Research Article

Comparative Study of Muscle Relaxant Activity of Midazolam with Diazepam in Male Albino Mice

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ABSTRACT

A muscle relaxant is a drug which affects skeletal muscle function and decreases the muscle tone. It may be used to alleviate symptoms such as muscle spasm, pain and hyperreflexia. Skeletal muscle relaxants are heterogeneous group of medications that refer to 2 major therapeutic groups: neuromuscular blockers and spasmolytics. The study was designed to assess the muscle relaxant activity of midazolam and compare the muscle relaxant activity of midazolam with diazepam using albino male mouse as experimental model. Diazepam and midazolam are centrally acting muscle relaxants that are frequently combined with opioids to produce calming and muscle relaxation before surgery. The white albino mice were selected, weighed and numbered. A total of 24 animals weighing about 30 grams were selected for the present study. They were divided into four groups each group consists of 6 mice. The animal was placed one by one on the rotarod more than one mouse at a time were placed. Initially free fall off reading was taken before administration of drugs. The free fall off time was noted when the mouse falls from the rotating rod. A normal untreated mouse generally falls off within 3-5 minutes. The reaction time was recorded after 30 minutes interval following administration of diazepam (2mg/kg) and midazolam (2mg/kg) administered separately through intraperitoneal mode of inoculation. Then reaction time was also recorded for 3mg/kg and 4mg/kg of Diazepam and midazolam. SAS package 24.0 version by applying one way ANOVA (Data analysis) and calculated the significance of drug. P value <0.05 indicates significant. The study was carried out in albino mice weighing 30 grams. Six mice were included in each group to study the muscle relaxant property in different concentrations such as 2, 3 and 4 mg/kg of diazepam, and midazolam. At 2mg/kg dose the percentage of fall of free ride time for diazepam was higher than midazolam. At 3mg/kg dose the percentage of fall of free ride time for diazepam and midazolam showed significant variations ('p'< 0.05) but diazepam has more muscle relaxant property than midazolam. The study also revealed that, midazolam at 4mg/kg dose was more potent than at lower tested drug concentrations and it reduces the muscle strength property. So diazepam is more potent drug than diazepam in terms of muscle relaxation activity. This study was carried out to evaluate the muscle relaxant property of midazolam which was compared with diazepam in mice by rotarod method test. It was found that the drug midazolam has good muscle relaxant action at higher concentrations but have less muscle relaxation activity than standard drug diazepam.

Keywords: Muscle relaxant activity, diazepam, Midazolam, Rotarod method.

INTRODUCTION

Muscle relaxant is an agent that reduces the contractility of muscle fibers. It may be used to relieve symptoms like muscle spasms, pain, and hyperreflexia. Muscle relaxants showed their pharmacologic effect centrally at the level of the spinal cord, the brainstem, or the cerebrum. Curare derivatives and succinyl choline compete with acetylcholine and block neural transmission at the myoneural junction. These drugs are using during anesthesia, in the management of patients undergoing mechanical ventilation, and in shock therapy, to reduce muscle contractions in pharmacologically or electrically induced seizures. The term muscle relaxant can be used to refer to two major therapeutic groups: neuromuscular blockers and spasmolytics[1-3]. Neuromuscular blocking agents are chemical substances that interferers locally with the transmission or reception of impulses from motor nerves to skeletal muscles[4]. They are used to induce muscle relaxation in anesthesia, endotracheal intubation, and electroshock therapy and as adjuncts in the treatment of tetanus, encephalitis, and poliomyelitis. They can cause bronchospasm, hyperthermia, hypotension, or respiratory paralysis and are used with caution, especially in patients with myasthenia gravis or with renal, hepatic, or pulmonary impairment and in elderly and debilitated individuals. During surgery, it is important to recognize that these agents prevent muscle movement but do not block the sensation of pain. Spasmolytics, also known as centrally acting muscle relaxants, are used to alleviate musculoskeletal pain and spasms and to reduce spasticity in a variety of neurological conditions[5]. Muscle relaxants are used to treat acute muscle spasms, spasticity from upper motor neuron syndrome, torticolis, orthopedic manipulations and low back or neck pain, fibromyalgia, tension headaches and myofascial pain syndrome[6]. There are 2 main categories of skeletal muscle relaxants:

