

Evaluation of seizure activity after phospho-diesterase and adenylate cyclase inhibition (SQ22536) in animal models of epilepsy

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Abstract

The role of adenylate cyclase (AC) inhibitor (SQ22536) was evaluated in the presence of PDE-5/6/8/10/11 and PDE-7 inhibitors such as dipyridamole and BRL-50481 in animal models of epilepsy. Seizures were induced in the animals by subjecting them to injection of chemical convulsant, pentylenetetrazole (PTZ) and maximal electroshock (MES). The study mainly comprises of the onset of seizures, mortality/recovery, percentage of prevention of seizures (anti-convulsant) and total duration of convulsive time. Present study mainly highlights the combined effects of AC inhibitor SQ22536 with dipyridamole as well as BRL50481 showed a good reduction ($P < 0.001$) in incidence of seizures, compared to SQ22536 and BRL50481 alone treated mice against PTZ (60 mg/kg, i.p.) model. The total convulsive time was prolonged significantly ($P < 0.01$) in SQ22536 alone treated (60.2%) and in with combination of SQ22536 with BRL50481 treated (27.4%) groups, compared to DMSO received group (100%). The study also demonstrates that SQ22536 alone, SQ22536 followed by dipyridamole and SQ22536 with BRL50481 greatly increased the anticonvulsant activity ($P < 0.01$, $P < 0.05$ and $P < 0.01$) along with higher protection 83.3%, 66.7% and 50% range respectively. SQ22536 with dipyridamole effectively ($P < 0.001$) decreased the MES (150 mA, 0.2 sec) induced convulsion, compared to SQ22536. The data shows that SQ22536 alone, SQ22536 followed by dipyridamole and SQ22536 with BRL50481 greatly increased the anti-convulsant activity ($P < 0.01$, $P < 0.01$ and $P < 0.01$) along with higher protection 83.3%, 50% and 66.7% range respectively in animals pre-treated with MES. The results suggest the possible involvement of SQ22536 alone and with presence of dipyridamole and BRL50481, delays the onset of seizure activity as well as prolongs the total duration of convulsive time in both models.

Keywords: Adenylate cyclase, PDE, SQ22536, dipyridamole, BRL50481, seizures

Introduction

Epilepsy is a common health problem and affects more than 50 million people worldwide, 5 million of them have seizures more than once per month (Porter, 1988). Approximately 5-10% of the population usually develops seizure at least once during their lifetime, with the highest incidence occurring in early childhood and late adulthood (Lowenstein, 2001). A seizure is a sudden change in behaviour characterized by changes in sensory perception (sense of feeling) or motor activity (movement) due to an abnormal firing of nerve cells in the brain. Epilepsy is a condition characterized by recurrent seizures that may include repetitive muscle jerking called convulsions. Epilepsy is a complex disease with diverse clinical characteristics that preclude a singular mechanism. One way to gain insight into potential mechanisms is to reduce the features of epilepsy to its basic components: seizures, epileptogenesis and the state of recurrent unprovoked seizures that defines epilepsy itself. A common way to explain seizures in a normal individual is that a disruption has occurred in the normal balance of excitation and inhibition. The fact that multiple mechanisms exist is not surprising given the varied ways the normal nervous system controls this balance. In contrast, understanding seizures in the brain of an individual with epilepsy is more difficult because seizures are typically superimposed on an altered

nervous system. The different environment includes diverse changes, making mechanistic predictions a challenge. Understanding the mechanisms of seizures in an individual with epilepsy is also more complex than understanding the mechanisms of seizures in a normal individual because epilepsy is not necessarily a static condition but can continue to evolve over the lifespan (Scharfman, 2007).

The cyclic adenosine 3', 5'-monophosphate (cAMP) plays a major role in the generation of seizure activity. An elevation in cAMP content has been reported in the cerebral cortex accompanying chemically induced epileptic activity (Walker *et al.*, 1973; Krivanek & Mares, 1977; Ferrendelli *et al.*, 1980). The adenylate cyclase (AC), an important transmembrane enzyme possesses certain activity in the brain which promotes the intracellular level of cAMP, from adenosine triphosphate (ATP) (Seamon *et al.*, 1981; Higashima *et al.*, 2002). In epileptic conditions the cAMP concentration in the cerebrospinal fluid is also elevated after an attack (Myllyla *et al.*, 1975). cAMP plays a key function by controlling a wide variety of cellular processes (Houslay *et al.*, 1998; Houslay, 2001) also which acts as a ubiquitous second messenger and modulator of signal transduction processes (Houslay, 1998). This cAMP is generated by the action of adenylate cyclase (Houslay & Milligan, 1997) and degraded by hydrolysis process, which is

regulated by a family of cyclic nucleotide phosphodiesterases (PDEs) (Conti & Jin 1999; Soderling & Beavo, 2000).

