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Evaluation of the anticonvulsant activity of the phosphodiesterase III inhibitor cilostazol in the animal model of epilepsy.

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ABSTRACT

The present study is objected to evaluate the anticonvulsant activity of the phosphodiesterase III inhibitor cilostazol in the animal model of epilepsy. Conventional anti-epileptic rodent models like Maximal Electric Shock (MES)-induced convulsions and Pentylenetetrazol (PTZ) induced convulsions were used. The animals were randomly divided into six groups, with six rats in every group. Here anti-epileptic activity of cilostazol with two different doses (10 mg/kg i.p and 20 mg/kg i.p) was compared with standard drug and standard drug + Cilostazol two different doses (10 mg/kg i.p and 20 mg/kg i.p). Cilostazol (20 mg/kg i.p) exhibited an anticonvulsant effect in MES-induced and PTZ induced convulsion models over the Control group and cilostazol (10 mg/kg i.p). Standard drugs were shown superiority in seizure suppression activity than cilostazol (20 mg/kg i.p). The time duration of onset of clonic convulsion and period of clonic convulsions in PTZ induced convulsion were increased and decreased respectively when compared to Standard drug + cilostazol both doses and standard drug alone. Phenytoin abolished convulsions induced by MES- convulsion model. So the present study established that cilostazol has low anticonvulsant efficacy in comparison with conventional drugs (Phenytoin and Sodium Valproate). The potentiating effect of cilostazol with standard drugs was also demonstrated.

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INTRODUCTION

The occurrence of at least one seizure with a perpetual alteration in the brain structure or function that increases the probability of approaching seizures is defined as Epilepsy (Fisher *et al.*, 2005). It affects around 50 million people worldwide with a direct consequence on physical well-being and issues like disruption to work, education, psychological stress and social stigma. It is an evidential contributor to global disease burden with an increased prevalence of mental health disorders including anxiety, depression, suicidal thoughts and mortality (WHO, 2020; Tellez-Zenteno *et al.*, 2007).

During the past three decades, over 15 new anti-epileptic drugs (AEDs) were introduced, although around 70–80% of patients with new-onset of epilepsy eventually enter remission with current AED and fail to control seizures in 20–30% of patients. No anticonvulsant drug has been shown to prevent the development of convulsions in patients before the first seizure rather than purely symptomatic suppression of seizures. These drugs are unable to prevent or reverse the development of drug-resistant epilepsy (Löscher *et al.*, 2013). So the development and discovery of new AEDs with new strategies came in to picture which can be useful in epilepsy, mental health. Cilostazol a phosphodiesterase-III inhibitor is approved for the treatment of intermittent claudication due to peripheral occlusive arterial disease as cilostazol is a thrombolytic agent and a vasodilator. It was reported additional BK Channel activation and adenosine based cAMP mechanism (Strandness *et al.*, 2002; Wu *et al.*, 2004; Sun *et al.*, 2002). BK Channels, adenosines are newer molecular targets for epilepsy (N'Gouemo, 2011). So the present study was conducted to evaluate the anticonvulsant activity of cilostazol in rodents with two different log doses (i.e. 10mg/kg, 20mg/kg) and standard drugs.

MATERIALS AND METHODS

Animals

The healthy wister strain albino rats of either sex weighing 150g-250g were obtained from the institutional animal house which was maintained under standard laboratory conditions (light period of 12 hrs/day and temperature $25^0C \pm 1^0C$) and included in the experiments. The present study got approved by the Institutional Animal Ethical Committee, and the animals were grouped into six groups, with six animals in each group.

Group I - Control - Normal saline

Group II - Standard (Phenytoin - 20 mg/kg i.p.)

Group III - Cilostazol (10 mg/kg i.p)

Group IV - Cilostazol (20 mg/kg i.p)

Group V - Standard (Phenytoin - 20 mg/kg i.p.) + Cilostazol (10 mg/kg i.p)

Group VI - Standard (Phenytoin - 20mg/kg i.p.) +Cilostazol (20 mg/kg i.p)

Cilostazol (Pure Chem private LTD.) was dissolved in DMSO (Pon Pure chemicals) which is a well tolerated (5ml/kg) non clinical vehicle by rats (Gad *et al.*, 2006). Other drugs used were analytical grade.

Maximal Electric Shock (MES) – induced convulsions

The traditional well-established method used to screen the anti-epileptic efficacy against primary and secondary generalised tonic-clonic seizures. Thirty minutes after administration of the drugs mentioned in the grouping, MES seizures were electrically induced by Electroconvulsiometer which was set at 60 Hz alternating current of 150 mA intensity for 0.2 seconds using moistened ear clip electrodes. Duration of various parameters like tonic flexion, tonic extension, clonic convulsions and righting reflexes were recorded. Reduction or suppression in the period of the above setting was taken as an efficacy measurement of the drugs (Medhi, 2010; Gupta, 2004; Kulakarni, 2007).

