Research Article

An Experimental Study of the Anticonvulsant Effect of Chlorpheniramine Maleate in Mice

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ABSTRACT

Objective: The need for the rational development of newer and adjuvant drugs to treat epilepsy has prompted this study of the potential anticonvulsant effect of chlorpheniramine maleate. **Method** The acute effect was studied in mice in single doses of 1 mg/kg, 2 mg/kg and 4 mg/kg of chlorpheniramine maleate and the chronic effect was studied in doses of 1 mg/kg and 4 mg/kg (administered daily for 21 days) using the maximal electroshock seizure and pentylenetetrazole-induced seizure models of epilepsy. Sodium valproate and normal saline were used as the standard and control, respectively. **Key findings**: For the acute study, in the maximal electroshock seizure model, the administration of 1 mg/kg of chlorpheniramine maleate resulted in the complete abolition of seizures in 33 percent of the mice, and this was increased to 67 percent with the administration of 4 mg/kg. In the pentylenetetrazole-induced seizure model, the administration of 1 mg/kg and 2 mg/kg chlorpheniramine maleate protected 33 percent of the animals from mortality, and 67 percent were protected with the administration of 4 mg/kg. For the chronic study, in the maximal electroshock seizure model, the administration of 1 mg/kg chlorpheniramine maleate resulted in the complete abolition of seizures in 40 percent of the mice and in 60 percent, with the administration of 4 mg/kg. In the pentylenetetrazole-induced seizure model, 50 percent of the mice were protected from mortality with 1 mg/kg chlorpheniramine maleate and 60 percent, with 4 mg/kg chlorpheniramine maleate. **Conclusion**: These findings indicate that chlorpheniramine maleate may be a good candidate as an add-on therapy for epilepsy.

Keywords: Abolition of seizure, add-on therapy, chlorpheniramine maleate, anticonvulsant, epilepsy, maximal electroshock, pentylenetetrazole.

INTRODUCTION

Epilepsy is a neurological disorder in which a person has two or more recurrent unprovoked seizures. Seizure is a paroxysmal event that is due to abnormal, excessive and hypersynchronous discharge from an aggregate of central nervous system neurons[1]**.** Epilepsy is the second most common disorder of the central nervous system after stroke, with an incidence rate of 0.3%–0.5% of the population worldwide [1]. Approximately 3% of the population is expected to have epilepsy some time during their lifetime[2]**.** Although various new drugs with their own unique advantages have been introduced, they have failed to provide satisfactory seizure control in as many as 25% of patients; their dose-related neurotoxicities and other side effects are a major limitation in their clinical use[3]**.** Thus, there is an ever-increasing need for research into the pathophysiology of epilepsy and for the development of newer drugs for treating epileptic seizures. Adenosine is an inhibitory modulator of brain activity, by acting on its receptors, mainly on A_1 receptors in the hippocampus, it exerts predominant inhibitory effects[4]. These inhibitory actions of adenosine can be used therapeutically to suppress seizures[5] and are considered important for maintaining postictal depression[6] and for

restoring the metabolic equilibrium following seizures.

Since epilepsy is a chronic disease that requires long-term management, oral administration of the investigational drug daily over a period of time may be more appropriate in determining its efficacy than a single dose. Hence, unlike previous experiments that have been conducted, this study aimed to evaluate the anti-epileptic effect of chlorpheniramine maleate based on chronic (repeated doses) in addition to acute (single dose) administration.

EXPERIMENTAL

This study was conducted in the experimental pharmacology laboratory of Vels university, Chennai, India. The study was duly approved by the Institutional Ethics Committee. The anticonvulsant profile of chlorpheniramine maleate (acute and chronic effects) was evaluated in two conventional experimental models of epilepsy: the maximal electroshock seizure (MES) test and the pentylenetetrazole (PTZ)-induced seizure test in mice.

Animals

Inbred albino mice (Swiss strain) of both genders weighing 20–30 g were used for the study. The mice were housed in groups of three to five in clean polypropylene cages in the laboratory environment at a temperature of 24°C–27°C, with cross-ventilation, a natural day/night cycle and free access to food and water. The mice were screened 24 hours prior to the study. Only the mice that showed all phases of convulsion with the maximal electroshock current were selected. In total, 30 mice were included in the acute experimental model. These were distributed into five groups of six mice each, of which three were males and three were females. Of the five groups, Group I served as a control, while Groups II, III and IV received the test drug and Group V received sodium valproate as a standard drug.

