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Chronotoxicological studies on dichlorphos in mice and humans

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Abstract—Chronotoxicological studies were performed with dichlorphos (DDVP) and its timing toxic effects on mice and humans. The circadian rhythms were revealed in the blood cholinesterase (ChE) activity in intact mice and normal persons, as well as in mice mortality treated at different daily times. The inverse relationship of the two rhythm suggested that the risk of exposure to DDVP might be much higher at evening hours than in other clocks of the day, and the disappearance of the ChE rhythms in DDVP-treated mice and DDVP-exposed workers implicated a disturbing effect of DDVP on the maintenance and regulation of the rhythmic mechanisms.

Keywords: circadian rhythm; cholinesterase; chronotoxicity; dichlorphos.

INTRODUCTION

A daily rhythm in the susceptibility of insects to certain pesticides has been known for some years (Polcik, 1964; Gosselink, 1967). The pest mortality produced by the pesticides was intimately related to the time point of day at which they were applied. In one experiment, the same dose of an organophosphorus pesticide, methylparathion, killed approximately 10 percent of the adult boll weevils treated at dawn but about 90 percent of those treated only 3 hours later (Cole, 1964). It would seem feasible to administer the pesticide in a regime which allowed it to exert the maximum killing effect and to make economy in amount of cost if application was correctly timed.

On the other hand, pesticides have been known as an important occupational and environmental hazard for human and animal health (Gupta, 1985; IPCS, 1986), and some of them, like dichlorphos and trichlorphon, were found to be chronopathogenic in animal studies (Nicolau, 1984). But whether exposure to these pesticides at different times of a day may lead to different biological responses in the organisms, either quantitatively or qualitatively, has received little attention so far.

The objective of this study was to determine the 24 hours variation in DDVP toxicity in mice and to reveal the circadian susceptibility-resistance cycle in humans so as to provide grounds for labour protection and data for further environmental studies.

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MATERIALS AND METHODS

materials

DDVP was provided by the Nanjing Electrochemical Plant, with a molecular weight of 220.98, specific gravity of 1.415 and purity of 80%. It was diluted with sesame oil before use. Dithio bis-nitrobenzonic acid (DTNB) from Zhejiang Academic Institute of Medicine. BALB/c mice from Animal Center at Nanjing Medical College.

Animal treatment

Male BALB/c mice of 18–20g were standardized with electric lamp light from 06:00 to 18:00 and darkness from 18:00 to 06:00, for at least sevendays, room temperature 20±2°C. Food and water were ad libitum. DDVP was given to mice orally at 6-hr intervals (02:00, 08:00, 14:00, 20:00) in concentration of 2/3 LD_{50} dosage (the preliminary trials found the LD_{50} to be 103 mg/kg). The mice were divided into groups with each of 20, and the number of deaths resulting within the initial 7 days was obtained.

Before the DDVP treatment, a group of 10 mice were sampled with each taken 10 μl of tail blood every 5 hours consecutively during the 24-hr period over 3 days for the determination of ChE activity. A modified Ch/DTNB procedure was applied according to Voss and Sachsse (Voss, 1970). The determination was repeated after the mice were to be taken DDVP for one week.

Human investigation

57 DDVP-exposed workers in the Najing Electrochemical Plant were observed, by being inquired about the exposure standing, clinical complaints and previous recordings of poisoning. The concentration of DDVP in the air of the workshop was measured. 30 of 57 workers were chosen as the observation group (15 male and 15 female), and 20 persons of non-exposure as the control group comparable for the variable of age and sex. Each person received a blood sampling from his or her finger (10μg) to have the ChE activity determined at the time points of 09:00, 13:00, 17:00 and 21:00 during a 24-hr span.

Statistical analysis

The chi-square test was used to determine the significance between the lowest and highest mortalities or ChE activities of different hours. Moreover, all data were fitted to a 24-hr cosine curve (Halberg, 1972). The single cosinor technique involves both an analytical method and a graphic presentation of the results. Estimates of mesor (a rhythm adjusted mean), amplitude (a measure of extent of predictable rhythmic changes), and acrophase (a measure of the rhythm's timing), with their statistical uncertainties, are produced with the help of a Zijin II computer system.

