# Delayed Effects of Daphnia Intoxication with Selective and Nonselective Inhibitors of Acetylcholinesterase

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Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 139, No. 1, pp. 77-78, January, 2005
Original article submitted February 25, 2004

Chronic experiments on successive generations of laboratory *Daphnia magna* culture demonstrated higher (compared to proserin) embryotoxicity of a new selective acetylcholinesterase inhibitor 1,3-bis[5-(diethyl-o-nitrobenzilammonio)pentyl]-6-methyluracyl dibromide (compound No. 547). The concentrations of proserin (neostigmine) and compound No. 547 not exceeding $^1/_{60}$ $LC_{50}$ (0.39 μmol/liter for compound No. 547 and 0.045 μmol/liter for proserin) were absolutely safe for the reproductive function of daphnia.

**Key Words:** *Daphnia; embryotoxicity; selective acetylcholinesterase inhibitor; ammonium uracyls*

Effects of long-term exposure to low concentrations of newly synthesized substances is an important problem of experimental biology. Cholinesterase inhibitors of organophosphorus and carbamate origin are toxic in extremely low doses, while the toxicity of onium inhibitors is lower for invertebrates [2]. A common drawback of cholinesterase inhibitors limiting their clinical use is narrow interval of their therapeutic (myoparalytical) effect ("pharmacological safety" calculated as $LD_{50}/ED_{50} \leq 3$), indicating that their effect on locomotor muscles is virtually nonselective.

We previously found a new class of highly selective and irreversible inhibitors of acetylcholinesterase (ACE; EC 3.1.1.7; $k^0=7.6\times10^8$-$3.5\times10^9$ $M^{-1}$ $min^{-1}$) among onium uracil derivatives [3] with $LD_{50}/ED_{50}>10$ [1].

Here we compared the toxicities of compound No. 547 (C-547), a selective ACE inhibitor, and proserin (neostigmine) under conditions of chronic experiment on *Daphnia magna*, an international test object of ecotoxicology.

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## MATERIALS AND METHODS

Effects of aqueous solutions of C-547 (1,3-bis[5-(diethyl-o-nitrobenzilammonio)pentyl]-6-methyluracyl dibromide) and proserin methylsulfate on the reproductive function of laboratory daphnia culture in 4 generations ($P\text{-}F_1\text{-}F_2\text{-}F_3$) was studied under standard conditions [4]. Toxic effects of quotes of 24-hour lethal concentration ($LC_{50}$) of C-547 (2.33 ($^1/_{10}$), 0.78 ($^1/_{30}$), and 0.39 μmol/liter ($^1/_{60}$)) and of proserin methylsulfate (0.27 ($^1/_{10}$, 0.09 ($^1/_{30}$), and 0.045 μmol/liter ($^1/_{60}$)) were studied. Each concentration was tested on 10 daphnia.

Experiments were carried out under conditions of single (parental generation; P) and chronic (generations $P\text{-}F_1\text{-}F_2\text{-}F_3$) poisoning. The duration of exposure of each generation was 21 days.

Survival, fertility, time of the first throw out, and quality of offspring served as the criteria of toxicity.

## RESULTS

Previous experiments showed 9-fold lower acute toxicity of C-547 for daphnia compared to proserin: $LC_{50}$=23.31 (17.61-28.32) and 2.72 (2.14-3.43) μmol/liter, respectively [1]. However, chronic experiment