A total of 40 mice were included in the chronic experimental model. These were distributed into four groups, each containing ten mice, of which five were male and five were female. Among these, Group I served as the control group, while Groups II and III received the test drug and Group IV received sodium valproate as a standard drug. For the preliminary dose selection study, six mice were tested for each level of dosage.

Dose and drug administration

Pure chemicals of chlorpheniramine maleate (Bafna Pharmaceuticals,Chennai, India), sodium valproate (Micronova Laboratory, Bangalore, Karnataka, India) and pentylenetetrazole (Genuine Chemicals Co, Mumbai, Maharashtra, India) were used in this study. The preparation and administration of drugs were conducted in the following way. The test drug, chlorpheniramine maleate, was dissolved in distilled water. The standard drug, sodium valproate, was dissolved in distilled water. Three test doses of 1 mg/kg, 2 mg/kg and 4 mg/kg chlorpheniramine maleate were administered in the acute study and two test doses of 1 mg/kg and 4 mg/kg chlorpheniramine were administered in the chronic study after the dose selection study was completed. This dose selection was done based on the findings of a preliminary study in which the anti-epileptic activity of chlorpheniramine maleate was observed in the MES test in increasing single doses of 1 mg/kg, 2 mg/kg, 4 mg/kg, 8 mg/kg and 16 mg/kg.

In the acute study, the drugs were administered intra peritonially in single doses at the maximum volume of 10 ml/kg one hour prior to the test procedure. In the chronic study, the drugs were administered intraperitonially at the maximum volume of 10 ml/kg once a day for 21 days. The mice were subjected to the test procedure one hour after the last dose on the 21st day.

Maximal electroshock (MES) test[8]

MES was induced in the mice using an electroconvulsiometer (Techno India Ltd, Ambala, Haryana, India). MES stimuli, comprising 0.2 seconds of rectangular positive pulses (48 mA at 60 Hz; pulse width 0.4 ms) were delivered through ear clip electrodes. Each mouse was administered the drug or normal saline (control) 30 minutes prior to receiving an electroshock. The anticonvulsant activity of the drug was evaluated based on its ability to protect (in %) against MES and decrease the duration of tonic hind limb extension, flexion and clonus in unprotected animals (i.e. only in animals in which seizures were not abolished).

Pentylenetetrazole(PTZ)-induced convulsion[8,9]

PTZ seizure was induced by administering a dose of 80 mg/kg PTZ intraperitoneally. Each mouse was administered the drug or normal saline (control) 60 minutes prior to the PTZ treatment. The mice were observed for the onset of clonus with a loss of the righting reflex, the onset of tonic hind limb extension and mortality. The anticonvulsant effect was evaluated based on the ability of the drug to prolong the duration of the latent period (i.e. the time taken for the onset of clonus with a loss of the righting reflex or tonic hind limb extension, whichever appeared first after the administration of PTZ) and its ability to decrease mortality.

Data analysis

The data is presented as mean ± standard error (SE). A comparison of the effect of the test drug with the control was done using one-way ANOVA followed by the post-hoc Bonferroni's test. The level of significance was measured using a two-tailed test. The various analyses were performed using the Statistical Package for the Social Sciences version 11.0 (SPSS India, Bangalore, Karnataka, India). A p-value < 0.05 was considered to be significant.

RESULTS

For the acute study, in the MES model (Table I), complete abolition of seizures was observed in 33%, 50% and 67% of mice in the groups administered 1 mg/kg, 2 mg/kg and 4 mg/kg chlorpheniramine maleate, respectively. In unprotected animals, a significant decrease in the duration of tonic hind limb extension was observed in the dosages of 2 mg/kg and 4 mg/kg chlorpheniramine maleate (6.67 ± 2.98 seconds at $\alpha = 0.01$ and 4.33 ± 2.75 seconds at $\alpha = 0.005$, respectively, as compared to 16.33 ± 0.56 seconds in the control group). A significant decrease in clonus was observed in the dosage of 4 mg/kg (1.33 ± 1.33 seconds at $\alpha = 0.01$ as compared to 10 ± 0.26 seconds in the control group). There was no significant decrease in the duration of tonic hind limb extension in the dosage of 1 mg/kg chlorpheniramine maleate. There was no significant effect in the flexion at any level of dosage. The maximal effect observed with chlorpheniramine maleate at any of the above dosages did not exceed that of sodium valproate in any of the above parameters.