RESULTS

Chronotoxicity of DDVP to mice

In mice taken 2/3 LD_{50} of DDVP, mortalities recorded for different subgroups ranged from 20% to 55%, corresponding to different daily administration times. The difference between the peak and nadir mortalities was 2.75 times as much, with statistical significance at $P < 0.05$. The timing of the maximum mortality occurred in mice group treated at 20:00, and the minimum mortality was observed at 08:00. By applying the single cosinor method to the data, a mortality rhythm was presented with a circadian oscillatory pattern (Fig.1). The parameters and the confident regions performed by the computer showed the acrophase of peak death time at −283 (240—325), the mesor of 31.25±0.18, and the amplitude of 18.15±0.58 (Fig. 2).

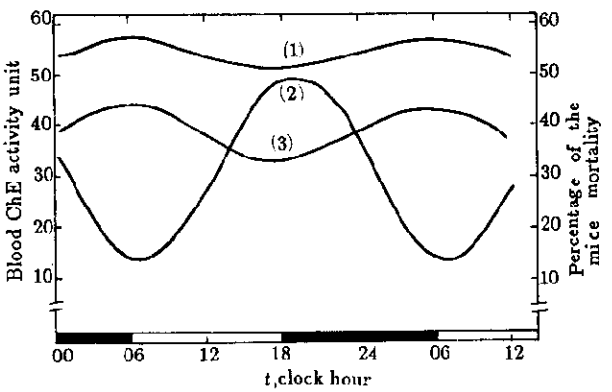

Fig. 1. Single consine curves fitting to data of blood ChE in normal persons (1) and mice (3), and mice mortality after DDVP treatment (2)

Circadian rhythm for mice blood ChE

The determination in the control group of intact mice showed a definite daily rhythm in the blood ChE activity. Under the standardized 12-hr photoperiod, the maximum value appeared at 03:00, while the minimum, at 18:00 (Table 1). When the data were pooled, there was a statistical fit ($P < 0.05$) to a 24-hr cosine curve. The acrophase of peak ChE activity time was -82 (67–98), the mesor was 37.37±0.10 and the amplitude was 5.35±0.24 (Fig. 2). Thus the data revealed a diurnal variation of the blood ChE in intact mice. For mice received DDVP, it was something different. In addition to the significantly lower ChE activities determined at each timepoint than those before the treatment, the DDVP-poisoned mice showed a non-rhythmic shift for their blood ChE in a 24-hr period. As shown in Table 1, the difference among each measuring value from the treated mice was obviously smaller compared with that of the intact mice.

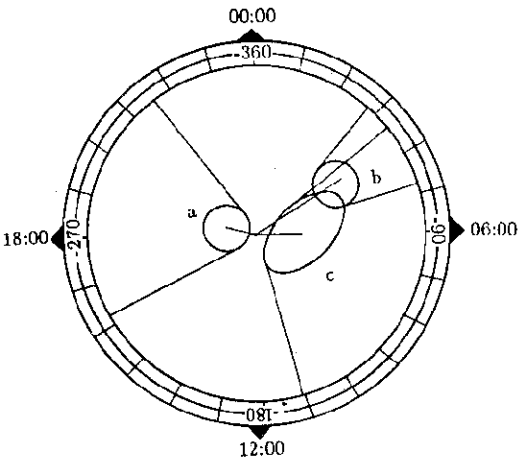

Fig. 2 Confident regions of circadian rhythms for mice mortality (a), blood ChE in intact mice (b) and normal persons (c).

Key to ellipses	N	PR	P	Mesor $\pm SE$	Amplitude $\pm SE$	Acrophase $\pm SE$
a	80	40	<0.05	31.25	18.15	−283
				±0.18	±0.58	240 325
b	40	46	<0.05	37.37	5.35	−82
				±0.10	±0.24	67 98
c	20	49	<0.05	52.99	2.55	−87
				±0.14	±0.70	47 158

Circadian rhythm for human blood ChE

The activities of blood ChE in DDVP-exposed workers were significantly low, ranging from 37 to 39 activity units (Table 1). This was not surprising because the DDVP concentration in the workshop air was so high that the maximum value measured at the working site exceeded the Chinese National Criteria by 25 times (7.6 mg/m^3). The cosinor fitting analysis resulted in a non-cosinusoidal waveform of the data from the workers. However, there was a statistical significant fit (P <0.05) for the 24-hr ChE activity in normal persons of the control group, with the mesor of 52.99±0.14, the acrophase at −87 (47–158), and the amplitude of 2.55±0.70 (Fig.

