The Effect of l-Ascorbate 1-(2-Hydroxyethyl)-4,6-Dimethyl-1,2-Dihydropyrimidin-2-One on the Regeneration of the Planarian Girardia tigrina

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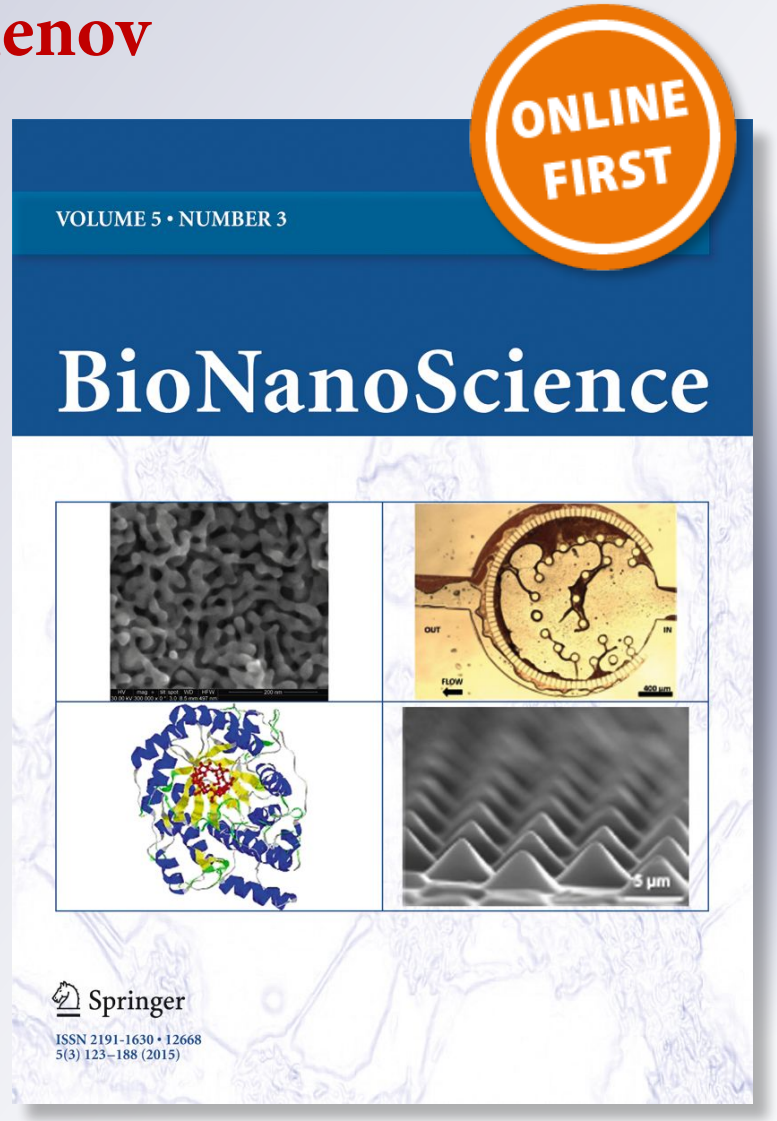

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The Effect of L-Ascorbate 1-(2-Hydroxyethyl) -4,6-Dimethyl-1,2-Dihydropyrimidin-2-One on the Regeneration of the Planarian *Girardia tigrina*

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Abstract The effect of L-ascorbate 1-(2-hydroxyethyl)-4,6-dimethyl-1,2-dihydropyrimidine-2-one, a co-crystal of Xymedon with ascorbic acid, derived from pyrimidine on the regeneration process of the planarian *Girardia tigrina* (Girard, 1850) has been investigated. Being a co-crystal of Xymedon with ascorbic acid, the preparation is characterized by the improved properties compared to Xymedon. The new preparation falls within the group of hepatoprotectors capable of liver regeneration in response to various destructive factors. It has been shown that the new preparation at different concentration levels can inhibit planarian head regeneration. Two combinations of the test substance were used in the work: a neat form of the preparation; a mixture of the preparation with Na2CO3 to reduce the acidity of the test substance. The neat preparation showed a statistically insignificant tendency to an increase in the regeneration of *G. tigrina* planarians. When mixed with Na2CO3, the preparation inhibited the regeneration of *G. tigrina* planarians. *G. tigrina* planarians is a popular model for studying regeneration processes and stem cell proliferation.

Keywords Regeneration · Planarians · Pyrimidine bases · Proliferation

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1 Introduction

Searching for new hepatoprotectors promoting liver cell proliferation has recently gained an increasing importance, because of various factors, such as liver dysfunction and disorders, infections, unhealthy feeding habits, and environmental pollution [1]. L-ascorbate 1-(2-hydroxyethyl)-4,6-dimethyl-1,2-dihydropyrimidine-2-one (below referred to as *preparation 1*), a co-crystal of Xymedon with ascorbic acid, is a new promising hepatoprotector synthesized in Russia. Xymedon has already proved its efficiency as a regenerative [2] and immunostimulatory preparation, especially as a hepatoprotector [3]. There are also data on the actoprotective (preparations that increase physical activity) and neuroprotective action of Xymedon derivatives [4–7]. In order to investigate the proliferative activity of *preparation 1*, we used a culture of the planarian *G. tigrina* (Plathelminthes, Turbellaria, Tricladida) as a test object. These planarians serve as a model that suits perfectly for studying regeneration processes and proliferation of stem cells [8]. The purpose of this paper is to analyze the proliferative activity of *preparation 1* at different concentrations on the regeneration of the planarian.