antispastic (such as baclofen or dantrolene) for conditions such as cerebral palsy and multiple sclerosis and antispasmodic agents for musculoskeletal conditions[7]. Evidence is extremely limited to support the use of antispastic agents for musculoskeletal conditions, for which an antispasmodic agent is typically more appropriate. Although musclerelaxant are actually classified into one group, the Food and Drug Administration (FDA) has approved just a few medications in this class to treats pasticity; the rest are approved to treat musculoskeletal conditions. There are benzodiazepines (BZD) which are sedative –hypnotics and also have muscle relaxant activity. They hasten the onset of sleep. At slight higher doses they induce sleep or hypnosis and increase the duration of sleep. They reduce anxiety and aggression and produce a calming effect. They produce CNS depression in a dose dependent manner. Midazolam is used as an IV anesthetic[8]. Benzodiazepines including diazepam, midazolam can be used as adjuvants to general anesthetics, but they carry the risk of prolonging respiratory depression which could be due to their longer half-lives. They reduce the muscle tone by a central action. They depress the spinal polysynaptic reflexes which maintain the muscle tone. Generally anxiety is associated with an increase muscle tone and may be responsible for the aches and pains in these patients. The muscle relaxation by BZDs adds to their beneficial effects in such patients. Benzodiazepines like Diazepam and midazolam are preferred in panic states and anxiety associated with organic disease. It possesses anxiolytic, anticonvulsant, hypnotic, sedative, skeletal muscle relaxant, and amnestic properties. Benzodiazepines bring about their effects through GABA by binding to the $GABA_A$ receptors and increases the frequency of chloride channel ion opening and enhances chloride ion conductance which brings the neuronal membrane hyperpolarization which reduces the synaptic transmission and cause CNS depression.

The present study was designed to assess the muscle relaxant activity of midazolam and compare the muscle relaxant activity of diazepam using albino male mouse as experimental model.

MATERIALS AND METHODS

In the present investigation, free fall off method was used to identify the central muscle relaxant effect (Muscle grip strength) of drug in male albino mice. The skeletal muscle relaxations together with taming or calming effect of Benzodiazepines reduce anxiety and tension. The loss of muscle grip is an indication of muscle relaxation. This effect can be easily studied in animals using rotarod. There has been recently some renewed thinking about the importance of Rotarod method[4]. Instead of just noting fall off time, more detailed observations should be made. It is important to observe the animal while it is walking on the Rotarod to see how many times the animal takes free ride. A free ride is defined as a revolution of the rod during which the animal holds on the rod, rather walks on it. There free ride could affect the calculations on drug responses. The difference in the fall off time from the rotating rod between the control and drug treated animal is taken as an index of muscle relaxation. The angle of the slope of the inclined plane or the rate of rotation of the rod should be adjusted such that a normal mouse can stay on the plane or on the rod for an appreciable period (3-5 min) of time.

Rota rod method

The white albino mice were selected, weighed and numbered. A total of 24 animals weighing about 30 grams were selected for the present study. They were divided into four groups each group consists of 6 mice. Animals which stay on the rotarod in between 2-5 minutes were included and others were excluded. An appropriate speed (15 rpm) on the rotarod is ideal and is used in the study. The rotarod is divided into several compartments; and the animal was placed one by one on the rotarod more than one mouse at a time were placed. Initially free fall off reading was taken before administration of drugs. The free fall off time was noted when the mouse falls from the rotating rod. A normal untreated mouse generally falls off within 3-5 minutes. The reaction time was recorded after 30 minutes interval following administration of diazepam (2mg/kg) and midazolam (2mg/kg) administered separately through intraperitoneal mode of inoculation. Then reaction time was also recorded for 3mg/kg and 4mg/kg of Diazepam and midazolam.