0007-4888/05/1391-0068 

**TABLE 1.** Fertility (Day 21; per female) under Conditions of Single and Repeated Poisoning with C-547 and Proserin (*M*±*m*)

| Generation | Concentration, challenge mode | | | | | |
|---|---|---|---|---|---|---|
| | $^1/_{10}$ $LC_{50}$ | | $^1/_{30}$ $LC_{50}$ | | $^1/_{60}$ $LC_{50}$ | |
| | single | repeated | single | repeated | single | repeated |
| C-547 | | | | | | |
| P | 82.0±8.7 | 82.0±8.7 | 68.8±6.5 | 68.8±6.5 | 74.8±8.2 | 74.8±8.2 |
| $F_1$ | 25.3±3.5* | 17.6±1.7* | 28.4±4.1* | 14.7±127*+ | 69.8±5.8 | 68.8±6.5 |
| $F_2$ | 28.7±2.7* | 0.0*+ | 34.7±3.8* | 0.0*+ | 66.8±4.6 | 65.8±5.9 |
| $F_3$ | 43.7±5.4* | 0.0*+ | 47.4±4.9* | 0.0*+ | 78.2±5.6 | 73.1±7.3 |
| Proserin | | | | | | |
| P | 74.1±14.4 | 74.1±14.4 | 68.1±6.9 | 68.1±6.9 | 78.4±9.3 | 78.4±9.3 |
| $F_1$ | 38.1±5.7* | 36.9±6.8* | 35.2±4.3* | 35.7±5.4* | 77.8±7.8 | 73.7±8.5 |
| $F_2$ | 37.9±6.3* | 35.5±4.7* | 36.3±4.6* | 36.6±5.0* | 76.9±7.6 | 69.7±6.9 |
| $F_3$ | 79.9±11.4 | 24.9±3.4*+ | 84.1±7.7 | 65.0±6.4 | 79.3±8.6 | 75.1±7.7 |

**Note.** Fertility of daphnia in the control: 75.8±10.4 (P), 71.7±10.7 ($F_1$), 70.6±6.9 ($F_2$), and 80.9±11.4 per female ($F_3$). $p<0.05$ compared to *control, +single poisoning.

demonstrated higher toxicity of C-547 in comparison with proserin. In generation P the survival rate on day 21 under conditions of single and repeated poisoning with C-547 ($^1/_{10}$, $^1/_{30}$ $LC_{50}$) did not differ from the control, while in generations $F_1$-$F_2$ 100% death of daphnia was observed on day 21 (there was no $F_3$). Proserin in equieffective concentrations was not toxic for P-$F_1$-$F_2$-$F_3$ generations, the survival rate not differed from the control. C-547 and proserin in a concentration of $^1/_{60}$ $LC_{50}$ lost chronic embryotoxicity (no mortality, abortive eggs, or stillborn daphnia).

Under conditions of single poisoning with $^1/_{10}$ and $^1/_{30}$ $LC_{50}$ fertility of survivors in generations $F_1$ and $F_2$ was impaired by both proserin and C-547 (Table 1). Under conditions of permanent exposure to C-547 ($^1/_{10}$ and $^1/_{30}$ $LC_{50}$) fertility suppression was more pronounced (no $F_2$ and $F_3$) than under conditions of proserin poisoning. The fertility of daphnia was restored in generation $F_3$ after single and repeated challenge with proserin ($^1/_{30}$ $LC_{50}$), but not with C-547 ($^1/_{30}$ $LC_{50}$).

On the other hand, despite the suppressive effect of C-547 on fertility, the time of the first throw out of young daphnia in surviving generations was retained at the level of the control (days 6-7). No cases with deformities of carapaces, antennas, and antennules in young $F_1$-$F_2$-$F_3$ daphnia caused by C-547 or proserin were detected.

Hence, C-547 and proserin in concentrations of $^1/_{10}$ and $^1/_{30}$ $LC_{50}$ exhibited embryotoxicity under conditions of 21-day chronic experiment. The concentrations of C-547 and proserin equal to $^1/_{60}$ $LC_{50}$ (0.390 and 0.045 μmol/liter, respectively) were safe for the reproductive function of daphnia.

The study was supported by the Russian Foundation for Basic Research (region; grant No. 03-04-96215), NIOKR AN RT Foundation (grant No. 03-3.8-209), and Program of the President of the Russian Federation “Leading Scientific Schools” (grant No. NSh-1063.2003.4).

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