Pentylenetetrazol (PTZ) induced convulsions

A widely accepted anti-epileptic model PTZ - induced convulsions, used to screen the anti-epileptics efficacy against petit- mal epilepsy, being a CNS stimulant can produce a jerky type of clonic convulsions in rats. The rats were injected with PTZ (70 mg/kg i.p) thirty minutes after test drug doses (10 & 20 mg/kg i.p) and standard drug (sodium valproate -200 mg/kg i.p), test drug plus standard drug doses. The onset and duration of clonic convulsions were recorded. The increase and decrease of onset and duration of clonic convulsions respectively taken as criteria for antiepileptic (Medhi, 2010; Gupta, 2004; Kulakarni, 2007).

Statistical Analysis

Values recorded were expressed as Mean + SD and were analysed by ordinary two way ANOVA followed by Tukey's multiple comparisons test. A P value of less than 0.05 was indicated as the existence of a significant difference.

RESULTS AND DISCUSSION

According to the results tabulated in the Table 1, Cilostazol (20 mg/kg i.p) exhibited statistically significant decrease in the MTDTF, MTDTE, MTDCC and MTDRF over Cilostazol (10 mg/kg i.p) (*** $p<0.001$), and Control groups (*** $p<0.001$). The results of Cilostazol (10 mg/kg i.p) did not demonstrate a significant decrease in MTDTF, MTDRF. Interestingly, it demonstrated decrease in MTDTE (** $p = 0.003$) and MTDCC (* $p =0.011$) over Control group. Convulsions induced by MES were ameliorated by Phenytoin, Phenytoin + Cilostazol (10 mg/kg i.p) and Phenytoin + Cilostazol (20 mg/kg i.p).

According to the results tabulated in the Table 2, Cilostazol (20 mg/kg i.p) demonstrated a statistically significant increase in onset of MTCC over Control group (*** $p<0.001$) and Cilostazol (10 mg/kg i.p) (*** $p<0.001$). Sodium valproate demonstrated

Table 1: Evaluation of the anticonvulsant activity of the Phosphodiesterase III inhibitor Cilostazol by Maximal Electric Shock (MES) – induced convulsions.

Groups	Mean time duration of tonic flexion MTDTF (Sec)	Mean time duration of tonic extension MTDTE (Sec)	Mean time duration of clonic convulsion MTDCC (Sec)	Mean time duration of righting reflex MTDRF (Sec)
Control	10.33± 2.80	21.33 ± 3.14	35.5 ± 3.56	5.58± 0.53
Phenytoin (20 mg/kg i.p)	0.17 ± 0.40	0.17± 0.40	0	0
Cilostazol (10 mg/kg i.p)	9.33± 1.50	18.17± 2.31	32.67 ± 1.75	3.78 ± 0.59
Cilostazol (20 mg/kg i.p)	5.5 ± 1.05	5.17 ± 1.17	27.33 ± 2.16	2.56 ± 0.2
Pheny + Cilostazol (10 mg/kg i.p)	0.17 ± 0.40	0	0	0
Pheny + Cilostazol (20 mg/kg i.p)	0	0	0	0

Table 2: Evaluation of the anticonvulsant activity of the Phosphodiesterase III inhibitor Cilostazol by Pentylenetetrazol(PTZ) - induced convulsions.

Groups	Onset of mean time for clonic convulsion MTCC (Sec)	Mean time duration of clonic convulsant MTDCC (Min)
Normal Saline	165.83 ± 8.1	29.05 ± 0.44
Sodium Valproate (200 mg/kg i.p)	446.16 ± 16.25	3.73 ± 0.57
Cilostazol (10 mg/kg i.p)	160.33 ± 11.12	26.84 ± 1.44
Cilostazol (20 mg/kg i.p)	227.33 ± 9.52	16.39 ± 1.41
Sod. Val + Cilostazol (10 mg/kg i.p)	476 ± 4.85	2.8 ± 0.35
Sod. Val+ Cilostazol (20 mg/kg i.p)	510 ± 8.87	1.24 ± 0.48

superior action over Cilostazol (20 mg/kg i.p) (*** p<0.001). Cilostazol (20 mg/kg i.p) + Sodium valproate demonstrated statistically a significant increase in onset of MTCC over other groups (*** p<0.001). Cilostazol (20 mg/kg i.p) demonstrated a significant decrease in MTDCC over Control group (*p=0.995). Cilostazol (20 mg/kg i.p) demonstrated decreases in MTDCC over Cilostazol (10 mg/kg i.p) but not statistically significant (p=0.154). Sodium valproate shown superior action over cilostazol (20 mg/kg i.p) (* p = 0.046). Sodium valproate + Cilostazol (20 mg/kg i.p) demonstrated a statistically significant difference with Cilostazol (10 mg/kg i.p) (*** p<0.001) and Cilostazol (20 mg/kg i.p) (** p = 0.009). Cilostazol (10 mg/kg i.p) did not demonstrate significant difference with control group in onset of MTCC and MTDCC over control group.