Statistical analysis

Statistical analysis done by SAS package 24.0 version by applying one way ANOVA (Data analysis) and calculated the significance of drug. ‘p’ value <0.05 indicates significant.

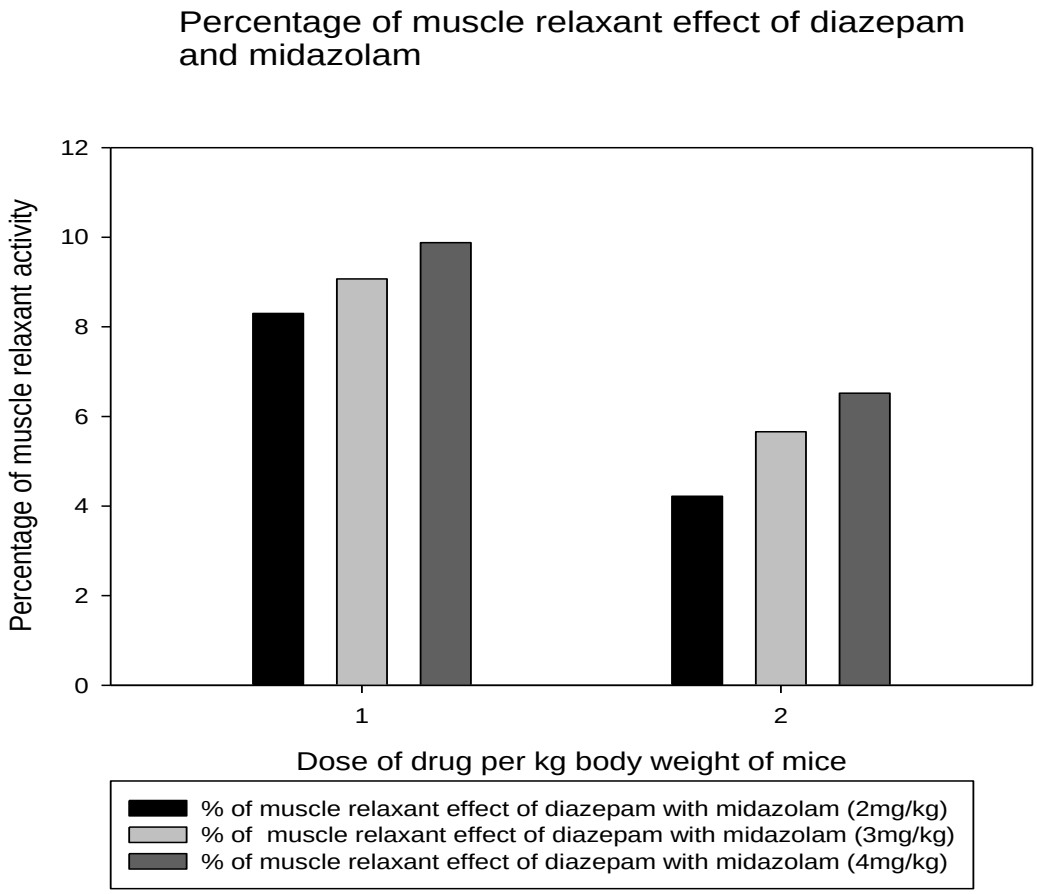
RESULTS

The present study was conducted in male albino mice weighing 30 grams. Six mice were included in each group to study the muscle relaxant property in different concentrations such as 2, 3 and 4 mg/kg of Diazepam, midazolam. For initial screening of a drug, mouse is one of the best animals as it is easy to handle and can be used repeatedly since the animal is not sacrificed by rotarod method. Second choice of animal is rat. Table-1 was the control group where the animals were treated only with 0.2ml normal saline. In the present study it was found that the percentage of fall of free ride time for Diazepam was 8.30% with 2mg/kg, 9.07% with 3mg/kg and 9.88% with 4mg/kg (Table 2). By applying one way ANOVA through regression analysis by using linear polynomial equation the results came for this was insignificant ($p \geq 0.05$). It was 4.22% percentage of free fall of ride with midazolam tested drug at 2mg/kg, 5.66% with 3mg/kg and 6.52% with 4mg/kg of midazolam when assessed by rotarod method (Table 3). By applying one way ANOVA through regression analysis by using linear polynomial equation the results came for this was insignificant ($p \geq$

0.05). At 2mg/kg dose the percentage of fall of free ride time for diazepam was 8.30% and for midazolam was 4.22% indicated that the standard drug diazepam showed high muscle relaxation activity than tested drug midazolam (Table 4).At 3mg/kg dose the percentage of fall of free ride time for Diazepam is 9.07% whereas for midazolam it was 5.66% (Table 5). The difference is slight higher but was more for Diazepam when compared with midazolam. Diazepam has more muscle relaxant property. Diazepam was more potent and it reduces the muscle strength property which was more than midazolam. At 4mg/kg the percentage of fall of free time for diazepam was 9.88% whereas for