PHARMACOLOGY AND TOXICOLOGY

Effect of Polymorphic Forms of Methyluracil on the Development and Lifespan of *Drosophila melanogaster*

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The effects of 6-methyluracil α- and β-forms (methyluracil and betamecil, respectively) on the development and lifespan of male and female drosophila were compared and their toxicity at various concentrations was examined. For the first time the dynamics of drosophila development was used for evaluation of biological properties of these compounds. Methyluracil is more toxic and more potent inhibitor of drosophila development than betamecil. Betamecil added to food throughout the life does not decrease its average lifespan.

Key words: *polymorphism; methyluracil; lifespan*

6-Methyluracil is a simple analogue of natural nucleic acid pyrimidine bases. Its possesses anabolic and anticatabolic activities and is widely used as a stimulator of reparative processes. 6-Methyluracil was shown to exist in two polymorphic α and β modifications: methyluracil (MU) and betamecil (BM), which differ in their structure both in the solid phase [1] and in solutions [3]. The structure of both modification was examined in detail [1]. In addition to their structure and physicochemical characteristics, 6-methyluracil modifications differ in membrane permeability, *in vivo* and *in vitro* antioxidant activity, and their effects on lipids and lipid-soluble free radical scavengers [2].

We examined the effect of MU and BM on the development and lifespan (LS) of *Drosophila melanogaster*, a usual model for gerontological studies [5,8].

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MATERIALS AND METHODS

Drosophila melanogaster D-32 line cultured at 25±1°C and 75% humidity was used in the study. *Drosophila melanogaster* was chosen as a model because of its short reproductive period (10-12 days) and LS (about 3 months).

The following procedure was used to determine toxic concentrations of 6-methyluracil polymorphic forms. Two female and 1 male flies were placed for 24 h into a tube containing nutrient medium for reproduction. Control and experimental groups were similar and formed simultaneously. After removal of parental flies the number of eggs was counted and the development of new generation was observed. Every day pupae and imago flies were counted which allowed to follow the dynamics of drosophila development. The ratio between the number of adult insects and eggs was used as an integral parameter of the development. Two-three hundreds lavae and pupae (20-25 tubes) per sample were used

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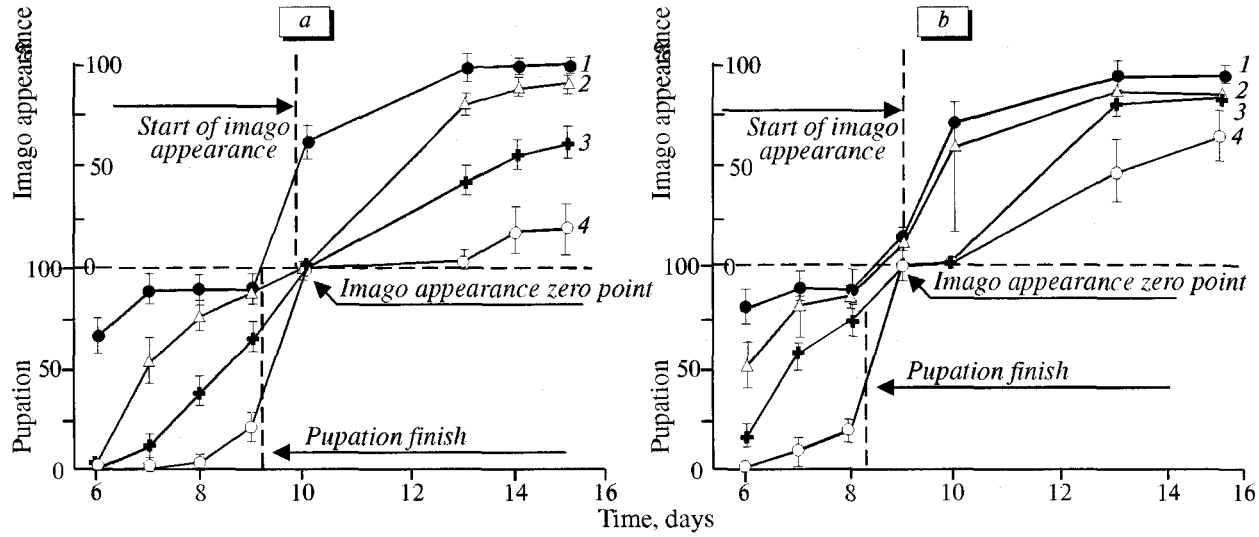