In the PTZ-induced seizure model (Table II), complete protection from PTZ-induced mortality was observed in 33% of the mice in the groups administered 1 mg/kg and 2 mg/kg chlorpheniramine maleate , and in 67% of the mice in the group administered 4 mg/kg chlorpheniramine maleate. In unprotected animals, a significant increase in the latent period was observed in the dosage of 4 mg/kg chlorpheniramine maleate (142.50 ± 18.35 as compared to 82.00 ± 7.36 seconds in the control group, at $\alpha = 0.05$). A significant increase in the duration of the latent period was not observed in the dosages of 1mg/kg and 2 mg/kg chlorpheniramine maleate. The maximal effect did not exceed that of sodium valproate at any dosage in any of the above parameters.

For the chronic study, in the MES model (Table III), complete abolition of seizures was observed in 40% and 60% of mice in the groups administered 1 mg/kg and 4

mg/kg chlorpheniramine maleate, respectively. In unprotected animals, a significant decrease in the duration of tonic hind limb extension was observed in the dosages of 1 mg/kg and 4 mg/kg chlorpheniramine maleate (6.9 ± 1.89 seconds at α = 0.01 and 4.8 ± 1.98 seconds at α = 0.005, respectively, as compared to 16.0 ± 0.47 seconds in the control group). A significant decrease in clonus was observed in the dosages of 1 mg/kg and 4 mg/kg (4.3 ± 1.21 seconds at α = 0.01 and 3.6 ± 1.61 seconds at α = 0.005, respectively, as compared to 10.6 ± 0.37 seconds in the control group). The decrease in the duration of flexion was significant only at the dosage of 4 mg/kg chlorpheniramine maleate (0.60 ± 0.25 seconds as compared to 1.45 ± 0.05 seconds in the control group, at α = 0.05). The maximal effect of chlorpheniramine maleate at any dosage did not exceed that of sodium valproate in any of the above parameters.

In the PTZ-induced seizure model (Table IV), complete protection from PTZ-induced mortality was observed in 50% and 60% of the mice in the groups administered 1 mg/kg and 4 mg/kg chlorpheniramine maleate, respectively. In unprotected animals, a significant increase in the latent period was observed at the dosage of 1 mg/kg and 4 mg/kg chlorpheniramine maleate (116.3 ± 9.60 seconds at α = 0.05 and 150.3 ± 14.02 seconds at α = 0.005, as compared to 63.61 ± 3.82 seconds in the control group). The maximal effect of chlorpheniramine maleate at any dosage did not exceed that of sodium valproate in the above parameter.

DISCUSSION

The aim of this study was to evaluate the anticonvulsant effects of chlorpheniramine maleate using the MES and PTZ-induced seizure models. In the MES model, complete abolition of seizure (protection) was observed in 67% of animals that were administered the highest dose (4 mg/kg) in the acute study, which was equal to that of sodium valproate. 60% of the animals were protected in the chronic study compared to 70% for sodium valproate. In unprotected animals, chlorpheniramine maleate decreased the duration of tonic hind limb extension and clonus in a dose-dependent manner in the acute study. However, the lowest dosage (1 mg/kg) on a single-dose administration did not show statistically significant effects with these parameters, whereas the data was significant after continuous administration of the same dosage for 21 days in the chronic study. This finding suggests that the efficacy of this drug improves with long-term administration. In the acute study of the PTZ-induced seizure model, chlorpheniramine maleate increased the latent period in a dose dependent manner. The lowest dosage of 1 mg/kg did not show significant effects in this parameter. However, the data was significant after continuous administration of the same dosage for 21 days in the chronic study. Despite this, the protection from mortality with the highest dose remained at 60%, as in the case of sodium valproate.

The effectiveness of chlorpheniramine maleate in the above epileptic models suggests the role of the blockade of A1 receptors in mediating anti-epileptic mechanisms[7]. Prior administration of chlorpheniramine maleate has resulted in significant protection against MES as well as PTZ-induced seizures in both the acute and chronic studies. However, the maximal effect did not exceed that of sodium valproate at any level of dosage. Hence, chlorpheniramine maleate can only be considered as an adjuvant drug rather than as an alternative to sodium valproate.

CONCLUSION

This study has supported and substantiated the hypothesis of the possible therapeutic role played by adenosine receptors in epilepsy. Further experimental and clinical studies are required on the administration of chlorpheniramine maleate concurrently with other standard anti-epileptic drugs in order to assess its efficacy as an adjuvant drug.

