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Behavioral and Pharmacological Effects of Benzodiazepines in Physiologically Active Mice: A Comparative Study among the Different Generic Forms of Benzodiazepines

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Abstract: Benzodiazepines (BZD) are a class of psychologically active drugs; increase the effect of the neurotransmitter gamma-aminobutyric acid (GABA) on the $GABA_A$ receptor. We aimed to investigate comparative behavioral and pharmacological activities of different generic of benzodiazepines by using albino Swiss mice model study. In this investigations, open field and EPM test was done. Regarding the experimental results, all drugs of benzodiazepines produced significant behavioral as well as pharmacological activities in both tests. Hence, data explained the therapeutic efficacy of benzodiazepines for anxiety and related neuropsychiatric disorders.

Key words: Benzodiazepines · $GABA_A$ receptor · Open field · EPM test and therapeutic efficacy

INTRODUCTION

Benzodiazepines (BZD), also called "benzos", are a class of psychologically active drugs whose center chemical structure is the fusion of a benzene ring and a diazepine ring (Fig. 1) [1]. They increase the effect of the neurotransmitter gamma-aminobutyric acid (GABA) on the $GABA_A$ receptor, resulting several pharmacological activities like sedative, hypnotic (sleep-inducing), anxiolytic (anti-anxiety), anticonvulsant and muscle relaxant properties [2, 3].

Typically, they categorized according to the half-life into three major groups such as short (<12 hr half-life), intermediate (12-24 hr half-life) and/or, long-acting (>24 hr half-life). Half-life defined as, the time essential for half of the drug to be metabolized into an inactive form. Short half-lives signify that the drug is cleared from the body more quickly; effects do not last as long. In contrast, longer half-lives indicate that the drug lasts much longer in the body. In the case of long-acting benzodiazepines where the dose may be lower and the frequency of doses is also lower. Long-acting benzodiazepines reduce the taking of many pills in a given

Fig. 1: Chemical structure of benzodiazepines

amount of time. The short and intermediate acting benzodiazepines are preferred for the treatment of insomnia while longer-acting benzodiazepines are suggested for the treatment of anxiety like disorder [4, 5]. In high doses, various shorter-acting benzodiazepines may also cause anterograde amnesia as well as dissociation. These properties make benzodiazepines useful in treating of numerous medical conditions like anxiety, insomnia, agitation, seizures, muscle spasms, alcohol withdrawal along with as a premedication for medical or dental procedures [5, 6]. The non-medical use

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of benzodiazepine drugs is known as misuse or abuse. In terms of recreationally, benzodiazepines are usually administered orally but sometimes they are taken intranasally or intravenously. Recreational use produces alike effects to alcohol intoxication [7, 8]. In research investigation on pentobarbital trained rhesus monkeys and benzodiazepines produced effects similar to barbiturates [9]. There are different benzodiazepines like alprazolam, bromazepam, brotizolam, chlordiazepoxide, clonazepam, clorazepate, clotiazepam, cloxazolam, diazepam, estazolam, etizolam, flunitrazepam, flurazepam, loprazolam, lorazepam, midazolam, nitrazepam, nordazepam, oxazepam, temazepam and triazolam [10]. In all over the country, these drugs must be dispensed according to the prescription of certified physician or medical. In this present study, we aimed to investigate comparative behavioral and pharmacological activities of different generic of benzodiazepines by using albino Swiss mice model study.

MATERIALS AND METHODS

Drugs and Dose Preparation: Different generic of benzodiazepine tablets name, diazepam (1mg), bromozepam (3mg), clonazepam (1mg) and midazolam (0.75mg) were collected from a medicine shop located in Bayezid thana of Chittagong district. After that, all tablets were crushed and making dose 1mg per kg of mice weight.

Animals: The Swiss Albino mice (male and female) weighing between 25 and 30g were collected from the well-known animal laboratory of Jahangirnagar University (JU), Savar, Bangladesh. The animals were housed under suitable laboratory conditions (relative humidity 55-65%, room temperature 23.0±2.0°C and 12 h light: dark cycle) and acclimatized for 7 days. The animals were fed with proper diet and water.

Open Field Test: This method was carried out as per procedure described by Gupta *et al*. [11]. The equipment of open field consists of a wooden field of half square meter with a series of squares alternatively highlighted in black and white colors. It had a wall of 50 cm height and was placed in a dimly lit room. Mice were treated with control, test drugs and after that, placed in the middle of the open field. Subsequently, the number of squares visited by the animals was counted for 3 minutes at 0, 30, 60, 90 and 120min after the treatments. The following formula was applied to calculate the percentage inhibition of movements:

Inhibition of Movements' (%): Mean No. of movements (control) - Mean No. of movements (test)/ Mean No. of movements (control) ×100

EPM Test: The EPM test was performed on the basis of formerly described method by Ali *et al*. [12]. The apparatus consists of two open arms (5 × 10 cm) and two closed arms (5 × 10 × l5 cm) radiating from a platform (5 × 5 cm) to form a plus-sign figure. The apparatus was situated 40 cm above the floor. The open arms edges were 0.5 cm in height to keep the mice from falling and the closed-arms edges were 15 cm in height. After the sixty minutes test drug was administered and each animal was individually placed in the center of the EPM and were allowed 5 min for free exploration. Subsequently, the number of open and enclosed arm entries and time spent on open arms were manually registered. Entry into an arm was, "as the point when the animal placed all four paws onto the arm".

