Influence of Cimetidine in Combination with Antiepileptic Drugs on Locomotor Activity in Mice

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The goal of the present study was to examine the effect of cimetidine, a histamine type 2 receptor antagonist given alone or together with one of the conventional antiepileptic drugs, carbamazepine, phenytoin, phenobarbital or valproate on the exploratory and spontaneous activity in mice after 1 or 7 days of experiment. Animal activity was registered electronically with the use of Digiscan analyser in relation to ambulatory and rearing activities, as well as total distance travelled by animals in 15 minute periods. Results showed that cimetidine given alone significantly decreased three variables of spontaneous motor activity (horizontal activity, total distance and vertical activity) in mice after single administration. Moreover, cimetidine co-administered with valproate (1 or 7 days), carbamazepine (1 day), phenytoin (1 day) or phenobarbital (7 days) significantly worsened spontaneous activity in mice. Likewise, impairment in horizontal and vertical explorative activity in mice was observed when cimetidine was injected with phenobarbital (1 day), carbamazepine (1 day), valproate (1 or 7 days) and phenytoin (1 day). It could be concluded that cimetidine has deleterious effect on locomotor activity of mice, especially in combination with the antiepileptic agents tested. Further studies are needed to elucidate the influence of cimetidine on patients with epilepsy.

Key words: Exploratory locomotor activity, spontaneous activity, antiepileptic drugs, cimetidine, drugs safety

Histamine is known to not only modulate immunological reactions, but also control feeding, locomotor behaviours or consciousness[1]. Unfortunately the role of histamine in the pathogenesis of epilepsy remains inconsistent. According to Wyngaarden and Seevers[2] histamine type 1 (H_1) receptor antagonists, the most commonly used antiallergic agents, may cause convulsions in healthy children. Similar findings were reported by Churchill and Gammon in adults with epilepsy[3]. Moreover, Gerald and Richter observed that antihistamine agents may increase susceptibility to clonic seizures in mice[4]. Additionally, Tuomisto and Tacke reported that histamine may lower maximal electroshock seizures (MES) in mice[5]. Scherkl *et al*. presented that a precursor of histamine, L-histidine, increases pentetrazol (PTZ)-induced seizure threshold in mice[6]. Centrally acting H_1 receptor antagonists (i.e. diphenhydramine, antazoline and pyrilamine) were previously shown to potentiate the risk of electroconvulsions[7,8] or chemoconvulsions in animals[9]. However the role of histamine type 2 (H_2) receptors in seizures pathogenesis is not well studied. Gerald and Richter reported that metiamide, a H_2 receptor antagonist may increase minimal seizure susceptibility in mice after both peripheral and intraventricular administration[4]. In a recent study published by Fukushima *et al*. H_2 receptor-null mice were shown to be significantly less prone to electrically induced seizures than wild-type mice[10]. Surprisingly, no difference was found between H_2 receptor-null mice and wild-type mice in evoking PTZ-induced seizures[10].

The aim of the present study was to analyse the effect of cimetidine, a histamine type 2 (H_2) receptor antagonist, after 1 and 7 d after administration on spontaneous or exploratory locomotor activity in mice, given alone or

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Accepted 09 May 2019
Revised 28 December 2018
Received 01 October 2018
Indian J Pharm Sci 2019;81(4):776-781

in combination with conventional antiepileptic drugs such as carbamazepine (CBZ), phenytoin (DPH), phenobarbital (PB) and valproate (VPA). CBZ, DPH, PB and VPA were administered at doses equal to their median effective dose (ED_{50}) against maximal electroshock in mice, while cimetidine was given at the dose of 20 mg/kg, which affected antiepileptic drugs efficacy, as shown in previous studies[11,12].

Adult male Swiss albino mice (weighing 22-26 g) were purchased from a licensed breeder (Dr T. Gorzkowska, Warsaw, Poland). Animals were kept in standard colony cages, temperature 23±2°, natural light-dark cycle, with food (Murigran pellets, Bacutil, Motycz, Poland) and tap water *ad libitum*. After 7 d of adaptation animals were randomly assigned into experimental groups (each consisted of 12 animals). Experiments were conducted between 10 am to 2 pm. Each animal was used only once. All experimental procedures were accepted by the Local Ethics Committee for Animal Experiments in Lublin, Poland.

Cimetidine, DPH and PB sodium were obtained from Polfa Warsaw, Poland. VPA magnesium (Dipromal) was procured from Polfa Rzeszow, Poland and CBZ (Amizepin) from Polfa Warsaw, Poland. VPA and PB were dissolved in distilled water, however cimetidine, DPH and CBZ were suspended in 1 % Tween 80 solution (Sigma St. Louis, MO, USA). All drugs were administered intraperitoneally (ip) in doses of 0.1 ml/g body mass volume, 30 min before the test.

