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EVALUATION OF INHIBITORY EFFECT OF DIPHENHYDRAMINE ON BENZODIAZEPINE DEPENDENCE IN RATS

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Abstract : Effect of diphenhydramine was investigated on withdrawal signs in lorazepam dependent rats. Physical dependence was produced by giving lorazepam admixed with the food in the following dose schedule: 10×4, 20×4, 40×4, 80×4 and 120×7 (mg/kg, daily x days). The parameters observed during the periods of administration of lorazepam and after its withdrawal were spontaneous locomotor activity (SLA), body temperature, reaction time to pain, foot shock aggression (FSA) and audiogenic seizures. Diphenhydramine was administered orally in the dose schedules of once daily (10, 20 and 40 mg/kg) and twice daily (5,10 and 20 mg/kg) in separate groups during the withdrawal period. The withdrawal signs observed in control group (without diphenhydramine) were hyperkinesia, hyperthermia, hyperaggression and audiognic seizures. Hyperkinesia and hyperthermia were blocked in all the groups of diphenhydramine–treated rats. FSA was inhibited only by diphenhydramine (10 and 20 mg/kg) given twice daily. Audiogenic seizures were completely blocked by once daily (20 and 40 mg/kg) as well as twice daily (20 mg/kg) doses of diphenhydramine. It may be concluded that diphenhydramine exerts a protective effects on benzodiazepine withdrawal syndrome.

Key words: physical dependence, rats, benzodiazepine, diphenhydramine, withdrawal syndrome

INTRODUCTION

The physical dependence on benzodiazepines, the most frequently prescribed group of anti-anxiety drugs, is now well–known. Several reports show that benzodiazepines produce physical dependence in man (1-3), and in different species of animals (monkey, dog and rodent) (4, 5). Most of the studies were confined to the development of physical dependence characterized by withdrawal syndrome. The important aspect of drug treatment of the withdrawal syndrome of benzodiazepines has not been looked into. We initiated studies in this direction with diphenhydramine because it was observed in our earlier study that the withdrawal syndrome of methaqualone was inhibited by diphenhydramine administered during the abstinence period (6). To explore the therapeutic potential of diphenhydramine in the treatment of benzodiazepine abstinence syndrome, the effect of different doses of oral diphenhydramine was evaluated on the withdrawal signs of lorazepam in the rats.

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METHODS

The study was conducted on albino rats of either sex (weighing 80-100 g). Each rat was fed daily with a diet comprising gram flour (9 g), milk powder (0.9 g) and glucose (0.1 g) mixed with 20 ml of water in deep containers to avoid spillage. It has been our observation that 20 ml of this diet is more than daily requirement. Water was available *ad libitum*.

Development of physical dependence on lorazepam :

In the present study, lorazepam was administered daily after uniformly mixing with the food as described by Yanura et al (7) for 23 days. The dose schedule of lorazepam used was 10x4, 20x4, 40x4, 80x4 and 120x7 (mg/kg, daily x days) (8). The average amounts of lorazepam consumed, calculated on the basis of daily food intake were: 8.1x4, 18.4x4, 32.9x4, 67.6x4 and 94.2x7 (mg/kg, daily x days). The withdrawal syndrome was observed after cessation of lorzepam administration. During the withdrawal period the rats were divided into groups of 10 each. One group did not receive diphenhydramine and served as control-withdrawal. The other groups received diphenhydramine daily by oral route during withdrawal period i.e. upto 10 days. On the basis of daily frequency of administration, there were two sets of diphenhydramine treated animals - (I) once daily (10, 20 and 40 mg/kg, p.o.), and (II) twice daily (5,10 and 20 mg/kg, p.o.). Each dose in both the sets was given to a separate group.

Following responses were observed prior to lorazepam administration (control-untreated) and during the periods of administration and withdrawal of lorazepam.

1. Spontaneous locomotor activity (SLA): SLA was recorded by photoactometer (9). The activity was counted for 5 min. after a period of 2 min acclimatization in each rat.

2. Pain response: The tail clip method was employed to study pain response (10). The time interval between the application of clip to tail and first biting of the clip by the rats was taken as reaction-time to pain. The rats which had initial reaction-time of more then 15 sec were excluded from the study. The cut off time was 60 sec in test groups.

3. Body temperature: The temperature was recorded by inserting the tip of thermometer into the rectum of rat for a period of 1 min.

4. Foot shock aggression (FSA): Aggression was induced by electric foot shock (2 mA; 5 shocks/sec) by Aggressometer to pairs of rats according to the method of Tedeschi et al (11). The paired rats were kept in close proximity to each other. The number of fighting bouts in upright posture were counted for a period of 1 min.

5. Audiogenic seizures: Audiogenic stimuli was given with the help of electric door bell fixed in a metal chamber for 30 sec to elicit the seizures (12).

Body weight and food intake were recorded daily in all the groups.

The drugs used were lorazepam (India, Cipla) and diphenhydramine (India, Park-Davis).

Significance of difference between the groups was determined by ANOVA (two way analysis of variance) and Student's 't' test for SLA, reaction time to pain, body temperature and FSA, and Chi-square test for audiogenic seizures.

RESULTS

(a) Lorazepam administration period: On the last day (i.e. day 23) of lorazepam administration count for SLA and FSA were

22±3.7 and 2±0.4 respectively, which were significantly lower then to those found on day 0. Reaction time to pain and body temperature were not affected significantly. Audiogenic seizures did not appear in any rat.

(b) Lorazepam withdrawal period: The maximal changes on significantly affected parameters during the withdrawal period in control and diphenhydramine treated groups are shown in Table I.