midazolam it was 6.52%.The difference was more for midazolam when it was compared with diazepam (Table 6). This study found that concentration of midazolam 4mg/kg body weight in mouse was more potent compared to the other tested concentrations and it reduces the muscle strength property. So diazepam was more potent than midazolam (Figure-1). By comparing the results of standard drug diazepam with test drug the results showed significant influence on muscle relaxation effect ('t' test = 15.58 and 'p' value = 0.004).

Table 1: Control (C) group- Treatment with 0.2 ml of Normal saline

Fall of free ride time				
S.No.	**Body weight (mice)**	**Before Normal saline (sec)**	**After Normal saline (sec)**	**Mean value**
1	30gms	282	280	278.15
2	30gms	260	260	
3	30gms	283	282	
4	30gms	290	290	
5	30gms	276	275	
6	30gms	280	280	



Formulae for % of fall of free ride time for 2mg/kg, 3mg/kg,4mg/kg of diazepam respectively.

$C\text{-}S_1/C$ =100;

$C\text{-}S_2/C$ =100;

$C\text{-}S_3/C$ =100

Formulae for % of fall of free ride time for 2mg/kg, 3mg/kg,4mg/kg of Midazolam respectively.

C-T1a/C =100;

C-T1b/C =100;

C-T1c/C =100

Figure 1: Percentage of muscle relaxant effect at the dose of 2mg/Kg, 3mg/Kg 4mg/Kg body weight in male albino mice

Table 2: Detection of muscle relaxant activity of Diazepam at the dose of 2mg/kg, 3mg/kg and 5mg/kg body weight (S_1, S_2, S_3) using Rotarod method in a mice weighing 30gms body weight

Fall of free ride time							
S No.	**Treatment**	**At 2mg/kg drug dosage (s_1)**		**At 3mg/kg drug dosage (s_2)**		**At 4mg/kg drug dosage (s_3)**	
Mice body weight 30gms		**Before Normal saline (sec)**	**After Normal saline (sec)**	**Before Normal saline (sec)**	**After Normal saline (sec)**	**Before Normal saline (sec)**	**After Normal saline (sec)**
1	Diazepam	280	240	278	235	280	230
2	Diazepam	260	225	260	220	260	215
3	Diazepam	280	235	280	230	280	220
4	Diazepam	280	240	278	234	275	225
5	Diazepam	275	230	275	228	280	230
6	Diazepam	278	238	280	237	278	235

Statistics: Nonlinear Regression; Equation: Polynomial, Linear (f=y0+a*x)