1, 2). It seemed that the circadian rhythm of blood ChE in normal people disappeared in the DDVP-exposed workers.

Table 1 Circadian determinations for blood ChE activity in mice and persons

		Time of day, h				
	N	03:00	08:00	13:00	18:00	23:00
DDVP-exposed mice	10	21±2.21*	20±1.58*	27 ±1.26*	19±0.95*	21±2.10*
Control group	10	44±0.95	37 ±0.95	39±1.26	27±0.95	39±0.95
		09:00	13:00	17:00	21:00	
DDVP-exposed workers	30	37±1.50**	39±1.27**	38±1.37**	37±1.52**	
Control group	20	55±1.65	52±1.18	51±1.10	51±1.47	

Note: Values are $X \pm SE$ of ChE activity units.
* Significantly different from control (P <0.05).
** Significantly different from control (P < 0.01).

DISCUSSION

The finding of the daily variation in DDVP toxicity, as indexed by mice mortality, indicated that the resistance of mice to DDVP varied considerably as a function of administration times. Under the experimental conditions, the maximum, mortality of mice occurred in the group poisoned at 20:00 hr, reflecting the comparatively higher susceptibility to DDVP around this hour. On other clocks of the day, the mice were more resistant by presenting the less death numbers. The daily rhythmic toxicity caused by drugs or environmental toxicants has been reported by some authors in recent years (Walker, 1981; Mitch, 1982). It is evident from the present study that the DDVP chronotoxicity can take place in insects as well as in mammalians.

The light-dark cycle of the environment has been considered as one of the strong synchronizers of rhythmic patterns in various functions of rodents (Philippens, 1976; Zatz, 1979). Some drugs were found to produce a maximum toxicity at the start of dark period (Friedman, 1972; Gall, 1977). For the moment it could be seen from the data that the maximum mortality in mice appeared soon after the beginning of darkness, and kept high during the first part of the dark. It is reasonable, therefore, to conclude that the DDVP toxicity to mice is not only

time-dependent but lightrelated as well.

But what happens during the first part of the dark? This is a time when many things are going on in rodents. For example, concentration of neurotransmitter acetylcholine (Ach) in central nervous system is low (Saito, 1975), some hormones and glucose level in serum are high (Takahashi, 1982), and the animals are beginning their daily activity and feeding. With respect to ChE, several studies were carried out and a diurnal fluctuation was reported with a nadir in the middle of the dark phase (Von Mayersbach, 1974; Owasoyo, 1980).

It was of interest to examine the difference between rhythms of the mice mortality and the ChE activity. When the two rhythms were put together, an inverse relationship emerged, that is, the time of maximum mice mortality coincided with that of nadir of the blood ChE activity, and vice versa (Fig. 1). This coincidence strongly implicated that the daily rhythm of DDVP toxicity might result from or at least depend on the circadian variation of ChE activity in the body.

Another interesting coincidence occurred in rhythms of mice and human blood ChE. Both rhythms presented approximately the same peak and nadir times. Considering the inverse relationship between DDVP toxicity and blood ChE activity found in mice, it can be inferable that at the time of maximum mice mortality, the organisms are in the state of most vulnerable to DDVP toxicity. Upon this basis, we suggest that people exposed to DDVP should pay more attention to exposure around evening hours since the risk of DDVP intoxication may be much higher during this time period than in other clocks of the day.

When combined this result with previous data on daily rhythmic susceptibility of insects to DDVP, a desirable spraying schedule should be worked out. Take the boll weevils as example. The insect showed a series of susceptibility peaks recurred about 6 hours at 03:00, 09:00, 15:00 and 21:00 in a 24-hr day (Cole, 1964). It would be optimal, thereby, for both the high insect mortality and the less risk of human intoxication if the working time around 09:00 should be preferred.

The disappearance of the circadian rhythm of blood ChE in DDVP-exposed mice and workers seems to reflect a disturbing effect of DDVP on the maintenance and regulation of the rhythm. However, the cause for this effect is not known. Because DDVP is a powerful inhibitor of the ChE, perhaps the sharply lowering of the levels of the enzyme may conceal its oscillation in a great degree, but disorders in the biological time structure, or disfunctions in ChE biosynthesis and catabolism may also be important factors.

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