PDE enzymes regulate the degradation of cAMP a product of the adenylate cyclase activation and could contribute to the pathophysiology of the seizure mechanisms. PDE enzymes are responsible for the hydrolysis of the cyclic nucleotides and therefore have a critical role in regulating intracellular levels of the second messengers cAMP, cGMP and hence cell function as well as downstream cell signalling in the various body systems (Maurice *et al.*, 2003). Recent evidence shows that the cyclic nucleotide phosphodiesterases exist in several molecular forms and that these isozymes are unequally distributed in various tissues (Jeon *et al.*, 2005). Twelve members of the PDE family have been identified and these can be further divided into 50 isoforms of subtypes and splice variants (Wallace *et al.*, 2005). Out of the twelve PDE gene families, PDE-5 & 6 belong to cGMP-specific (Francis *et al.*, 1990; Loughney *et al.*, 1998; Wang *et al.*, 2001) PDE-7 & 8 are cAMP-specific (Michaeli *et al.*, 1993; Soderling *et al.*, 1998), PDE-10&11 related with cGMP-sensitive and dual specificity (Loughney *et al.*, 1999; Yuasa *et al.*, 2000). Clinical signs of epilepsy arise from the intermittent, excessively synchronized activity of group of neurons. Different neurotransmitters and neuro-modulators are known to play a significant role in the system of excitation (Fisher & Coyle, 1991).

The present study examines the role of adenylate cyclase in the presence of cyclic nucleotide phosphodiesterase-5/6/7/8/10/11 inhibitors in the generation of seizure threshold. We used pharmacological tools like SQ-22536 (adenylate cyclase inhibitor), Dipyrindamole (PDE-5/6/8/10/11 inhibitor) and BRL-50481 (PDE-7 inhibitor) to block and attenuate the effects of PDE and evaluate the effect on chemical convulsant and maximal electroshock induced seizures in mice and rats.

Materials and methods

Either sex of Swiss Albino mice weighing between 24-26 g and Wistar strain rats weighing between 160-220 g were utilized for this study. The animals were placed randomly and allocated to treatment groups in polypropylene cages with paddy husk as bedding. Animals were housed at temperature of $24 \pm 2^{\circ}\text{C}$ and relative humidity of 30-70%. A 12:12 dark: light cycle was followed during the experiments. All the animals were allowed free access to water *ad libitum* and fed with standard commercial pelleted rat chow (M/s. Hindustan Lever Ltd., Mumbai). All the experimental procedures and protocols used in this study were reviewed by the institutional animal ethical committee and were in accordance with the guidelines of the CPCSEA.

Drugs and chemicals

The following drugs and chemicals were used for conducting this study. 10% w/v of dimethyl sulfoxide

(DMSO) Sigma, USA, gabapentin (Micro labs Ltd., Bangalore, India), SQ22536 (Sigma, USA), zonisamide (Sun Pharma, Mumbai, India), dipyrindamole (Tocris Bioscience, UK), BRL50481 (Tocris Bioscience, UK) and except gabapentin and zonisamide, other drugs are soluble in DMSO, gabapentin and zonisamide are soluble in sterile water for injection.

A. Chemoshock method

Pentylentetrazole (PTZ) or metrazol (MTZ) induced seizure model in mice

Swice Albino mice were divided into 7 groups with six animals (n=6) in each. Treatment protocol and group description is mentioned as follows:

Group-I : Mice served as solvent control, received 10 % w/v of DMSO (5 ml/kg, i.p).

Group-II : Mice received gabapentin (2.5 mg/kg, i.p) treated as positive control.

Group-III : Mice received SQ22536 (1nmol/kg, i.p) an adenylate cyclase inhibitor.

Group-IV : Mice received dipyrindamole (2 mg/kg, i.p) a PDE-5/6/8/10/11 Inhibitor.

Group-V : Mice received BRL50481 (2mg/kg, i.p) a PDE-7 inhibitor.

Group-VI : Mice received SQ22536 (1nmol/kg, i.p) along with dipyrindamole (2 mg/kg, i.p) combination of adenylate cyclase inhibitor and PDE-5/6/8/10/11 inhibitor.

Group-VII: Mice received SQ22536 (1 nmol/kg, i.p) along with BRL50481 (2 mg/kg, i.p) combination of adenylate cyclase inhibitor and PDE-7 inhibitor.

All the drugs were administered intraperitoneally 30 min prior to the administration of pentylentetrazole (60 mg/kg, i.p). The animals were observed for 1 h by placing in a separate cage. The onset time of various phases of convulsions like action, jerky movement, convulsions and recovery/mortality were noted in seconds as per (Yemitan & Salahdeen, 2005; Salahdeen & Yemitan, 2006) method.