Analysis of Variance (ANOVA): Uncorrected for the mean of the observations:

	DF (Degree of freedom)	**SS (Sum of squares)**	**MS (Mean square)**
Regression	2	306106.6260	153053.3130
Residual	4	168.3740	42.0935
Total	6	306275.0000	51045.8333

Corrected for the mean of the observations:

	DF	**SS**	**MS**	**F (Fischer test)**	**P (Probability value)**
Regression	1	102.4593	102.4593	2.4341	0.1937 (Insignificant)
Residual	4	168.3740	42.0935		
Total	5	270.8333	54.1667		

Table 3: Detection of muscle relaxant activity of Midazolam at the dose of 2mg/kg, 3mg/kg and 5mg/kg body weight (T_{1a}, T_{1b}, T_{1c}) using Rotarod method in a mice weighing 30gms body weight

Fall of free ride time							
SNo.	**Treatment**	**At 2mg/kg drug dosage (T_{1a})**		**At 3mg/kg drug dosage (T_{1b})**		**At 4mg/kg drug dosage (T_{1c})**	
Mice body weight 30gms		**Before Normal saline (sec)**	**After Normal saline (sec)**	**Before Normal saline (sec)**	**After Normal saline (sec)**	**Before Normal saline (sec)**	**After Normal saline (sec)**
1	Midazolam	275	245	280	240	275	235
2	Midazolam	280	250	275	245	280	240
3	Midazolam	278	246	278	250	290	245
4	Midazolam	290	262	280	240	278	230
5	Midazolam	280	248	290	242	285	240
6	Midazolam	285	258	285	244	280	242

Statistics: Nonlinear Regression; Equation: Polynomial, Linear (f=y0+a*x); Analysis of Variance (ANOVA): Uncorrected for the mean of the observations:

Analysis of Variance: Uncorrected for the mean of the observations:

	DF (Degree of freedom)	SS (Sum of squares)	MS (Mean square)
Regression	2	341848.5651	170924.2826
Residual	4	65.4349	16.3587
Total	6	341914.0000	56985.6667

Corrected for the mean of the observations:

	DF	SS	MS	F	P
Regression	1	77.8984	77.8984	4.7619	0.0945 (Insignificant)
Residual	4	65.4349	16.3587		
Total	5	143.3333	28.6667		

Table 4: Comparison of percentage of fall of free ride time at effective dosages (2mg/kg) of Midazolam with Diazepam on Rotarod Method

Name of the drug	Dose	Percentage of fall of free ride time	Mean	Standard deviation	Standard error	P-value
Diazepam	2mg/kg	8.30%	255.05	7.84	3.20	P <0.05 Significant
Midazolam	2mg/kg	4.22%	266.4	5.35	2.18	

Table 5: Comparison of percentage of fall of free ride time at effective dosages (3mg/kg) of Midazolam with Diazepam on Rotarod Method

Name of the drug	Dose	Percentage of fall of free ride time	Mean	Standard deviation	Standard error	P-value
Diazepam	3mg/kg	9.07%	252.92	7.65	3.12	P <0.05 Significant
Midazolam	3mg/kg	5.66%	262.4	5.35	2.18	

Table 6: Comparison of percentage of fall of free ride time at effective dosages (4mg/kg) of Midazolam with Diazepam on Rotarod Method

Name of the drug	Dose	Percentage of fall of free ride time	Mean	Standard deviation	Standard error	P-value
Diazepam	4mg/kg	9.88%	250.67	7.84	3.20	P <0.05 Significant
Midazolam	4mg/kg	6.52%	260.00	5.35	2.18	

