ORIGINAL ARTICLE

Effect of artesunate on maximal electroshock and pentylenetetrazole-induced seizures in albino mice

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Artemisinin-based combination therapies are highly efficacious, and they are now listed as first-line therapies for uncomplicated malaria in most countries where malaria is endemic. Neurotoxicity of artemisinins is a growing concern. However, no studies have reported its antiepileptic or epileptogenesis potential, hence the present study was undertaken to explore the activity of artesunate in experimentally induced seizures in rodent models. Artesunate at doses 36.4 and 72.8 mg/kg respectively significantly reduced the duration of the hind limb extensions (3.033±1.493 and 2.033±1.383, respectively) when compared to the control (P<0.0001) in the maximal electroshock-induced seizure model. However, no significant decrease was noted in the duration of clonic convulsions in a pentylenetetrazole-induced seizure model indicating lack of activity in petit mal epilepsy. The results of the present study indicate that artesunate at both the doses employed showed a significant anticonvulsant activity in the maximum electroshock-induced seizure model suggesting its potential utility in the management of generalized tonic-clonic seizures and partial seizures. Further studies regarding its mechanism of action are warranted.

Key words: Anticonvulsant, artesunate, neurotoxicity, pentylene tetrazole-induced seizures and maximum electroshock-induced seizures

INTRODUCTION

Malaria is a leading cause of mortality and morbidity in developing areas of the world, and remains a major public health problem in endemic regions.[1] Resistance to available drugs is increasing, creating a need for new drugs that are well tolerated and simple to use. In the face of this ominous situation, artemisinin and its derivatives (artesunate, artemether, arteether, and dihydroartemisinin) have given renewed hope for combating resistant malaria.[2,3] These drugs have gained considerable prominence in the chemotherapy of both uncomplicated and severe falciparum malaria by demonstrating high activity against multidrug-resistant falciparum strains with low toxicity profiles.

Artesunate is a semi-synthetic derivative of artemisinin, the active compound of the Chinese herb *A. annua* which consists of the sodium succinyl salt of dehydroartemisinin.[4] Artesunate and its active metabolite dihydroartemisin are potent blood schizonticides, highly effective against multi-drug-resistant strains of *Plasmodium falciparum* and hence widely used for the treatment and management of malaria.[5]

Neurotoxicity is the greatest concern regarding artemisinins because the administration of high doses in laboratory animals has led to severe and irreversible changes in the brain. Several studies have shown that high doses of artesunate can produce selective damage to brainstem centres, gait disturbances in mice and rats[6-8] and loss of spinal cord and pain response mechanisms in animals.[8,9] Others showed some varying degree of cell clustering, cellular hypertrophy, and intercellular vacuolations in the stroma of the superior colliculus of artesunate-treated animals.[10]

Various studies have reported the central properties of the essential oil and the crude ethanol extract from aerial parts of *A. annua* L,[11] from which artesunate have been derived. One study reported the GABA–A agonistic activity of two flavonoids present in the plant *A. herba–alba*[12] which closely resembles *A. annua* plant from which artesunate has been derived.

The pharmacological actions of artesunate on the central nervous system have yet to be explored in detail. So in this present study, the effect of artesunate on seizures was evaluated to help in understanding the epileptogenic/antiepileptic activity of artesunate, which

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is being widely used in hospitals for chloroquine-resistant falciparum malaria.

MATERIALS AND METHODS

Animals

Healthy adult Swiss albino mice of either sex weighing 20–25 g and Wistar albino rats of either sex weighing 150–200 g were used in the study. These animals were housed in groups of 6 mice per cage and 3 rats per cage with dust free rice husk as a bedding material under laboratory conditions with controlled environment of temperature 25°±2°C, humidity (60%±10%) and 12 h light/dark cycle as per CPCSEA guidelines. The experiments were carried out during 1200–1400 h. Animals had free access to food and water ad libitum. The animals were acclimatized with laboratory conditions one week prior to experimentation. The animals were fasted overnight before the experiment. The study protocol was approved by the Institutional Animal Ethics Committee.

Drugs

Pentylenetetrazol (Sigma, USA), Artesunate (Zydus Cedilla, India), phenytoin (Parke Davis, India) and sodium valproate (Cabbott Ltd, India) were used in this study. The drugs were dissolved in normal saline for injection and administered in a volume of 5 ml/kg to both rats and mice. The doses were selected by extrapolating from the human dose, with help of the conversion table mentioned in Ghosh.[13]

Assessment of Anticonvulsant Activity

Electrically-induced seizures: Maximal electroshock (MES)-induced seizures (MES) in rats.