B. Maximal electroshocks (MES) method for rats

Wistar strain rats were divided into 7 groups with six animals (n=6) in each. Treatment protocol and group description is mentioned as follows:

Group-I: Rats served as solvent control, received 10 % w/v of DMSO (3.5 ml/kg, i.p).

Group-II: Rats received zonisamide (35 mg/kg, i.p.), treated as positive control.

Group-III : Rats received SQ22536 (0.7 nmol/kg, i.p) an adenylate cyclase inhibitor,

Group-IV: Rats received dipyrindamole (1.4 mg/kg, i.p) a PDE-5/6/8/10/11 inhibitor.

Group-V: Rats received BRL50481 (1.4 mg/kg, i.p) a PDE-7 inhibitor.

Group-VI: Rats received SQ22536 (0.7 nmol/kg, i.p) along with dipyrindamole (1.4 mg/kg, i.p) combination of adenylate cyclase inhibitor and PDE-5/6/8/10/11 inhibitor.

Group-VII: Rats received SQ22536 (0.7 nmol/kg, i.p) along with BRL50481 (1.4 mg/kg, i.p) combination of adenylylase inhibitor and PDE-7 inhibitor.

All the drugs were administered intraperitoneally 30 min prior to the electroshock. The electroshock was induced in animals by passing a current of 150 mA for 0.2 sec duration through electroconvulsimeter (Techno India) using corneal electrodes. The incidence of seizures, tonic limb flexion, tonic extensor, clonus, stupor and recovery / mortality of the animals were observed and tabulated as per Achliya *et al.* (2005).

data obtained from experiments conducted with PDE-5/6/7/8/10/11 inhibitors along with adenylylase activator and inhibitor on chemoshock such as PTZ (60 mg/kg, i.p) induced seizures in mice. The highlights of the findings are the data obtained with combination of AC inhibitor, SQ22536 and dipyridamole which showed a good reduction ($P < 0.001$) in onset of action, jerky movements and convulsion against PTZ induced seizures in mice when compared to SQ22536 alone received group of animals (Fig. 1, 2 & 3). The combination of SQ22536 and PDE-7 inhibitor, BRL50481 received mice

Table 1. Effect of drugs on pentylenetetrazole induced seizures in mice.

Treatment groups	Drug name	Total duration of convulsion (Sec)	% change from control (Convulsive time)	Mortality (%)	Protection (%)	Significance
I	10% DMSO	212.50	100	83.3	16.7	--
II	Gabapentin	275.00	29.4	33.3	66.7	$P < 0.01$
III	SQ22536	340.05	60.2	16.7	83.3	$P < 0.01$
IV	Dipyridamole	240.31	13.2	50.0	50.0	NS
V	BRL50481	210.40	0.9	66.7	33.3	NS
VI	SQ22536+Dipyridamole	260.18	22.5	33.3	66.7	$P < 0.05$
VII	SQ22536 + BRL50481	270.42	27.4	50.0	50.0	$P < 0.01$

The group of mice ($n=6$) were injected with 60 mg/kg, i.p. of PTZ for induction of convulsion and the total convulsive time was estimated. A value of $P < 0.05$ was considered significant Vs DMSO group, $NS = P > 0.05$. All the drugs were administered intraperitoneally. The drugs used were administered in the following doses. DMSO (5 ml/kg, i.p.), Gabapentin (2.5 mg/kg, i.p.), SQ22536 (1 nmol/kg, i.p.), Dipyridamole (2 mg/kg, i.p.) and BRL50481 (2 mg/kg, i.p.). (One way ANOVA followed by Dunnett's test compared with DMSO treated mice)

Statistical analysis

All the results were expressed as mean \pm SEM. One way analysis of variance (ANOVA) followed by Tukey-Kramer multiple comparisons test was applied. The statistical analysis of the data in order to compare the inter group differences and one way analysis of variance (ANOVA) followed by Dunnett's test was also used. To compare with DMSO treated group the estimation of total duration of convulsion time in seconds and percentage of change from control were analysed in Table 1 and 2. P values < 0.05 were considered as statistically significant.

showed a significant ($P < 0.001$) decrease in seizure activity when compared to SQ22536 and BRL50481 alone treated mice (Fig. 1, 2 & 3). The overall highlights of Fig. 1, 2 and 3 explicit the individual effect of AC inhibitor, SQ22536 which delays the onset of action of seizures as well as prolongs the total duration of convulsive time (Table 1).

Table 1 summarizes the total duration of convulsion, percentage change from control, mortality and protection in incredible levels of percentage. The total convulsive time was prolonged significantly ($P < 0.01$) in SQ22536

Table 2. Effect of drugs on maximal electroshock induced seizures in rats.