DISCUSSION

8-Chloro-6-(2-fluorophenyl)-1-methyl-4H-imidazo[1,5-a][1,4]benzodiazepine (midazolam, Ro 21-3981, Dormicum) is an imidazobenzodiazepine whose salts are soluble and stable in aqueous solution. It has a quick onset and, due to rapid metabolic inactivation, a rather short duration of action in all species studied. Midazolam has a similar pharmacologic potency and broad therapeutic range as diazepam. It produces all the characteristic effects of the benzodiazepine class, i.e., anticonvulsant, anxiolytic, sleep-inducing, muscle relaxant, and "sedative" effects. The magnitude of the anticonflict effect of midazolam is smaller than that of diazepam in rats and squirrel monkeys, probably because a more pronounced sedative component interferes with the increase of punished responses. Previous studies suggested that the CNS depression and non-specific muscle relaxation effect can reduce the response of motor coordination. Increased muscle tone is common feature of anxiety states in human and may contribute to the aches and pains including headache often troublesome in anxious patients. The relaxant effect of benzodiazepines may therefore be clinically useful. A reduction of muscle tone appears to be possible without appreciable loss of coordination. Skeletal muscle relaxants are used to treat two different types of conditions like spasticity from upper motor neuron syndrome and muscular pains or spasms from peripheral musculo-skeletal conditions. In this study centrally acting skeletal muscle relaxants diazepam, midazolam are used and muscle relaxant activity of midazolam is compared with diazepam. The study was carried out in albino mice weighing 30 grams. Six mice were included in each group to study the muscle relaxant property in different concentrations such as 2, 3 and 4 mg/kg of diazepam, and midazolam. At 2mg/kg dose the percentage of fall of free ride time for diazepam was more than midazolam. At 3mg/kg dose the percentage of fall of free ride time for diazepam and midazolam showed slightly higher variations with diazepam having more muscle relaxant effect than midazolam. The study also revealed that, midazolam at 4mg/kg dose was more potent and reduces the muscle strength activity. So diazepam is more potent than midazolam. This study was carried out to evaluate the muscle relaxant property of midazolam which was compared with diazepam in mice by rotarod method test. It was found that the drug midazolam has good muscle relaxant action at 4mg/Kg body weight concentration. Veena *et al*. (2015) studied the centrally acting skeletal muscle relaxants diazepam and its muscle relaxant activity. The study was carried out in albino mice weighing 40gms. They found that diazepam demonstrated muscle relaxant property and considered to have maximum muscle relaxant property may be due to high lipid solubility[4]. In 1956, Dunham and Miya suggested that the skeletal muscle relaxation induced by a test compound could be evaluated by testing the ability of mice or rats to remain on a revolving rod. This forced motor activity has subsequently been used by many investigators. The dose which impairs the ability of 50% of the mice to remain on the revolving rod is considered the endpoint. By this test the muscle relaxant potency in a series of compounds such as the benzodiazepines (Vogel *et al*) have been performed[9]. Midazolam has a pharmacokinetic advantage over other BZDs with sedative doses (0.10–0.07 mg kg−1 intravenously) that have a peak effect in 2 min that is sustained for approximately 30 min. The incidence of thrombophlebitis following intravenous administration of midazolam is

much lower compared to diazepam because of the pH-dependent ring opening of the diazepine ring of midazolam.

CONCLUSION

The present study was carried out to compare the muscle relaxant activity of midazolam, and Diazepam given in different concentration in experimental model in rotarod test in albino mice. Diazepam and midazolam were given in concentration of 2mg/kg, 3mg/kg and 4mg/kg body weight in rotarod method for each mouse in each group respectively. It was found that diazepam and midazolam produced central muscle relaxant effect when assessed by rotarod test. On inter drug comparison of all the two drugs it was found that midazolam has more muscle relaxant property on increased concentration i.e. 4 mg/kg body weight similarly like the standard drug diazepam upon increasing the drug concentrations when assessed by rotarod but at different tested concentrations 2, 3 and 4 mg/kg diazepam has more muscle relaxant property than midazolam. Thus, it was found that diazepam demonstrated increased muscle relaxant activity compared to midazolam at four different tested concentrations of drug.

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