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CHRONOTOXICITY OF CYCLOPHOSPHAMIDE UNDER DIFFERENT CONDITIONS OF LIGHT AND DARKNESS

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UDC 615.277.3.099"52"

KEY WORDS: chronotoxicity; biological rhythms; cyclophosphamide.

Various substances, including chemotherapeutic preparations, differ in their therapeutic and toxic action when administered at different times of the 24-h period [1, 5]. Data have been published to show that the toxicity of cyclophosphamide varies at different times of day and night [6, 8-11, 14]. Mice are known to be most resistant to sub- and superlethal doses of cyclophosphamide at midnight and 6 a.m. [2-4]. During desynchronosis, caused by keeping animals in continuous darkness or, in particular, in continuous daylight, the character of the circadian rhythm of toxicity may vary, and in some cases it may flatten out [7, 12, 13].

This paper describes the study of the chronotoxicity of cyclophosphamide during the 24-h period in animals kept under conditions of natural alternation of daylight and darkness or in continuous illumination.

EXPERIMENTAL METHOD

Two series of experiments were carried out in which mature noninbred male albino mice weighing 21-26 g were used. In series I 360 mice were divided into four groups, with 90 animals in each group, and were kept under conditions of natural light and darkness and with free access to food and drink. In series II, 144 mice also were divided into four groups with 36 animals in each group and kept for 14 days under isolation in a closed (with no windows) but well ventilated room, in continuous artificial light (average about 60 lx) and with food and water *ad lib*. During the first days when animals of this series were kept under conditions of continuous daylight, they showed increased excitability, and some mice had bites on their tail, in agreement with observations made by other workers [12] on animals kept in the same way. Cyclophosphamide (USSR origin), in a dose of 900 mg/kg body weight, calculated individually for each mouse, was injected intraperitoneally 4 times a day at 6 p.m., midnight, 6 a.m., and noon. Death of the animals was recorded every 30 min during the 24-h period. Toxic effects of cyclophosphamide were determined by observing the survival rate of the experimental animals. The results were subjected to statistical analysis by the chi-square test.

EXPERIMENTAL RESULTS

In series I the animals died 4 h after injection of cyclophosphamide. As will be clear from Fig. 1, at all times of the investigation more animals survived when the drug was injected at midnight and 6 a.m. than when it was injected at 6 p.m. and noon. The differences became significant ($P < 0.05$) 7 h after injection, between injections at midnight (82% of mice survived), at 6 p.m. (67%), and at noon (55%). At later times of observation the differences still remained, and after 24 h their significance after injections at midnight (22%) and 6 a.m. (21%) compared with injection at 6 p.m. (10%) was real or close to reality ($P \leqslant 0.05$). Later this tendency continued. Consequently, mice become chronoresistant to the toxic action of cyclophosphamide and reach their peak of resistance at midnight and 6 a.m., with a minimum at 6 p.m. These results agree with data in the literature [2-4]. However, they do not confirm the results of investigations [8, 10] in which the maximum and minimum of toxicity of cyclophosphamide were observed at different times of the 24-h period.

Department of Biology, N. A. Semashko Moscow Medical Stomatologic Institute. (Presented by Academician of the Academy of Medical Sciences of the USSR F. I. Komarov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 100, No. 10, pp. 483-485, October, 1985. Original article submitted December 21, 1984.

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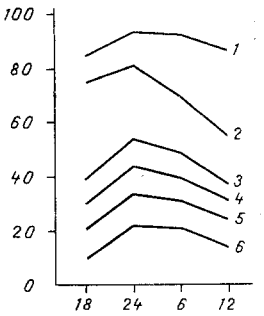

Fig. 1

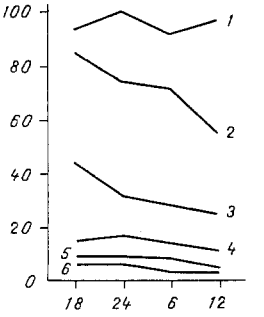

Fig. 2

Fig. 1. Circadian rhythm of toxicity in animals during natural alternation of daylight and darkness. Abscissa, time of injection of cyclophosphamide; ordinate, percent of surviving animals. 1-6) 6, 7, 9, 10, 12, and 24 h respectively after injection.

Fig. 2. Toxicity of cyclophosphamide in animals kept in continuous daylight. 1-6) 2, 3, 4, 7, and 8 h respectively after injection. Remainder of legend as to Fig. 1.

Our results are not in full agreement with certain other data [6, 11]. One reason for this disagreement may be that in the investigations cited above the animals were kept under conditions of fixed artificial illumination (12 h of daylight, 12 of darkness). Nevertheless, our data agree fully with the results of investigations [14, 15] on the embryotoxicity of cyclophosphamide, in which the maximal number of surviving fetuses (which are known to have the maternal biological rhythms) occurred at midnight and 2 a.m., and the minimal number at 9 a.m. to 6 p.m. In these experiments the animals also were kept under natural conditions of alternation of daylight and darkness. Our data also correlate well with the results of a study of the toxicity of a compound similar to cyclophosphamide, namely ifosfamide [15], according to which the number of surviving mice reached a maximum at 5 a.m. and a minimum at 1 and 5 p.m.

In the experiments of series II the animals died as early as 30 min after injection of cyclophosphamide. As Fig. 2 shows, the character of the graphs showing the percentage of surviving animals differed from the experiments of series I. A significantly higher ($P < 0.05$) rate of survival of the animals was observed 3 h after injection of the drug at 6 p.m. (85%) than after its injection at noon (55%). After 4 h the character of the curve was very similar: after injection at 6 p.m, there were 44%, and after injection at noon 25% of surviving animals; however, these differences are not significant ($P > 0.05$). During later observations the curves tended to equalize and differences at the corresponding points were not significant. The largest number of mice died, incidentally, between 2 h 30 min and 4 h 30 min after injection of cyclophosphamide: in the case of injection at 6 p.m. 73.5% of the animals died during this interval, 80% at midnight, 75% at 6 a.m., and 72.2% at noon, i.e., the percentage of animals dying during this period was very similar and did not differ statistically significantly. After injection at 6 p.m. 100% mortality among the animals was observed after 11 h, 97.1% of animals died 13 h after injection at midnight, and the remaining two mice died after 19 h; 100% mortality was observed 8 h 30 min after injection at 6 a.m. and 11 h after injection at noon. The numerical differences are not significant. It can be concluded from these results that, starting with the 4th hour of observation after injection of the drug, mortality of the animals at the various times was relatively uniform in character.

Comparison of the results of the experiments of series I and II shows that the circadian rhythm of cyclophosphamide toxicity observable in animals kept under conditions of natural alternation of daylight and darkness was reversed in experiments with continuous illumination during the first few hours of observation, but later was leveled out. These results confirm the view that the conditions of illumination are one of the leading factors synchronizing biological rhythms in animals. When this "time detector" is blocked, desynchronosis develops, as is confirmed not only by disappearance of the rhythm of cyclophosphamide

chronotoxicity, but also by a fall in the animal's general resistance to its action, reflected in earlier death of the experimental animals.

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