2 Material and Methods

The experiments were performed on *Girardia tigrina* (Plathelminthes, Tricladida) asexual freshwater planarians. The planarians were obtained at the Institute of Cell Biophysics RAS, Pushchino, Russia. The planarians were precultured in fish tanks at the temperature of 26 °C and fed with dipteran larvae. The planarian specimens used in the experiments had the body length of 10–11 mm and were kept in starvation for 7 days. Regeneration was initiated by amputation of the head end in the area of eyes. A

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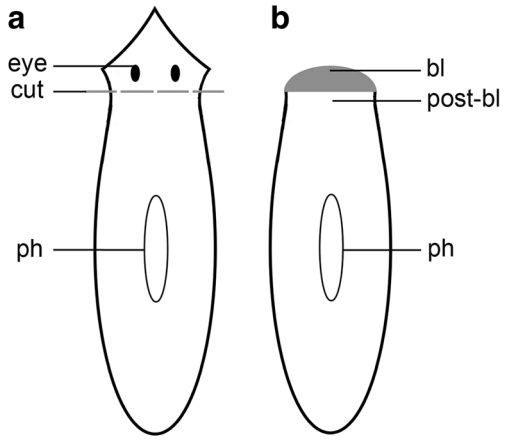

Fig. 1 Decapitation scheme of planarians *G. tigrina*: eye - eyes, cut-line of transection, ph - pharynx, bl - blastema, post-bl - post blastema area

total of 750 planarian specimens took part in the experiments. The preparation was introduced into the glasses with water following the process of decapitation. All procedures were carried out in at least three replications for each concentration level. For each of the determined values, the result was averaged over 30 animals in the experiment (n = 30) and control (n = 30) groups.

G. tigrina planarians undergo regeneration by developing a regeneration bud (blastema). The blastemal growth was estimated by the method of vital computer morphometry [8–10] based on registration of a photocontrast between old (pigmented) and novel (without pigment) body parts. The area of the whole body (S) and blastema (s) was measured with the help of the obtained images. The regeneration rate was calculated by the following formula: R = s/S (where R is the regeneration index, which is an indirect indicator of cell proliferation. The effect was estimated as a difference (%) between values of the regeneration index (R) in the control and experimental variants [11]. The photo registration of blastemal formation were obtained using a Carl Zeiss.V12 stereo microscope. To determine the total body area of the planarians and the area of the blastema, the Carl Zeiss Zenblue edition program was used. The video recording of the creeping planaria was performed in the maximally straightened state. Then, we selected one frame, and the error in the repeated determination of the area should be less than 1%.

Statistical processing of the data was carried out with the help of the Past Ver. 3.11 (Paleontological Statistics) software package. The significance of differences in the regeneration index between the experimental and control groups was determined by the method of multiple comparisons (Kruskall-Wallis H-test), as well as by the method of pairwise comparisons (Mann–Whitney U test).

The investigation was divided into two stages: studying the effect of *preparation 1* alone and when mixed with sodium carbonate buffer (Na2CO3) [12] to reduce the acidity of the preparation up to the neutral value (pH = 7.0).