Fig. 1. Effects of methyluracil (a) and betamecil (b) on the dynamics of *Drosophila melanogaster* development. *1*) control; *2, 3* and *4*) drugs in concentrations of 0.2, 0.3 and 0.5%, respectively. The developmental period is divided into larval and pupal stages, therefore 100% of larval stage indicating 100% pupation corresponds to the pupal stage and shows the absence of imago forms. The values on the curves are given with two-fold standard error of the mean.

to provide sufficient number of observations for LS studies.

To determine LS and estimate gerontoprotective properties of MU and BM, the flies were placed to tubes with nutrient meduim containing test drugs in the corresponding concentrations. The age of the parental flies (10 males and 10 females) was about 5 days. Imago flies were selected 12 h after hatching, anesthetized with ether, and divided into males and females. Thus, all females were virginal, though fertilization does not affect the LS. Thereafter, the flies were placed into tubes (10 flies in each) containing the second type of medium and the tubes were closed with sterile cotton plugs. The medium was changed every other day by putting the flies into tubes with fresh medium. Simultaneously living flies were counted without anesthesia. The populations of 120 flies were used for the study of MU and BM effects on LS. Similar population was used as the control. Two successive generations of flies were used to examine the effect of these compounds on LS.

Two types of nutrient media — for the developmental stage and for adult flies, were used. The developmental medium consisted of 120 g living yeast, 100 g sugar, and 15 g agar per 1 liter water. The second medium was composed of 100 g corn groats, 50 g sugar, 60 g treacle, and 4.5 g agar per 1 liter water. Living yeast of the first medium were killed by 2 h boiling, thereafter agar and sugar were added. Hot media were poured into 100×25 mm glass tubes and a drop of living yeast suspension was added on the surface of cold medium. Before use the tubes were kept for 24 h at room temperature.

Aqueous solutions of MU and BM were added to the nutrient medium of experimental groups. The final concentration of these compounds in the medium ranged from 0.05 to 0.5% of total weight. Because both drugs have limited solubility, the solutions were heated to 80°C prior to addition to the warm medium.

The Kolmogorov—Smirnov's test was used for the comparison of LS curves. The significance of differences was estimated by Student's *t* test.

RESULTS

Figure 1 shows the development of larvae in the medium containing MU and BM. The dynamics of drosophila development in the presence of various MU

TABLE 1. Mean Lifespan of *Drosophila melanogaster* (days, *M±m*)

Drug	Drug concentration in the medium, weight %						Control	
	0.05		0.1		0.15			
	females	males	females	males	females	males	females	males
BM	51.0±1.65	50.2±1.23	49.6±1.16	49.2±1.51	49.1±1.46	47.8±1.46	51.0±1.47	49.8±1.53
MU	54.0±1.73	52.0±1.33	48.0±1.53	42.8±1.14	43.4±1.23	35.8±1.17	55.1±1.67	56.7±1.47

concentrations showed that larvae were most sensitive to various compounds, because some medium components could initiate lethal processes at this developmental stage (Fig. 2). The pupal stage was less sensitive. Different sensitivity to test compounds can be explained by principle differences between the larval and pupal stages. It is known that the main feature of the pupal stage is the predominance of cell differentiation and the formation of imago organs and tissues. On the contrary, larval stage is characterized by predominance of growth and proliferation. The inhibition of the larva development results from a decrease in the growth rate. Nevertheless, the size of larvae treated with varying concentrations of test drugs before pupation did not differ from the control. A pronounced toxic effect of MU and BM in concentrations of 0.3 and 0.5% was observed. MU was more toxic than BM and being added to the nutrient medium caused significant ($p<0.05$) death of larvae.