Treatment groups	Drug name	Total duration of convulsion (Sec)	% change from control (convulsive time)	Mortality (%)	Protection (%)	Significance
I	10% DMSO	236.50	100	100	--	--
II	Zonisamide	285.00	20.6	33.3	66.7	$P < 0.05$
III	SQ22536	335.00	41.7	16.7	83.3	$P < 0.01$
IV	Dipyridamole	260.81	10.3	33.3	66.7	NS
V	BRL50481	283.45	19.9	83.3	16.7	$P < 0.05$
VI	SQ22536 + Dipyridamole	290.40	22.8	50.0	50.0	$P < 0.01$
VII	SQ22536 + BRL50481	330.47	39.7	33.3	66.7	$P < 0.01$

The group of rats ($n=6$) were subjected to 150 mA (0.2 sec) electroshock and total convulsive time was estimated. A value of $P < 0.05$ was considered significant Vs DMSO group, $NS = P > 0.05$. All the drugs were injected intraperitoneally. The drugs used were administered in the following doses. DMSO (3.5 ml/kg, i.p.), zonisamide (35 mg/kg, i.p.), SQ22536 (0.7 nmol/kg, i.p.), Dipyridamole (1.4 mg/kg, i.p.) and BRL50481 (1.4 mg/kg, i.p.). (One way ANOVA followed by Dunnett's test compared with DMSO treated rats).

Results

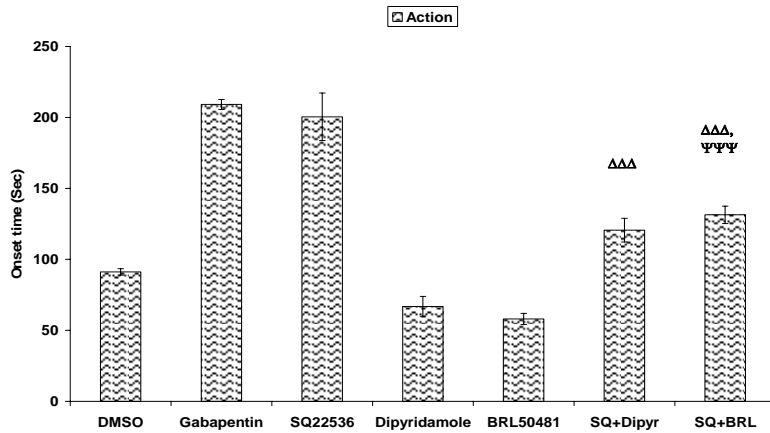
Evaluation of onset of seizures

A. Chemoshock method

Pentylenetetrazole (PTZ) or Metrazol (MTZ) induced seizure model in mice: Fig. 1, 2 and 3 summarizes the

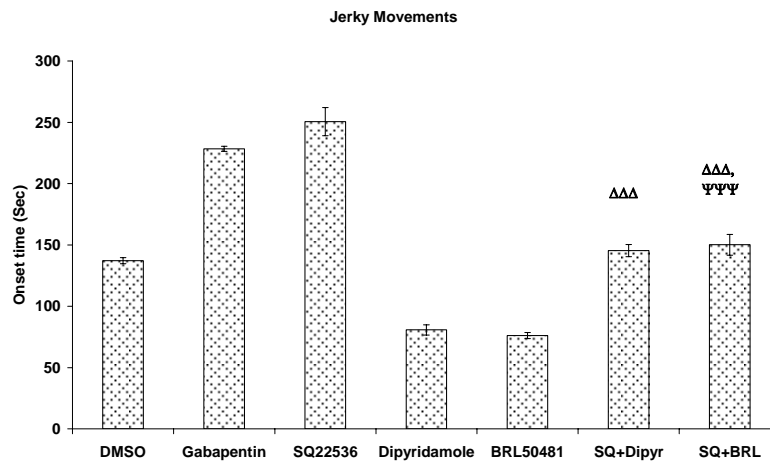
alone treated (60.2%) and combination of SQ22536 with BRL50481 treated (27.4%) groups compared to DMSO received group (100%). The data shows that 83.3% and 66.7% of protection of animals were noticed in SQ22536 and i.p injection of SQ22536 followed by dipyridamole

Fig. 1. Effect of PDE-5/6/7/8/10/11 inhibitors along with adenylate cyclase inhibitor on chemoshock seizures in mice.



Data represented as mean \pm SEM (n=6), which represents onset time of action phase of convulsion in seconds. Treatments were given 30 mins prior to chemical convulsant injection of PTZ (60 mg/kg, i.p). $\Delta\Delta\Delta$ Denotes $p < 0.001$ compared with SQ22536 received group, $\Psi\Psi\Psi$ denotes $p < 0.001$ compared with BRL50481 received group (One-way ANOVA followed by Tukey-Kramer multiple comparisons test).

Fig. 2. Effect of PDE-5/6/7/8/10/11 inhibitors along with adenylate cyclase Inhibitor on chemoshock seizures in mice.