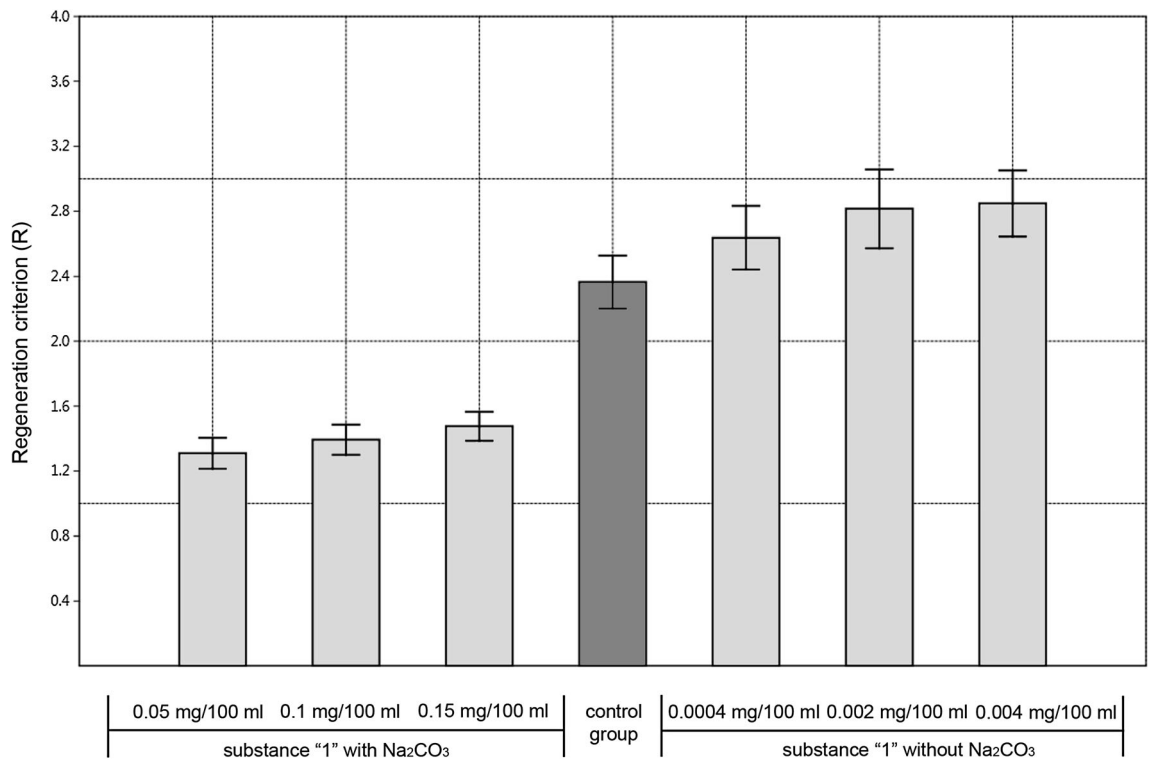

Fig. 2 Regeneration index for various concentrations of preparation 1 (mean value ± standard error)

Table 1 The comparison of the regeneration index for planarians at different concentration levels of preparation 1 + Na2CO3 and in the control group, based on the Mann-Whitney *U* test. Above the diagonal line—the index value, below the diagonal line—the level of significance

	0.05 mg/100 ml	0.1 mg/100 ml	0.15 mg/100 ml	control group
0.05 mg/100 ml		150	111	67
0.1 mg/100 ml	0.38840		128	70
0.15 mg/100 ml	0.07458	0.41870		77
Control group	> 0.001	> 0.001	> 0.001	

3 Results and Discussion

The first stage of the investigation was to find the concentration (CL50) of *preparation 1* that is lethal for *G. tigrina* planarians. For all stage of the investigation of lethal concentration, we analyzed the same levels of *preparation 1* and *preparation 1* + Na2CO3 as studied previously for Xymedon [2]. For this purpose, the following concentrations of *preparation 1* were analyzed: 0.3, 0.1, 0.04, 0.003, and 0.001 mg/100 ml. The concentrations of 0.3, 0.1, and 0.04 mg/100 ml turned out to be lethal for planarians. All planarians survived at the concentrations of 0.003 and 0.001 mg/100 ml.

To determine the lethal concentration of *preparation 1* mixed with sodium carbonate buffer (Na2CO3), we analyzed the concentrations: 0.1, 0.2, and 0.3 mg/100 ml. The concentration of 0.3 mg/100 mL turned out to be lethal for *G. tigrina* planarians. As mentioned above, sodium carbonate (Na2CO3) was used to reduce the acidity of *preparation 1*. The control group was represented by planarians that were decapitated and kept in clean water afterwards (Fig. 1).

Experiment 1: *Preparation 1* The following concentrations of *preparation 1* were used: 0.002 mg per 100 ml, 0.004 mg per 100 ml, 0.0004 mg per 100 ml (Fig. 2). It was demonstrated with the help of the method of multiple comparisons that there are no significant differences between the experimental and control groups ($H = 2.927$, $p = 0.4$).

The concentration levels of *preparation 1* at this stage of the experiment were low (CL50 based on the results of the analysis). According to the regeneration index, they were almost equal to those in the control group. We associate this with an increased acidity of the solution due to the presence of ascorbic acid in the compound. Notably, similar results (unpublished data) were obtained in the course of analogous studies with the use of cell cultures.

Experiment 2: *Preparation 1* + Sodium Carbonate Na2CO3 To estimate the effect of *preparation 1* + Na2CO3, the following concentrations were used: 0.15, 0.1, and 0.05 mg/100 ml (Fig. 2). The analysis by the method of multiple comparisons proved significant differences ($H = 30.45$, $p < 0.001$). The pairwise comparison (U) of the regeneration index values revealed significant differences between various concentrations of preparation 29D + Na2CO3 and the control group (Table 1). Therefore, *preparation 1* with sodium carbonate inhibits the process of regeneration in planarians.

4 Conclusions

The previous studies proved that *preparation 1* (a co-crystal of Xymedon with ascorbic acid) is a more effective hepatoprotector [4] than Xymedon. This study analyzes the effect of the new *preparation 1* on alternative model objects in the study of regeneration. It was found that *preparation 1* at low concentration levels (0.002, 0.004, and 0.0004 mg/100 mL) does not influence the regeneration of *G. tigrina* planarians. When *preparation 1* with Na2CO3 buffer is used, the process of planarian regeneration is inhibited.

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The images of the planarians were obtained using microscopes of Interdisciplinary Center for Analytical Microscopy of Kazan Federal University.

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