A comparison of the dynamics of larva and pupa development shows that both MU and BM in concentrations of 0.2, 0.3 and 0.5% significantly delayed the development of flies, though the effect of MU was more pronounced. MU and BM in concentrations of 0.05 and 0.1% were not toxic and did not delay the development of flies, therefore the corresponding curves are not presented on Fig. 1. The development of the insects fed the medium containing toxic MU and BM concentration (0.3 and 0.5%) was delayed by 2 days. The development of flies on the medium containing 0.2% MU was delayed by 1 day compared to the medium with BM. Besides, the appearance of the imago insects developing on MU-containing medium was significantly slower during the first day (10th developmental day, Fig. 1) as compared to flies treated with BM. The concentration of 0.2% of MU and BM was not toxic because 100% pupation was observed.

The average LS in the control and experimental (MU- and BM-treated flies) groups is presented in Table 1. Differences between LS values in the control group can be explained by its fluctuations in the successive generations of flies [8]. It should be noted that males were more sensitive to MU: even the lowest examined concentration (0.05%) significantly decreased ($p<0.05$) male LS compared to the control. BM had similar effect on males and females. The increase in BM concentration from 0.1 to 0.15% did not decrease male LS, while MU under the same conditions decreased it by 16%. The revealed differences can be attributed to lower BM toxicity.

Thus, high concentrations of 6-methyluracil isoforms decreased both the average and maximum LS, thus producing a negative effect on the whole fly population. At the same time, MU accelerated ageing to a greater extent than BM. The differences in LS between males treated with 0.15% MU or BM and controls were 28 and 12 days, respectively.

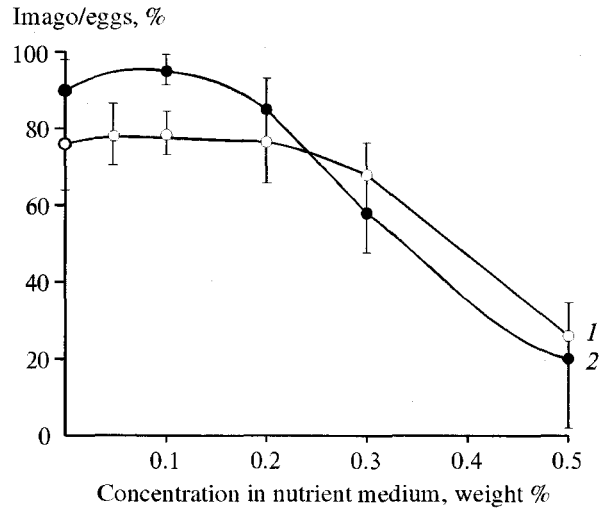

Fig. 2. Betamecil (*1*) and methyluracil (*2*) toxicity during larval stage.

The mechanism of MU and BM effect on the LS of experimental flies can be connected with inhibition of the antioxidant system. Both forms are known to affect, though to a different degree, the regulation of lipid peroxidation (LPO). Aging can be associated with LPO-induced change in lysosome membrane integrity [9]. The observed differences between the effects of MU and BM on the development and LS of drosophila confirm the data on different biologic activity of these two crystal forms [2]. We have earlier showed that BM decreased the content of free radical scavengers and modulated the concentration of vitamin E in rat liver and heart. It was also shown that MU possesses no antiradical activity and even decreases the concentration of active antiradical agents, thus promoting LPO in membranes [2]. Enhanced LPO is most typical of cell membranes in old animals [7]. On the other hand, BM showed antioxidant activity of 26.8% tocopherol equivalents [2]. This property of BM makes it less toxic for the development and LS of flies.

In the present study we used the concentrations that significantly exceeded therapeutic doses. Since even high BM concentrations did not decrease average LS, it is quite possible that its lower doses can decelerate aging. The revealed BM properties can be of particular interest, because the effects of a number of pharmacological agents on LS are not studied yet. Thus, BM has significant advantages over MU especially during long-term application of these drugs in pulmonology, physiology, surgery, gynecology, and oncology.

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