The results on the different parameters are:

(i) Spontaneous locomotor activity (SLA): There was significant difference in the SLA count between control-withdrawal and diphenhydramine groups. The control-withdrawal group showed 87% increase in SLA (hyperkinesia) from the SLA count of control-untreated while there was decrease in SLA (hyperkinesia) in diphenhydramine treated rats once daily (30-69%) as well as twice daily (33-47%) in comparison to control-untreated.

TABLE I: The effects of different dose schedules of diphenhydramine on withdrawal signs : hyperkinesia, hyperthermia, hyper-aggression and audiogenic seizures of lorazepam.

Groups	SLA count Mean±SE	Body temp C^0 Mean±SE	FSA count Mean±SE	Audiogenic seizures incidence %	Duration (days)
1. Control-untreated	64±4.8	37.6±0.3	4±0.6	0	-
2. Control-withdrawal	120±7.2@	39.3±0.2@	7±0.9@	30@	9
3. Diphenhydramine treated (mg/kg, p.o.)					
Once daily					
10	45±5.8@*	37.7±0.3*	8±1.1@	20@	3
20	33±4.5@*	38.1±0.2*	10±1.3@	0*	-
40	20±3.9@*	37.9±0.3@*	9±0.8@	0*	-
Twice daily					
5	81±7.1*	38.1±0.3*	7±0.7@	30@	7
10	43±4.9@*	37.8±0.2*	2±0.3@	20@	6
20	34±2.8@*	37.6±0.4*	2±0.1@*	0*	-

@ = P <0.01 as compared with control–untreated.
* = P <0.01 as compared with control–withdrawal.
The significance of difference between the means of two groups was determined by Student's t test.
SLA : spontaneous locomotor activity
FSA : foot shock aggression
Data in the table show the maximal effect.

The maximal effects (peak withdrawal) appeared on days 2-4 and then gradually declined to level of control-untreated within 10 days. The ANOVA test showed that variations between the control-untreated, control-withdrawal and diphenhydramine treated groups for SLA, body temperature and FSA were highly significant ($F = 8.2 - 69.4$; $P < 0.05 - 0.001$) while there was no significant variation among animals ($F = 0.73$, $P > 0.05$).

(ii) Body temperature: The body temperature was significantly different in control and diphenhydramine groups. A significant rise in temperature (hyperthermia) was observed in control-withdrawal group. The maximal rise in the temperature was 1.7°C. The body temperature did not rise in rats treated with

diphenhydramine either once or twice daily schedules.

(iii) Foot shock aggression (FSA): The control showed 75% increase in fighting counts (hyperaggression) from control-untreated. The hyperaggressive response was present in rats received diphenydramine once daily (100-125% increase) while twice daily administration of diphenhydramine (10 and 20 mg/kg) produced 50% decrease (hypoaggression) as compared to control-untreated.

(iv) Audiogenic seizures: In control group 30% of rats developed audiogenic seizures. The seizures appeared from the day 1 of lorazepam withdrawal and persisted till day 9. The audiogenic seizures were blocked in the once daily diphenhydramine group; 20 and 40 mg/kg doses afforded 100% protection against audiogenic seizures whereas with 10 mg/kg dose audiogenic seizures appeared in 20% rats but there was a significant reduction in the duration of seizures (only upto 3 days) as compared to that of control group (upto 9th day). In rats treated twice daily with diphenhydramine, no significant protection could be afforded with doses 5 and 10 mg/kg. However, 20 mg/kg dose given twice daily protected the animals completely from the audiogenic seizures induced by lorazepam withdrawal.

There were no significant differences in the reaction time to pain, body weigtht and food intake between the control and diphenhydramine groups.

DISCUSSION

The withdrawal signs of lorazepam - hyperkinesia, hyperthermia, hyperaggression and audiogenic seizures, were significantly attenuated by different dose schedules of diphenhydramine given orally during the abstinence period of lorazepam. The oral doses of diphenhydramine tested in the present study, however, differed in the extent of their success. Both the dose schedules of diphenhydramine, once daily as well as twice daily, were effective against hyperkinesia and hyperthermia. However, once daily dose schedule could not affect hyperaggression but was successful in blocking the audiogenic seizures. The twice daily administration of diphenhydramine showed inhibition of these two withdrawal signs - hyperaggression and audiogenic seizures only in higher doses. Analyses of these results on the basis of either total daily intake or frequency of administration of diphenhydramine does not yield a definite pattern. The total daily intake of 20 mg/kg daily inhibited hyperaggression without affecting audiogenic seizures whereas the same amount of diphenhydramine given in single administration the outcome was opposite i.e. blockade of audiogenic seizures without suppression of hyperaggression. The dose 20 mg/kg twice daily was more effective than 40 mg/kg twice daily was the most effective one as it suppressed completely all the withdrawal signs of lorazepam.

It is difficult to explain the possible mechanism underlying the inhibitory influence of diphenhydramine on benzodiazepine dependence. The presumption that diphenhydramine might be acting as substitute to lorazepam following its withdrawl is unlikely because there is no report indicating the cross tolerance between benzodiazepine and diphenhydramine (13). The existing knowledge on the neurochemical basis of benzodiazepine dependence is scanty (3, 4). A detailed study on central neurotransmitter mechanism involved in benzodiazepine dependence may be able to

answer to question - how does diphenhydramine diminish withdrawal signs of lorazepam? The study suggested the potential of diphenhydramine in the drug therapy of benzodiazepine dependence.

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