Animals activity was examined by the Digiscan animal activity monitor system (Omnitech Electronics, Columbus, OH, USA). Each monitor had a Plexiglas open field box (41×41×32 cm) with a grid of infrared beams mounted horizontally every 2.5 cm and vertically every 4.5 cm. Photocells were placed on the wall opposite to each photo-beam and were activated when animal interrupted a beam. Each box was divided into four quadrants (20×20×32 cm) by an acrylic cross. Mice were placed and tested in the opposite quadrant of each unit (i.e. two mice per box). Photocells from each activity box were connected with Digiscan analyser, which transmitted beam breaks (activity data) to the computer. During this study the pattern of beam interruptions was recorded and analysed by IBM-PC compatible computer. Monitoring system recorded interruptions from each infrared beam at 100 Hz frequency. Any beam interruption was reported as an activity score. Concomitant interruption of two or more beams separated by at least one second was recorded as movement score. All data were collected during two consecutive 15-min periods. Data were saved every 15 min into computer. Three types of activity were recorded: two variables of horizontal activity (total distance in centimetre travelled and number of separated horizontal animal movements with a minimum one second break) and vertical activity (total number of interrupted photo-beams according to sensors).

Day before the experiments, animals were habituated to laboratory procedures and tests began immediately after ip administration of the vehicle. Next day mice were examined under the same conditions. Prior to the test, animals were deprived of food for 24 h. Antiepileptic drugs were administered at doses equal to their ED_{50} values against MES-induced seizures. Mice received analysed substances at times scheduled for the electroconvulsive test, according to Swiader *et al*.[7]. Each mouse immediately after drug administration was transferred into activity chamber. Tests were performed twice and lasted 15 min each. First record was categorized as exploratory activity test, the second one was defined as spontaneous mice activity.

Animals received a single dose of cimetidine (1 or 7 d) and one of examined antiepileptic drugs at the time prior to tests described above. Antiepileptic agents were tested at the time of their maximal anticonvulsant activity according to previous studies[8,9], whereas cimetidine's maximal activity time was determined experimentally by Swiader *et al*.[12].

Animals received a single or repeated dose of cimetidine as ip injection and one of tested antiepileptic agents at the time prior to tests described above. Antiepileptic drugs were analysed at the time of their peak anticonvulsant activity according to previously published studies. Adversely, cimetidine's time of maximal activity was determined experimentally[12]. Results of animal activity were subjected to Kruskal-Wallis test (non-parametric ANOVA test) followed by Dunn's test.

Cimetidine given alone (at a dose of 20 mg/kg) for 1 or 7 days did not modify mice motor activity, i.e. horizontal activity, total distance or vertical activity. Interestingly, cimetidine significantly decreased mice vertical activity when co-administered for 1 day with VPA or DPH, but increased it when given with PB (Table 1). In contrast, cimetidine after 7 d of administration did not affect the vertical activity in mice when given together with antiepileptic drugs

(Table 2). Additionally, it was observed that cimetidine significantly impaired horizontal activity in mice receiving every tested antiepileptic drug for 1 d. On the contrary, cimetidine significantly increased horizontal activity of mice receiving VPA (at the dose of 257 mg/kg) for 7 d. Moreover, the increase in total distance has been shown in animals treated with single dose of VPA (287 mg/kg), PB (22.2 mg/kg and 24.7 mg/kg dose) and cimetidine in combination with PB (Table 1). However CBZ (8.9 mg/kg) co-administered with cimetidine significantly decreased total distance in tested mice (Table 1). After 7 d of experiment an increase in total distance in mice has been reported after administration of VPA and PB, but adding cimetidine did not change it (Table 2).

Administration of cimetidine (20 mg/kg) and DPH alone significantly impaired vertical activity in mice after single administration, whereas PB increased it (Table 3). Similar reduction in vertical activity was observed when cimetidine was co-administered with DPH. Moreover, single cimetidine administration was shown to decrease the total distance travelled by animals, similar to DPH and VPA or combined treatment

TABLE 1: EFFECT OF CIMETIDINE (1 DAY TREATMENT) ON EXPLORATORY LOCOMOTOR ACTIVITY IN MICE

Drug (mg/kg)	Horizontal activity		Vertical activity
	Movement	Total distance	Movement
Vehicle	2247±256	1116±228	306±54
Cimetidine (20)	1895±132	685±63	226±31
VPA (287)	2624±231	2166±156	212±26
VPA (265)	2162±199	1680±232	215±40
VPA (265)+cimetidine (20)	1187±131$^{\#\#}$	984±106	44±5.7$^{**\#\#}$
PB (24.7)	3368±159	2338±125**	446±33
PB (22.2)	3129±186	2275±185**	487±53
PB (22.2)+cimetidine (20)	2915±144	1960±183	272±32
DPH (9.9)	1838±208	662±87	152±29
DPH (10.9)	2080±119	1142±32	201±41
DPH (10.9)+cimetidine (20)	1373±166$^{**\#\#}$	808±129	131±16$^{\#\#}$
CBZ (14.5)	1811±388	1063±264	221±28
CBZ (8.9)	2108±265	1029±66	363±37
CBZ (8.9)+cimetidine (20)	1607±198	799±61	263±12