Data represented as mean \pm SEM (n=6), which represents onset time of jerky movement phase of convulsion in seconds. Treatments were given 30 mins prior to chemical convulsant injection of PTZ (60 mg/kg, i.p). $\Delta\Delta\Delta$ Denotes $p < 0.001$ compared with SQ22536 received group, $\Psi\Psi\Psi$ denotes $p < 0.001$ compared with BRL50481 received group (One-way ANOVA followed by Tukey-Kramer multiple comparisons test).

treated groups against PTZ induced seizures in mice. The results show that there was an increase in seizure activity (0.9%) in BRL50481 treated alone animals. Apart from these highlighted points, the author would like to discuss few things from the data obtained (data not shown), Fig 1, 2 and 3 expresses the action of animals against PTZ induced seizures as follows, gabapentin treated group showed significant ($P < 0.001$) reduction in onset of action and jerky movements of seizures, when compare to all

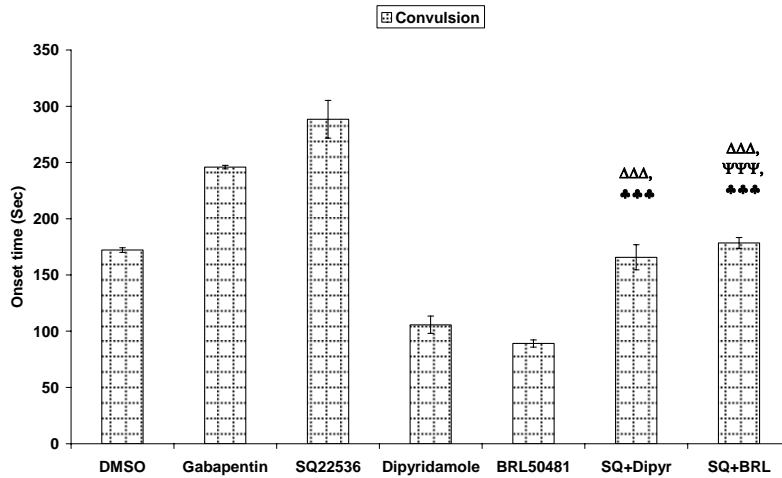
groups except SQ22536 (NS). The data shown in Table 1 also demonstrates that i.p administration of SQ22536 (1 nmol/kg, i.p) greatly increased the anticonvulsant activity ($P < 0.01$) along with higher protection (83.3%) range. Simultaneously, the combined effect of SQ22536 with exogenously administered BRL50481 (2 mg/kg, i.p) and SQ22536 with dipyridamole (2 mg/kg, i.p) showed a significant ($P < 0.01$ & $P < 0.05$) anti-convulsant activity with moderate protection (50% & 66.7%) range respectively (Table 1). A similar trend was noted in the results obtained from SQ22536 received groups explicit mild reduction ($P < 0.05$) in convulsion compared to gabapentin. SQ22536, dipyridamole and BRL50481 treated groups showed a significant reduction ($P < 0.001$) in jerky movements against DMSO received mice (data not shown).

Maximal electroshocks (MES) method for rats

Fig. 4, 5, 6 and 7 illustrate the data obtained from experiments conducted with maximal electroshock induced seizures in rats. It is evident from the data displayed in fig. 4, 5 and 6 that combination of AC inhibitor, SQ22536 and dipyridamole effectively ($P < 0.001$) decreased the tonic limb flexion, tonic extensor and clonus stage of convulsion, compared to SQ22536 alone treated rats. The same significant level ($P < 0.001$) was obtained in SQ22536 combined with BRL50481, instead of dipyridamole (Fig. 4, 5 & 6). The overall highlights of fig. 4, 5, 6 and 7 explicit the BRL50481 alone received group, potentiates the seizure activity against MES induced convulsion. Emphasis was also seen on the independent effect of AC inhibitor, SQ22536 in delaying the onset of seizure activity (Fig. 4, 5, 6 & 7) as well as prolonging the total duration of convulsive time (Table 2).