In the electrically induced seizure experiment, the MES method described previously by Swinyard was employed.[14] The animals were chosen by preliminary screening. Rats which showed extension of hind limb were included in the study. The animals were divided into four groups of six rats each. Group Ia received normal saline (1 ml/kg body weight) and served as control. Groups IIa and IIIa received the test drug artesunate at a dose of 36.4 mg/kg I.P and 72.8 mg/kg I.P., respectively. Group IVa received the standard drug Phenytoin (25 mg/kg I.P.). The drugs and normal saline were given one hour prior to induction of convulsions. The seizures were induced by maximal electroshock in albino rats with the help of an electroconvulsiometer (Inco Electroconvulsiometer model #100-3) by passing a current of 150 mA for 0.2 s using ear clip electrodes. The animals were observed for the extensor phase as well as its duration and post-ictal depression. The duration of tonic extension of hind limb was used as end point, i.e. prevention or a decrease in the duration of hind limb extension was considered as a protective action.

Pentylenetetrazole-induced Seizures in Mice

The albino mice were selected 2 weeks prior to conducting the experiment by injecting the pentylenetetrazole, a standard convulsing agent in a dose of 30 mg/kg subcutaneously in the scruff of neck. Only those mice which showed clonic convulsions within 30 min during preliminary examination were chosen for the present study.

The animals were divided into four groups of six rats each. Group Ib received normal saline (1 ml/kg body weight I.P.) and served as control. Groups IIb and IIIb received the test drug artesunate at a dose of 36.4 and 72.8 mg/kg I.P., respectively. Group IVb received the standard drug, sodium valproate (75 mg/kg I.P.). PTZ (30 mg/kg, S.C.) was administered after 1 h of administration of the test drug and normal saline. Animals were observed for 30 min after injection of PTZ.

The anticonvulsant property of artesunate in this model was assessed by its ability to delay the onset of myoclonic spasm and clonic convulsions. Protection against PTZ-induced convulsions and percentage of mortality was measured.

Statistical Analysis

The data were expressed as mean±S.E.M or percentage. One-way analysis of variance (ANOVA) followed by the *post hoc* Dunnett's multiple comparison test was used for data expressed in mean±E.M, using sigma stat software (Version 2.0, jandel scientific Inc., USA). Differences between means were considered to be significant at $P<0.01$, whereas the *z*-test was applied for the data expressed in percentage and in this case the '*P*' value less than 0.05 was considered significant when compared to control.

RESULTS

Maximal Electroshock-induced Seizure

Maximal electroshock produced hind limb tonic extension seizures (HLTE) in all the animals used. The vehicle-treated rats showed tonic hind limb extension for a total duration of 14.441±0.643 s. Artesunate at doses 36.4 and 72.8 mg/kg respectively significantly reduced the duration of the hind limb extensions ($P<0.0001$) when compared to the control as shown in Table 1. The standard antiepileptic drug, phenytoin completely inhibited the MES-induced tonic

Table 1: Effect of artesunate on MES seizures

Drug treatment	Dose (mg/kg)	Duration of hind limb extension (seconds) (mean±SEM) *n*=12
Normal saline		14.441±0.643
Artesunate (A1)	36.4	3.033±1.493**
Artesunate (A2)	72.8	2.033±1.383**
Phenytoin (P)	25.0	0±0.000**

One-way ANOVA; Values are expressed as mean±SEM; **$P<0.0001$ = Considered highly significant; MES seizures – Maximal electroshock-induced seizure

seizures in all the animals used. There was a significant absence of mortality in artesunate and phenytoin groups. However, two deaths were reported in the control group.

Pentylenetetrazole-induced Seizure

Pentylenetetrazole produced tonic seizures in all the animals used. At both doses (36.4 and 72.8 mg/kg), artesunate did not affect the duration of convulsions to any significant extent [Table 2]. The standard antiepileptic sodium valproate however showed a significant decrease in the mean duration of convulsions (P<0.0001). Five and four cases of mortality were reported in the control group and artesunate-treated groups, respectively. No mortality was observed in the sodium valproate group.