**P<0.01 vs. vehicle, $^{\#\#}$P<0.01 vs. drug. Valproate (VPA), phenobarbital (PB), phenytoin (DPH) and carbamazepine (CBZ) were administered ip 30 min before the test. Cimetidine in a single dose was given ip 30 min before the test. Data are expressed as means±SD, n=12, Kruskal-Wallis with Dunn's post-hoc test

TABLE 2: EFFECT OF CIMETIDINE (7 DAYS TREATMENT) ON EXPLORATORY LOCOMOTOR ACTIVITY IN MICE

Drug (mg/kg)	Horizontal activity		Vertical activity
	Movement	Total distance	Movement
Vehicle	2369±154	1151±136	419±38
Cimetidine (20)	2845±185	1378±134	617±87
VPA (274)	2151±168	1627±185	232±44
VPA (257)	2329±86	1579±119*	251±32**
VPA (257)+cimetidine (20)	1635±315	1144±257	655±89$^{\#\#}$
PB (23.9)	3522±186**	2581±379**	643±40**
PB (31.3)	4480±596**	3640±864**	621±139
PB (31.3)+cimetidine (20)	5587±385	5240±323	999±78
DPH (9.5)	1735±261	759±187	260±40
DPH (10.9)	1905±137	982±92	382±42
DPH (10.9)+cimetidine (20)	1976±142	1097±101	417±34
CBZ (14.5)	1815±199	1071±153	284±46
CBZ (13.6)	1741±405	1136±182	407±56
CBZ (13.6)+cimetidine (20)	2001±462	1108±397	357±169

**P<0.01 vs. vehicle, $^{\#\#}$P<0.01 vs. drug. Valproate (VPA), phenobarbital (PB), phenytoin (DPH) and carbamazepine (CBZ) were given ip 30 min before the test. Cimetidine was given ip 30 min before the test. Data are expressed as means±SD, n= 12, Kruskal-Wallis with Dunn's post-hoc test

of cimetidine and VPA (Table 3). Cimetidine, CBZ, VPA and DPH alone or in combination with cimetidine and CBZ, VPA or DPH significantly decreased mice horizontal activity after a single administration (Table 3).

After 7 d of experiment, VPA and CBZ injected alone decreased mice vertical activity as well, whereas PB increased it (Table 4). Interestingly, when cimetidine was administered in combination with VPA and PB animals' vertical activity was significantly increased (Table 4).

Total distance travelled by mice treated with DPH as well as cimetidine with DPH for 7 d was significantly decreased compared to control group (Table 4), however PB alone or combination of cimetidine and VPA or PB caused opposite effect. Additionally, VPA and DPH alone or co-administration of cimetidine with DPH significantly impaired animals' horizontal movements after 7 d of treatment, whereas PB alone or co-administration of cimetidine with VPA or PB increased it (Table 4).

H_2 receptors are highly expressed in the central nervous system[13]. The possible role of histamine receptor antagonists on epileptic activity in animals and humans was recently analysed. It was reported that cimetidine, a H_2 receptor antagonist up to the dose of 100 mg/kg did not raise the threshold for PTZ-

TABLE 3: EFFECT OF CIMETIDINE (1 DAY TREATMENT) ON SPONTANEOUS ACTIVITY IN MICE

Drug (mg/kg)	Horizontal activity		Vertical activity
	Movement	Total distance	Movement
Vehicle	1226±141	1960±183	272±32
Cimetidine (20)	558±76**	100±19**	26±1.7 **
VPA (287)	2037±257	1598±179**	149±6
VPA (265)	1562±164	1333±114**	137±11
VPA (265)+cimetidine (20)	1248±126	1061±76**	78±11**##
PB (24.7)	1175±173	546±90	268±21
PB (22.2)	1555±173	500±87	203±16
PB (22.2)+cimetidine (20)	1102±132	488±56	265±39
DPH (9.9)	348±30**	42±7**	9.5±0.8**
DPH (10.9)	809±68	365±40	79±14**
DPH (10.9)+cimetidine (20)	570±98**	312±60	121±9
CBZ (14.5)	1305±120	518±91	212±18
CBZ (8.9)	1085±163	372±56	130±17
CBZ (8.9)+cimetidine (20)	1132±99	436±36	103±10**