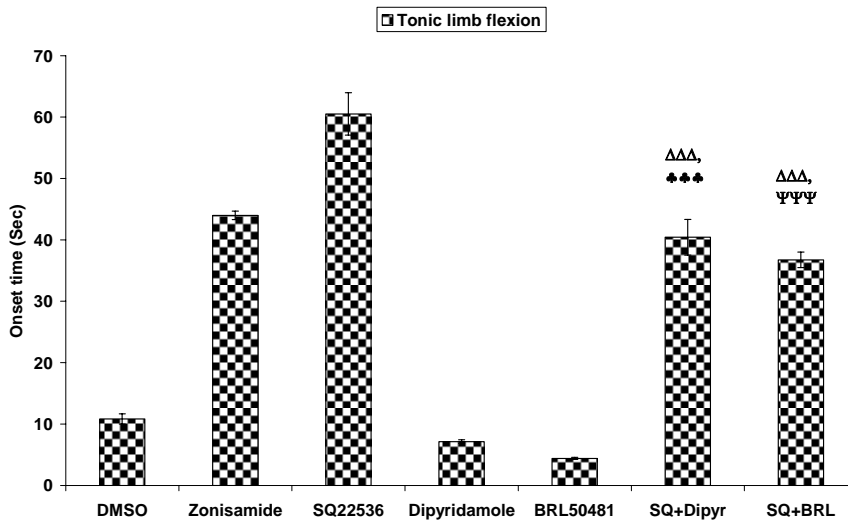
Table 2 demonstrated the total duration of convulsion, percentage change from control, mortality and protection in marked levels of percentage. The total convulsive time was long lasting significantly ($P < 0.01$) in SQ22536 alone treated (41.7%) and combination of SQ22536 with BRL50481 treated group increase significantly ($P < 0.01$) the duration of convulsion (39.7%), compared to DMSO received group (100%). The data showed that 83.3% and 66.7% of protection of animals were noticed in SQ22536 and i.p injection of SQ22536 followed by BRL50481 treated groups against MES induced seizures in rats. From Table 2 it was evident that there was a significant increase in seizure activity (10.3%) when dipyridamole treated alone. Apart from these highlighted points, the author would like to discuss few things from the data obtained (data not shown) the action of animals against MES induced seizures. Gabapentin, SQ22536, SQ22536 with

Fig. 3. Effect of PDE-5/6/7/8/10/11 inhibitors along with adenylate cyclase inhibitor on chemoshock seizures in mice.



Data represented as mean \pm SEM (n=6), which represents onset time of convulsion phase in seconds. Treatments were given 30 mins prior to chemical convulsant injection of PTZ (60 mg/kg, i.p.). $\Delta\Delta\Delta$ Denotes $p<0.001$ compared with SQ22536 received group, $\Psi\Psi\Psi$ denotes $p<0.001$ compared with BRL50481 received group, \clubsuit and $\clubsuit\clubsuit\clubsuit$ denotes $p<0.05$ and $p<0.001$, respectively, compared with dipyridamole received group and ns denotes non significant (One-way ANOVA followed by Tukey-Kramer multiple comparisons test).

Fig. 4. Effect of PDE-5/6/7/8/10/11 inhibitors along with adenylate cyclase inhibitor on maximal electroshock induced convulsions in rats.



Data represented as mean \pm SEM (n=6), which represents onset time of tonic limb flexion phase of convulsion in seconds. Treatments were given 30 mins prior to maximal electroshock (150 mA, 0.2 sec). $\Delta\Delta\Delta$ denotes $p<0.001$ compared with SQ22536 received group, $\Psi\Psi\Psi$ denotes $p<0.001$ compared with BRL50481 received group, $\clubsuit\clubsuit\clubsuit$ denotes $p<0.001$ compared with dipyridamole received group (One-way ANOVA followed by Tukey-Kramer multiple comparisons test).

dipyridamole, SQ22536 with BRL50481 treated groups showed significant ($P<0.001$) reduction in onset of tonic limb flexion phase of convulsion, when compare to DMSO. Simultaneously, the individual effect of SQ22536 and dipyridamole received groups showed a significant

($P<0.001$) reduction in tonic extensor phase of convulsion, against gabapentin treated group. Table 2 reveals that i.p administration of SQ22536 (0.7 nmol/kg, i.p) greatly enhances the anti-convulsant activity ($P<0.01$) along with higher protection (83.3%) range. At the same time, the combined effect of SQ22536 with exogenously administered BRL50481 (1.4 mg/kg, i.p) and SQ22536 with dipyridamole (1.4 mg/kg, i.p) showed a significant ($P<0.01$ and $P<0.01$) anti-convulsing activity with judicious protection (66.7% and 50%) range respectively (Table 2.).

