SC, was determined. The total number (N) of survived SC in all crypts on the intestinal cross-section was calculated by the formula: N=A{-ln[(A-B)/A]}, where A is the number of crypts on intestinal cross-section in intact mice and B is the number of crypts survived after irradiation.

Analysis of intestinal preparations from 5 non-irradiated mice showed that in intact animals the number of crypts per section is 116.7±1.4. We examined 10 sections of the intestine from each irradiated and non-irradiated animal, more than 1000 crypts for each control mouse and 5000 crypts for the experimental group consisting of 5 mice were analyzed.

The number of mice survived after irradiation in a supralethal dose of 12.9 Gy was determined daily. Each of 6 groups consisted of 12 animals. Reliability of differences was evaluated using χ^2 test [6].

The data are presented as mean±standard deviation. The differences between the mean yields of SC in irradiated animals were statistically analyzed using Newman—Keuls and Dunnett tests [1].

RESULTS

It was found that 1,2-DMH, similarly to its metabolite azoxymethane [11], considerably (by 1.6 times) increased the number of survived crypts per intestinal section, and hence, the number of intestinal epithelial SC (IESC, Table 1).

Taking into account the data that survival of SC in critical cell systems determines postradiation lifespan of experimental animals irradiated in supralethal doses [3], we carried our experiments on evaluation of the effect of carcinogen on animal survival during the acute stage of radiation damage. The mean lifespan of animals receiving 1,2-DMH before irradiation in a dose of 12.9 Gy increased from 4.7 to 6.7 days (Table 2), which confirms close relationship between survival of IESC located in crypts and serving as a natural reserve of regenerative processes in damaged epithelial tissue [3] and survival of irradiated animals.

Non-specific COX inhibitor indometacin had no effect on animal lifespan. However, administration of indometacin to animals receiving 1,2-DMH before irradiation reduced their lifespan to a level observed in the control group (12.9 Gy without protection). These results indirectly suggest that COX are involved into the improvement of IESC survival by carcinogen 1,2-DMH.

For additional control, we studied the stimulatory effect of LPS on IESC survival [7]. Both LPS and carcinogen considerably improved the mean lifespan of experimental animals to 5.4 days. Indometacin inhibited the radioprotective effect of LPS to the control level. These data indirectly confirmed the results of evaluation of crypt survival after treatment with LPS and indometacin [10].

E. coli. LPS, a component of cell wall of Gram-negative bacteria, was also used in evaluation of the effect of DMH on the formation of endogenous splenic colonies formed by survived and proliferating CFU-S-8. Both SMH regioisomers (1,1-DMH and 1,2-DMH) increased the yield of CFU-S-8 in the spleen of irradiated animals by 2.5 and 5.3 times (Table 3). The weight of the spleen insignificantly increased. As expected, LPS produced an

TABLE 1. Radioprotective Effect of 1,2-DMH (*M*±*m*)

Treatment	Number of viable crypts	Number of survived SC
Control (12.9 Gy)	15.2±1.7	16±2
1,2-DMH+12.9 Gy	23.6±3.3	26±5*

Note. Here and in Table 2, 3: *p<0.05 compared to the control.

TABLE 2. Mean Lifespan of Experimental Mice

Treatment	Mean lifespan, days
Control (12.9 Gy)	4.7±0.4
12.9 Gy+indometacin	4.0±0.2
LPS+12.9 Gy	5.4±0.4*
DMH+12.9 Gy	6.7±0.5*
LPS+12.9 Gy+indometacin	3.7±0.2
1,2-DMH+12.9 Gy+indometacin	3.7±0.2

appreciable radioprotective effect and increased the number splenic endogenous colonies formed by CFU-S-8 by 6 times.

The data obtained on intestinal crypts suggest that COX-1 is involved into the mechanism of stimulation of IESC survival [11]. An attempt was undertaken to determine the degree of the effect of nonspecific inhibitor of COX on the radioprotective effect of DMH for hemopoietic stem cells. Indometacin considerably increased the yield of CFU-S-8 in irradiated animals to 4 colonies per spleen (vs. 1.5 in irradiated controls, Table 3), which agrees with published data [9]. Combined treatment with DMH and indometacin also increased the yield of CFU-S-8, but this increase did not exceed the sum of individual effects of each preparation (Table 3). Similar additive effect on the yield of CFU-S-8 was observed after combined treatment with LPS and indometacin. Thus, in contrast to IESC, additional treatment with indometacin did not weaken the radioprotective effect of DMH or LPS on hemopoietic stem cells.

The following conclusions can be made. 1,2-DMH stimulate *in vivo* survival of hemopoietic SC and IESC. Similarly to the effect of LPS, this effect was observed when the carcinogen was administered 1 day before irradiation. Treatment with 1,2-DMH before irradiation in supralethal doses inducing death of IESC prolonged animal lifespan. Nonspecific COX inhibitor abolished this effect probably by reducing survival of hemopoietic SC due to inhibition of prostaglandin E_2 [10,11]. Carcinogen 1,2-DMH improved survival of hemopoietic SC. Inhibition of COX with indometacin did not abolish this effect. The radioprotective effect of 1,2-DMH and LPS on stem cells in the presence of indometacin was a sum of individual effects of these preparations and indometacin.

Thus, 1,2-DMH, a representative of hydrazines, well-known activators of a wide spectrum of redox-active hemoproteins and flavoprotein enzymes [13], can also stimulate survival of radiation-damaged hemopoietic stem cells. It can be hypothesized that this effect is determined by blockade of thanatogenic mechanisms driving SC with damaged genetic apparatus to apoptosis. At the same time, various types of SC of adult organism differ by their participation in processes of production of reactive oxygen species.

TABLE 3. Effect of DMH Analogs, LPS, and Indometacin on the Yield of Splenic Endogenous Colonies Formed by CFU-S-8 Survived after γ-Irradiation in a Dose of 6.5 Gy (*M*±*m*)

Treatment	Weight of spleen, mg	Mean number of endogenous colonies
Control (6.5 Gy)	31.8±7.7	1.5±1.5
1,2-DMH+6.5 Gy	35.7±3.7	4.0±1.3*
1,2-DMH+6.5 Gy+ indometacin	42.8±8.6	6.9±4.7*
LPS	44.3±9.2	9.0±2.6*
LPS+6.5 Gy+ indometacin	47.1±14.1	11.6±7.4*
Indometacin	29.9±4.9	4.0±1.3*
1,1-DMH+6.5 Gy	32.8±2.1	8.0±0.8*

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