**P<0.01 vs. vehicle, ##P<0.01 vs. drug. Valproate (VPA), phenobarbital (PB), phenytoin (DPH) and carbamazepine (CBZ) were given ip 30 min before the test. Cimetidine in a single dose was given ip 30 min before the test. Data are expressed as means±SD, n=12, Kruskal-Wallis with Dunn's post-hoc test

TABLE 4: EFFECT OF CIMETIDINE (7 DAYS TREATMENT) ON SPONTANEOUS ACTIVITY IN MICE

Drug (mg/kg)	Horizontal activity		Vertical activity
	Movement	Total distance	Movement
Vehicle	1540±100	621±85	266±40
Cimetidine (20)	1554±136	571±84	348±85
VPA (274)	755±114**	446±91	77±11**
VPA (257)	991±151	628±90	111±10**
VPA (257)+cimetidine (20)	2125±185##	1388±183**##	311±32##
PB (23.9)	2441±212**	1969±201**	507±37**
PB (31.3)	3555±375**	2900±632**	625±61**
PB (31.3)+cimetidine (20)	5368±608**	4915±800**	1178±41**##
DPH (9.5)	1295±76	309±27	167±20**
DPH (10.9)	1799±153	737±89	343±35
DPH (10.9)+cimetidine (20)	1516±112	406±65##	213±36##
CBZ (14.5)	1098±98**	441±46	193±16
CBZ (13.6)	1541±200	599±81	285±58
CBZ (13.6)+cimetidine (20)	1181±125	674±75	280±64

**P<0.01 vs. vehicle, ##P<0.01 vs. drug. Valproate (VPA), phenobarbital (PB), phenytoin (DPH) and carbamazepine (CBZ) were given ip 30 min before the test. Cimetidine was given ip 30 min before the test. Data are expressed as means±SD, n=12, Kruskal-Wallis with Dunn's post-hoc test

induced seizures in mice[12]. Moreover, given for 1 day at 20 mg/kg dose cimetidine significantly increased anticonvulsant properties of ethosuximide (lowered its ED_{50} from 134 to 103 mg/kg) against PTZ-induced seizures and increased its plasma concentration[12]. Additionally, after 1 or 7 d of administration cimetidine (20 mg/kg) did not affect anticonvulsant properties of VPA, clonazepam and PB against PTZ-induced seizures. Cimetidine given for 7 days also did not modify the free plasma levels of VPA, PB and clonazepam[12], assuming that pharmacokinetic interactions are less possible with these antiepileptic drugs.

In another study cimetidine (20 mg/kg) after 14 d of administration significantly increased antiepileptic effect of CBZ against MES-induced convulsions in mice, however, 1 and 7 d treatment showed no effect[11]. Contrary to that, cimetidine decreased anticonvulsant properties of PB after 7 and 14 d of administration[11]. Also after 14 d of treatment cimetidine lowered PB brain concentration and increased both plasma and brain CBZ concentration. Long-term memory impairment after cimetidine co-administration with antiepileptic drugs in mice was also reported[11]. In contrast, cimetidine (up to 40 mg/kg) did not change the threshold for aminophylline-induced seizures in mice[9].

Results of the present study indicated that cimetidine may impair both spontaneous and exploratory activity in mice. H_2 receptor antagonist tested in these studies led to horizontal or vertical movement impairment in mice receiving CBZ, DPH, VPA or PB. Additionally, cimetidine alone or administered with antiepileptics might affect total distance in mice. Spontaneous activity in mice could be as well disturbed after cimetidine given alone or together with tested antiepileptic drugs. Contrary to these results, Leza *et al*. reported that cimetidine enhanced the locomotor activity in mice in a dose-dependent manner, without affecting amphetamine-induced stimulation[14]. Since cimetidine potentiated buprenorphine evoked hyperactivity an involvement of opioid receptors in H_2 receptors antagonists was proposed[14]. However in the study of Leza *et al*. antihistamine agents were used at a dose of 1 and 10 mg/kg, whereas in the present study a dose of 20 mg/kg was tested, which could have significantly produced a different impact on the locomotor activity of mice.

Observed effects could be due to peripheral drug administration. Despite poor blood-barrier penetration, among all H_2 receptor antagonists, cimetidine was found in the central nervous system and reported to evoke neuropsychiatric effects such as mental confusion[15].

In summary, the H_2 receptor antagonist cimetidine should be carefully considered in patients with diagnosed epilepsy. Co-administration of cimetidine with antiepileptic drugs could have clinical implications, which needs further studies.

Acknowledgements:

This study was supported by the grant no P05A 044 18 from the State Committee for Scientific Research, Warsaw, Poland.

Conflict of interest:

Authors declare no conflicts of interest.

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