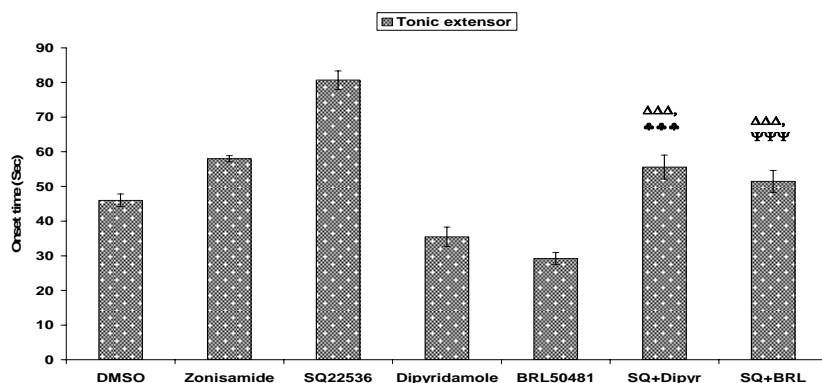
Discussion

The data obtained from this study showed that pre-treatment with adenylate cyclase inhibitor, SQ22536 alone and along with the PDE-5/6/7/8/10/11 inhibitors such as dipyridamole and BRL 50481, potentiated the anticonvulsant activity against the PTZ and MES induced convulsions as depicted in Fig. 1-7. PDE-5/6/8/10/11 inhibitor, dipyridamole is an adenosine transport inhibitor, which acts mainly in two ways: (i) by increasing cyclic nucleotides as a result of the inhibition of phosphodiesterase (especially type 5, which is cGMP dependent) (Lugnier *et al.*, 1986) and (ii) by increasing extracellular levels of adenosine (Roos & Pleger, 1972) which leads to the activation of adenylate cyclase (Gresele *et al.*, 1986) to convert adenosine into cAMP. Secondly, it also inhibits cGMP-phosphodiesterase, increasing the amount of intracellular cGMP which may augment the downstream signalling effects of nitric oxide (NO), a vasodilator and inhibitor of platelet aggregation (Gamboa *et al.*, 2005; Liao, 2007). Dipyridamole also increases cAMP by inhibiting the cellular uptake of adenosine (Roos & Pleger, 1972). Our results support these findings in such a way that this combination showed a good reduction ($P<0.001$) in induction of seizure activity against PTZ and MES induced seizures in animals when compared to SQ22536 alone received group of animals (Fig. 1-7).

SQ22536 is a specific adenylate cyclase (AC) inhibitor (35) which was employed to inhibit the activity of AC. Recent study explains that SQ22536 abolished the elevation of cAMP (Gao & Usha Raj, 2001). Our study also explains that the BRL50481

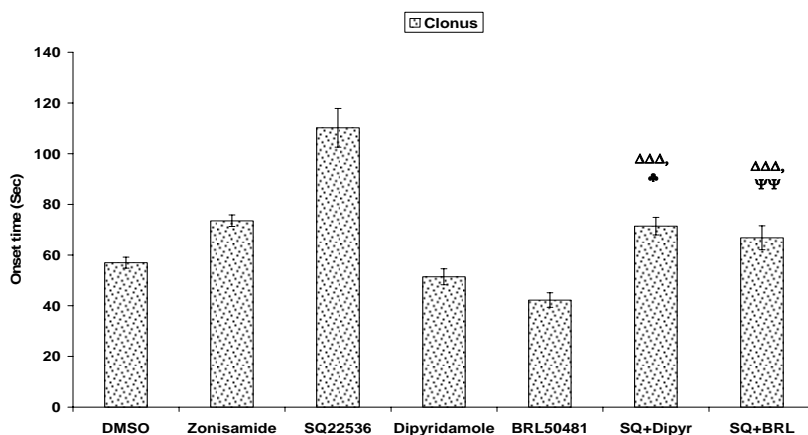
showed a quick onset of seizure responses with increase in the mortality range in both animal models of epilepsy and this shows the potential role of this agent for therapeutic purpose. Murray (1990) discovered that

Fig. 5. Effect of PDE-5/6/7/8/10/11 inhibitors along with adenylate cyclase inhibitor on maximal electroshock induced convulsions in rats.



Data represented as mean \pm SEM ($n=6$), which represents onset time of tonic extensor phase of convulsion in seconds. Treatments were given 30 min prior to maximal electroshock (150 mA, 0.2 sec). $\Delta\Delta\Delta$ Denotes $p<0.001$ compared with SQ22536 received group, $\Psi\Psi\Psi$ denotes $p<0.001$ compared with BRL50481 received group, $\clubsuit\clubsuit\clubsuit$ denotes $p<0.001$ compared with dipyridamole received group (One-way ANOVA followed by Tukey-Kramer multiple comparisons test).

Fig. 6. Effect of PDE-5/6/7/8/10/11 inhibitors along with adenylate cyclase inhibitor on maximal electroshock induced convulsions in rats.



Data represented as mean \pm SEM ($n=6$), which represents onset time of clonus phase of convulsion in seconds. Treatments were given 30 mins prior to maximal electroshock (150 mA, 0.2 sec). $\Delta\Delta\Delta$ Denotes $p<0.001$ compared with SQ22536 received group, $\Psi\Psi$ denotes $p<0.01$ compared with BRL50481 received group, \clubsuit denotes $p<0.05$ compared with dipyridamole received group (One-way ANOVA followed by Tukey-Kramer multiple comparisons test).