Statistical Analysis: All data were expressed as mean ± standard error of mean (S.E.M.). Statistical comparisons were performed using one-way ANOVA followed by Tukey/Tukey-Kramer (equal/unequal observations). The values obtained were compared with the control group and considered statistically significant when $p<0.001$, $p<0.01$ and $p<0.05$. All statistical analysis was performed using MaxStat Lite 3.60 version software.

Ethical Acceptance: The study protocol was approved by the Planning and Development committee (Grant No. Pharmacy P&D 68/09-15), Department of Pharmacy, International Islamic University Chittagong, Bangladesh.

RESULTS AND DISCUSSION

This study was aimed to investigate comparative behavioral and pharmacological activities of four different generic (diazepam, bromozepam, clonazepam and midazolam) of benzodiazepines by using albino Swiss mice model study. Among the four benzodiazepines, diazepam is long acting, bromazepam and clonazepam are considered as intermediate acting and midazolam is short acting benzodiazepines. But, diazepam and midazolam have rapid onset of action within 15 minutes where as, bromazepam and clonazepam have intermediate onset of action within 15-30 minutes [13, 14]. The open-field was done by recording spontaneous locomotor activity of mice to illuminate the sedative effects of benzodiazepines. In this methodology, any agent with capable to sedation

Table 1: Effect of Benzodiazepine on open field test

Treatment	Dose (mg/kg)	Number of squares crossed (% of inhibition) 0 min	30 min	60 min	90 min	120 min
Control	1% Tween 80 in water	102±0.31	91±0.70	82.60±0.51	66.6±1.07	51.20±0.58
Diazepam	1	98.8±1.53[a] (3.13)	31.60±0.92[a] (65.27)	26.40±0.51[a] (68.03)	17±0.70[a] (74.47)	11.80±3.74[a] (77.98)
Bromozepam	1	77.2± 2.78[a] (24.31)	21.60±0.67[a] (76.26)	11.20±0.73[a] (86.44)	7.40±0.24[a] (88.88)	5.4±0.24[a] (51.49)
Clonazepam	1	65.4± 4.57[a] (35.88)	44.4±6.58[a] (51.20)	29.80±4.25[a] (63.92)	14.60±3.88[a] (78.07)	9.6±2.42[a] (83.58)
Midazolam	1	63.20±4.43[a] (38.03)	36.80±2.47[a] (59.58)	31.20±2.78[a] (62.22)	23.80±3.41[a] (64.26)	17±2.04[a] (66.66)

Values are presented as the Mean ± SEM (n = 5) [a]$p< 0.001$

Table 2: Effect of the Benzodiazepine on the number of entries and time of the spent in open and closed arm in elevated maze test

Treatment	Dose (mg/kg)	Responses No. of open arm entries	Time spent in open arm (mins.)	No. of closed arm entries	Time spent in closed arm (mins.)
Control	1% Tween 80 in water	0.40±0.49	1.20±0.49	2.2±0.49	286.80±2.17
Diazepam	1	5.20±0.37[b]	35.40±2.48[b]	19.20±1.15[a]	216.2±4.1[a]
Bromozepam	1	5.6±1.16[a]	57±14.54[a]	3.20±0.37	222±12.51[a]
Clonazepam	1	6.2±0.37[a]	156.8±2.39[a]	9.20±0.37[c]	132.4±0.87[a]
Midazolam	1	1.6±0.24	274±2.91[a]	0.60±0.24	0.064±0.026[b]

Values are presented as the Mean ± SEM (n = 5) and[a]$p< 0.001$, [b] $p <0.01$, [c] $p < 0.05$

will cause a decrease in the number of movements, interpreted as a decrease in curiosity of the new environment [15, 16]. According to the results of this investigation, all generic of benzodiazepines were capable to suppress locomotor activity. The maximum suppression of locomotor activity in zero minute was done by midazolam. Sixty minutes (60) and ninety (90) minutes later maximum suppression achieved by bromazepam but one hundred twenty (120) minutes later clonazepam suppressed maximally. In all cases the probability values calculate comparing with control and these were less than 0.001 (p<0.001). This capability of benzodiazepines recommends all drugs are endowed with central nervous system depressant activity (Table 1).

The EPM is one of the most widely validated tests and is highly responsive to investigate the activity of both anxiolytic and anxiogenic drugs acting at the gamma aminobutyric acid type A (GABAA)-benzodiazepine complex [17]. In EPM, typical mice will normally wish to spend a lot of their allotted time in the closed arms and this preference appears to reflect an aversion towards open arms that is generated by the fears of the open spaces. The increases open arm explorations are considered as anxiolytic. Furthermore, the reverse holds true for anxiogenics [18, 19]. In the drugs of diazepam and bromazepam induced an anxiolytic-like effect in mice, as it increased open arm entries and the time spent in the open arms of the EPM when compared to the control animals. Conversely, clonazepam and midazolam produced anxiogenic-like effect as, closed arms entries as well as, the time spent in the closed arms were high in the EPM. In all cases benzodiazepines produced statistically significant activities except, number of closed arm entries for bromazepam (Table 2).

CONCLUSION

In conclusion, all drugs of benzodiazepines produced the significant behavioral as well as pharmacological effects in open field test. The benzodiazepines also produced mixed like effects in EPM test. This preliminary study might be helpful for researcher those involved in the field of neuroscience.

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