Cilostazol is a phosphodiesterase IIIa inhibitor, in the present study cilostazol (20 mg/kg i.p) exhibited an anticonvulsant effect in MES induced and

PTZ induced convulsion models over the Control group and cilostazol (10 mg/kg i.p). There was a significant difference between Standard drugs and cilostazol (20 mg/kg i.p). Standard drugs were shown superiority in seizure suppression activity than cilostazol (20 mg/kg i.p). On the other hand, the time duration of onset of clonic convulsion and period of clonic convulsions in PTZ induced convulsion were increased and decreased respectively when compared to Standard + Cilostazol both doses and Standard drug alone. Phenytoin abolished convulsions induced by MES – convulsion model. The anticonvulsant activity of cilostazol may be due to inhibiting degradation of cyclic AMP, activation of BK_{ca} (the large-conductance Ca^{2+} -activated K^{+} channel) which are regulated by calcium ion levels in the cells and adenosine uptake inhibition. One of the best approaches to anti-epileptic activity can be gain by a slight change of seizure threshold levels by targeting K channels (Wolfart and Laker, 2015). BK_{ca} channels are abundantly present on glutamatergic presynaptic nerve terminals and also mossy fibres where they can organise glutamate release under the circumstance of disproportionate neuronal activity (Kaczorowski *et al.*, 1996). These are recruited only following conditions associated with massive Ca^{2+} ions accumulation in presynaptic nerve terminals like seizures (Hu *et al.*, 2001). BK_{ca} channel activation leads to hyperpolarisation induced inhibition of glutamate. Downregulation or loss of function of BK_{ca} channels alpha subunits was reported in the cortex as well as the hippocampus during the chronic phase of generalised tonic-clonic seizure and temporal lobe epilepsy (Young *et al.*, 2003). Penitrem, a BK_{ca} channel blocker, was reported as a trigger of epilepsy and status epilepticus (Young *et al.*, 2003). Neuroprotection can also be obtained by BK_{ca} channel opening in mitochondria of neuron by stimulation of neuronal respiration and decreases reactive oxygen species (ROS) thereby have importance in seizure-induced lesions (Rong *et al.*, 1999). Chin-Wei Huang et al., study results suggested that zonisamide mediated anti-epileptic action could be partly associated with the direct stimulation of BK_{Ca} channels expressed in hippocampal neurons inanition to its I_{na} channel blockade. Cilostazol inhibits adenosine uptake which interns lead to anticonvulsant activity by adenosine based mechanism.Adenosine release is more after the exuberant neuronal activity of convulsions as a neuromodulator (Dulla *et al.*, 2009). It is believed that increased levels of adenosine are due to a feedback mechanism that limits seizure intensity (Ilie *et al.*, 2012). Anticonvulsant activity of adenosine is due to A_1R receptors and potentate other standard anti-epileptic drugs (Tosh *et al.*, 2012). Angelatou, F. et, al., study showed A_1R receptor up-regulation in Seizures induced by pentylenetetrazol (PTZ) in mouse cortex, hippocampus, thalamus. Gleiter C.H et al., research showed that electroconvulsive shock-induced (ECS) seizure also increased the number of A_1R receptors in the cortex. Some regions overall excitatory glutamatergic transmission has been reduced, and a little effect on GABA was reported (Tosh *et al.*, 2012). Adenosine based mechanism also produces a neuroprotective effect.

CONCLUSIONS

The study exposed anticonvulsant activity of cilostazol (20 mg/kg i.p) against MES, PTZ induced convulsion animal models. Cilostazol (20 mg/kg i.p) was produced anticonvulsant activity when compared to control and cilostazol (10 mg/kg i.p). Between cilostazol (10 mg/kg i.p) and control, there was no significant difference. The potentiating effect of cilostazol with sodium valproate also demonstrated. Further research should be carried to evaluate anticonvulsant activity being there is an ambiguity.

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Conflict Of Interest

No interest in divergence.

REFERENCES

Dulla, C. G., Frenguelli, B. G., Staley, K. J., Masino, S. A. 2009. Intracellular Acidification Causes Adenosine Release During States of Hyperexcitability in the Hippocampus. *Journal of Neurophysiology*, 102(3):1984–1993.

Fisher, R. S., van Emde Boas, W., Blume, W., Elger, C., Genton, P., Lee, P., Engel, J. 2005. Epileptic Seizures and Epilepsy: Definitions Proposed by the International League Against Epilepsy (ILAE) and the International Bureau for Epilepsy (IBE). *Epilepsia*, 46(4):470–472.