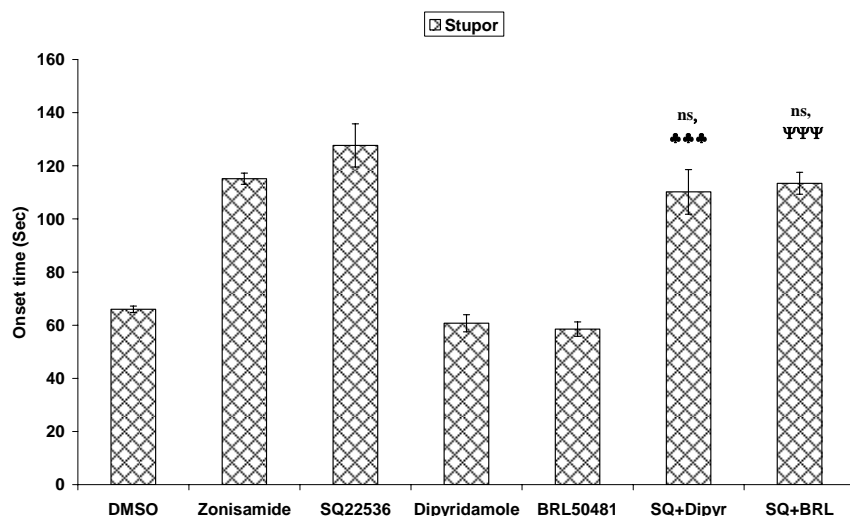
adenylate cyclase assay reveals the direct effect of AC activator providing the net effect of measurement of cAMP production by AC and cAMP degradation by PDEs (37). In mammalian cells, AC consists of at least 10 isoforms (Sunahara *et al.*, 1996) some isoforms are stimulated by Ca^{2+} -calmodulin and inhibited by calmodulin antagonists (Mons *et al.*, 1998). Since the decrease in cAMP was largely based on usage of SQ22536, acting predominantly by Ca^{2+} -calmodulin dependent (Sunahara *et al.*, 1996). Recent study shows

that SQ22536 abolished the elevation of cAMP content by iloprost (a prostaglandin I_2 analog) in guinea-pig which supports our findings (Turcato & Clap, 1999).

BRL50481 is a selective inhibitor of PDE-7, a novel subtype of PDE that is expressed in a number of cell types, including T lymphocytes. There are at least two genes coding for PDE7, each with several splice variants (Adkinson, 2008). Two PDE7 genes (PDE7A & PDE7B) have been identified in humans (Gardner *et al.*, 2000; Hetman *et al.*, 2000). Li *et al.* (1999) suggested that PDE 7 may modulate human T-cell function. PDE7 is highly expressed in brain regions, including the hippocampus and olfactory bulb (Miro *et al.*, 2001; Irisarri *et al.*, 2005). The distribution of PDE 7A3 is largely unknown, but it has been found in human T-lymphocytes (Glavas *et al.*, 2001) and may also be present in many PDE7A1-expressing cells as both transcripts are probably regulated by the same promoter (Torras-Llort & Azorin, 2003). In contrast, PDE7B is abundant in the brain, liver, heart, thyroid glands, and skeletal muscles, but it is not found in leukocytes (Gardner *et al.*, 2000). Our study reports concurrence with combined effects of SQ22536 with exogenously administered BRL50481 (1.4 mg/kg, i.p) and SQ22536 with dipyridamole (1.4 mg/kg, i.p) showing a significant ($P<0.01$ and $P<0.01$) anticonvulsant activity with judicious protection (66.7% & 50%) range respectively against MES model as depicted in Table 2. Fig. 4, 5, 6 and 7 illustrates the PDE-7 inhibitor, BRL50481 showed a marked ($P<0.01$) decrease in onset of tonic extensor phase of convulsion in MES model of epilepsy. The total convulsive time was prolonged significantly ($P<0.01$) in SQ22536 alone treated (60.2%) and combination of SQ22536 with BRL50481 treated (27.4%) groups, compared to DMSO received group (100%) as in Table 1.

Thus, in conclusion the study reflects the individual effect of adenylate cyclase (AC) inhibitor, SQ22536 delay the onset of action of seizures as well as prolonging the total duration of convulsive time in both PTZ and MES models of epilepsy. The SQ22536 greatly increased the anti-convulsant activity along with higher percentage protection range of animals in both models of epilepsy. Further studies can be conducted using specific neuronal cell lines and elucidating the exact signal transduction mechanisms responsible for anti-convulsant effects.

Fig. 7. Effect of PDE-5/6/7/8/10/11 inhibitors along with adenylyl cyclase inhibitor on maximal electroshock induced convulsions in rats.



Data represented as mean \pm SEM (n=6), which represents onset time of stupor phase of convulsion in seconds. Treatments were given 30 mins prior to maximal electroshock (150 mA, 0.2 sec). $\Psi\Psi\Psi$ denotes $p < 0.001$ compared with BRL50481 received group, *** denotes $p < 0.001$ compared with dipyridamole received group and ns denotes non significant (One-way ANOVA followed by Tukey-Kramer multiple comparisons test).

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