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Table I: Acute study: maximal electroshock seizure (MES) test (n = 6 in each group)

Treatment group	Dose (ml/kg)	Mean ± SE(sec)			Abolition of seizures †%
		Flexion	THLE	Clonus	
Control (normal saline)	10	1.33 ± 0.17	16.33 ± 0.56	10 ± 0.26	0
Chlorpheniramine maleate	1	0.92 ± 0.30	9.67 ± 3.24	6.5 ± 2.14	33
	2	0.75 ± 0.33	6.67 ± 2.98*	4.33 ± 1.98	50
	4	0.50 ± 0.32	4.33 ± 2.75 **	1.33 ± 1.33*	67
Sodium valproate	100	0.50 ± 0.32	4.17 ± 2.64	2 ± 1.29	67

*Significant at α= 0.01, **Significant at α = 0.005 compared to the control group.
†Denotes the abolition of the tonic hind limb extension and is considered as the end point of the test.
THLE: tonic hind limb extension; SE: standard error

Table II. Acute study: pentylenetetrazole-induced test (n = 6 in each group)

Treatment group	Dose (ml/kg)	Mean latent period ± se(sec)	Mortality (%)
Control (normal saline)	10	10 82.00 ± 7.36	83.33
Chlorpheniramine maleate	1	100.67 ± 8.81	67
	2	123.50 ± 8.43	67
	4	142.50 ± 18.35*	33
Sodium valproate	100	142.50 ± 18.35*	33

*Significant at α = 0.05 compared to the control group.
SE: standard error

Table III. Chronic study: maximal electroshock seizure test (n = 10 in each group)

Treatment group	Dose (ml/kg)	Mean ± SE(sec)			Abolition of seizures †%
		Flexion	THLE	Clonus	
Control (normal saline)	10	1.45 ± 0. 05	16.0 ± 0.47	10.6 ± 0.37	0
Chlorpheniramine maleate	1	0.90 ± 0.24	6.9 ± 1.89**	4.3 ± 1.21**	40
	4	0.60 ± 0.25*	4.8 ± 1.98 ***	3.6 ± 1.61***	60
Sodium valproate	100	0.45 ± 0.23	3.7 ± 1.88	2.0 ± 1.02	70

*Significant at α = 0.05, **Significant at α = 0.01, ***Significant at α = 0.005 compared to the control group.
†Denotes the abolition of the tonic hind limb extension and is considered as the end point of the test.
THLE: tonic hind limb extension; SE: standard error.

Table IV. Chronic study: pentylenetetrazole-induced seizure test (n = 10 in each group)

Treatment group	Dose (ml/kg)	Mean latent period ± se(sec)	Mortality (%)
Control (normal saline)	10	63.61 ± 3.82	100
Chlorpheniramine maleate	1	116.3 ± 9.60*	50
	4	150.3 ± 14.02**	40
Sodium valproate	100	167.3 ± 16.86	40

*Significant at α= 0.05, **Significant at α = 0.005 compared to the control group. SE: standard error

REFERENCES

1. Lowenstein DH. Seizure and epilepsy. In: Braunwald E, Fauci AS, Hauser SL, et al, eds. Harrison's Principles of Internal Medicine. 15th ed. New York: McGraw-Hill, 2001;2354-69.
2. Annegers JF. The epidemiology of epilepsy. In: Wyllie E, ed. The Treatment of Epilepsy: Principles and Practice. 2nd ed. Baltimore: Williams & Wilkins, 1997:165-72.
3. Schwartzkroin PA. Wyler AR. Mechanisms underlying epileptiform burst discharge. Ann Neurol 1980;7:95-107.
4. Johansson B. Hyperalgesia, anxiety, and decreased hypoxic neuroprotection in mice lacking the adenosine A1 receptor. Proc Ntl Acad Sci. USA 2001;98:9407-12.
5. Huber A. grafts of adenosine – releasing cells suppress seizures in kindling epilepsy. Proc Ntl Acad Sci. USA 2001;98:7611-16.
6. Etherington LA, Frenguelli BG, Endogenous adenosine modulates epileptiform activities in rat hippocampus in a receptor subtype - dependent manner. Eur. J Neurosci 2004;19:2539-50.
7. Manjunath S, Pranav kumar M and Sakhare. Adenosine and adenosine receptors: a newer therapeutic perspective. Indian j pharmacol 2009:41:97-105.
8. Francisco A Oliveira, Reinaldo N de Almeida, Maria de FaÂtima V Sousa, JoseÂ M Barbosa-Filho, Silmara A Diniz and Isac A de Medeiros. Anticonvulsant properties of N-salicyloyltryptamine in mice. Pharmacology, Biochemistry and Behavior. 2001;68: 199±202.
9. David N, Johnson A, Steve H, White B, Ewart A, Swinyard B, Timothy E, Albertson C, Brian F and Kilpatrick A. AHR-12245: A potential anti-absence drug. Epilepsy Res.1991;8:64-70.