Gad, S. C., Cassidy, C. D., Aubert, N., Spainhour, B., Robbe, H. 2006. Nonclinical Vehicle Use in Studies by Multiple Routes in Multiple Species. *International Journal of Toxicology*, 25(6):499–521.

Gupta, S. K. 2004. *Drug screening methods. 2nd edition*. Jaypee Brothers Medical Publishers (P) Ltd.

Hu, H., Shao, L.-R., Chavoshy, S., Gu, N., Trieb, M., Behrens, R., Laake, P., Pongs, O., Knaus, H. G., Ottersen, O. P., Storm, J. F. 2001. Presynaptic Ca2+-Activated K+Channels in Glutamatergic Hippocampal Terminals and Their Role in Spike Repo-

larization and Regulation of Transmitter Release. *The Journal of Neuroscience*, 21(24):9585–9597.

Ilie, A., Raimondo, J. V., Akerman, C. J. 2012. Adenosine Release during Seizures Attenuates GABAA Receptor-Mediated Depolarization. *Journal of Neuroscience*, 32(15):5321–5332.

Kaczorowski, G. J., Knaus, H. G., Leonard, R. J., McManus, O. B., Garcia, M. L. 1996. High-conductance calcium-activated potassium channels; Structure, pharmacology, and function. *Journal of Bioenergetics and Biomembranes*, 28(3):255–267.

Kulakarni, S. K. 2007. *Hand book of experimental pharmacology. 4th edition.*, volume 131 of *133*. Vallabh Prakashan Publishers.

Löscher, W., Klitgaard, H., Twyman, R. E., Schmidt, D. 2013. New avenues for anti-epileptic drug discovery and development. *Nature Reviews Drug Discovery*, 12(10):757–776.

Medhi, B. 2010. Practical Manual of Experimental and Clinical Pharmacology. *Jaypee Brothers Medical Publishers (P) Ltd*, pages 191–200.

N'Gouemo, P. 2011. Targeting BK (big potassium) channels in epilepsy. *Expert Opinion on Therapeutic Targets*, 15(11):1283–1295.

Rong, Y., Doctrow, S. R., Tocco, G., Baudry, M. 1999. EUK-134, a synthetic superoxide dismutase and catalase mimetic, prevents oxidative stress and attenuates kainate-induced neuropathology. *Proceedings of the National Academy of Sciences*, 96(17):9897–9902.

Strandness, D. E., Dalman, R. L., Panian, S., Rendell, M. S., Comp, P. C., Zhang, P., Forbes, W. P. 2002. Effect of Cilostazol in Patients with Intermittent Claudication: A Randomized, Double-Blind, Placebo-Controlled Study. *Vascular and Endovascular Surgery*, 36(2):83–91.

Sun, B., Le, S. N., Lin, S., Fong, M., Guertin, M., Liu, Y., Tandon, N. N., Yoshitake, M., ichi Kambayashi, J. 2002. New Mechanism of Action for Cilostazol: Interplay Between Adenosine and Cilostazol in Inhibiting Platelet Activation. *Journal of Cardiovascular Pharmacology*, 40(4):577–585.

Tellez-Zenteno, J. F., Patten, S. B., Jetté, N., Williams, J., Wiebe, S. 2007. Psychiatric Comorbidity in Epilepsy: A Population-Based Analysis. *Epilepsia*, 48:2336–2344.

Tosh, D. K., Paoletta, S., Deflorian, F., Phan, K., Moss, S. M., Gao, Z.-G., Jiang, X., Jacobson, K. A. 2012. Structural Sweet Spot for A1Adenosine Receptor Activation by Truncated (N)-Methanocarba Nucleosides: Receptor Docking and Potent Anticonvulsant Activity. *Journal of Medicinal Chemistry*, 55(18):8075–8090.

WHO 2020. epilepsy factsheet. *World Health Organization*.

Wolfart, J., Laker, D. 2015. Homeostasis or channelopathy? Acquired cell type-specific ion channel changes in temporal lobe epilepsy and their antiepileptic potential. *Frontiers in Physiology*, 6:168–168.

Wu, S.-N., Liu, S.-I., Huang, M.-H. 2004. Cilostazol, an Inhibitor of Type 3 Phosphodiesterase, Stimulates Large-Conductance, Calcium-Activated Potassium Channels in Pituitary GH3Cells and Pheochromocytoma PC12 Cells. *Endocrinology*, 145(3):1175–1184.

Young, K. L., Villar, D., Carson, T. L., Imerman, P. M., Moore, R. A., Bottoff, M. R. 2003. Tremorgenic mycotoxin intoxication with penitrem A and roquefortine in two dogs. *Journal of the American Veterinary Medical Association*, 222(1):52–53.