Table 2: Effect of artesunate on PTZ seizures

Treatment	Dose (mg/kg)	Duration of convulsions (seconds) (mean±SEM) n=12
Normal saline		12.161±0.55
Artesunate	36.4	19.46±2.34†
Artesunate (A2)	72.8	17.01±1.81†
Sodium valproate (V)	75.0	1±0.65**

One-way ANOVA; **P<0.0001 = Considered highly significant; †P>0.05 = Considered not significant; PTZ seizures – Pentylenetetrazole-induced Seizure

DISCUSSION

Artemisinins have emerged as the leading drugs for malaria. Neurotoxicity is the greatest concern regarding artemisinins because the administration of high doses in laboratory animals has led to severe and irreversible changes in the brain.[15] One study comparing oral artesunate versus quinine-tetracycline in acute malaria reported the occurrence of convulsions in one patient after two doses of artesunate; this has not been reported in previous clinical trials of the drug.[16] However, other studies have reported that the incidence of convulsions with artesunate are less when compared to quinine.[17] Hence, this study was designed to explore the anticonvulsant effects, if any of artesunate in electric shock and PTZ-induced seizures in rodents.

The maximal electroshock test is the most widely used animal model in antiepileptic drug discovery because seizure induction is simple and the predictive value for detecting clinically effective antiepileptic is high. This method identifies the drug with activity against generalized tonic-clonic seizures and partial seizures using clinically established antiepileptic drug. The pharmacology of acute maximal electroshock dose not differ from the pharmacology of generalized tonic-clonic seizures in genetic models with chronic epilepsy.[18] It has often been stated that antiepileptic drugs that block MES-induced tonic extension act by blocking seizure spread. Moreover, MES-induced tonic extension can be prevented either by drugs that inhibit voltage-dependent Na+ channels, such as phenytoin, valproate, felbamate and lamotrigine, or by drugs that block glutamatergic excitation mediated by the *N*-methyl-daspartate (NMDA) receptor, such as felbamate.[19]

The results of the present study indicate that artesunate at both the doses employed showed a significant anticonvulsant activity in the MES-induced seizure model. Thus, the anticonvulsant activity exhibited by Artesunate shows that it could have blocked the seizure spread by inhibiting either Na+ channels and/or glutamatergic excitation through NMDA receptors. The study also suggests its potential utility in the management of generalized tonic-clonic seizures and partial seizures.

By contrast, the pentylenetetrazole test represents a valid model for human generalized myoclonic and also absence seizures In general, compounds with anticonvulsant activity in the petit mal epilepsy are effective in pentylenetetrazole-induced seizure model.[20] The observations emanated in the present study indicate that no significant protection was observed in the artesunate-treated groups against PTZ-induced seizures and hence its lack of activity against PTZ-induced seizures suggests that is unlikely to be useful in petit mal epilepsy. Several studies[21,22] have shown that NMDA receptor antagonists are more effective in antagonizing MES-induced seizures, as compared to PTZ/picrotoxin-induced seizures. Hence, the role of artesunate on NMDA receptors need to be further explored.

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REFERENCES

1. Breman JG, Alilio MS, Mills A. Conquering the intolerable burden of malaria: What's new, what's needed: A summary. Am J Trop Med Hyg 2004;71(2 Suppl):1-15.
2. Hein TT. An overview of the clinical use of artemisinin and its derivatives in the treatment of falciparum malaria in Vietnam. Trans R Soc Trop Med Hyg 1994;88S:7-8.
3. Harinasuta T, Karbwang J. Qinghaosu: A promising antimalarial. J Am Med Assoc South East Asian edn 1994;34:7-8.
4. Ittarat W, Udomsangpeth R, Chotivanich KT, Looareesuwan S. The effects of quinine and artesunate treatment on plasma tumor necrosis factor levels in malaria-infected patients. Southeast Asian J Trop Med Public Health 1999;30:7-10.
5. van Agtmael MA, Cheng-Qi S, Qing JX, Mull R, van Boxtel CJ. Multiple dose pharmacokinetics of artemether in Chinese patients with uncomplicated falciparum malaria. Int J Antimicrob Agents 1999;12:151-8.
6. Nontprasert A, Pukrittayakamee S, Dondorp AM, Clemens R, Looareesuwan S, White NJ. Neuropathologic toxicity of artemisinin derivatives in a mouse model. Am J Trop Med Hyg 2002;67:423-9.

7. Nontprasert A, Nosten-Bertrand M, Pukrittayakamee S, Vanijanonta S, Angus BJ, White NJ. Assessment of the neurotoxicity of parenteral artemisinin derivatives in mice. Am J Trop Med Hyg 1998;59:519-22.
8. Genovese RF, Petras JM, Brewer TG. Arteether neurotoxicity in the absence of deficits in behavioral performance in rats. Ann Trop Med Parasitol 1995;89:447-9.
9. Dayan AD. Neurotoxicity and artemisinin compounds: Do the observations in animals justify limitations in clinical use? Med Trop (Mars). 1998;58(3 Suppl):32-7.
10. Eweka AO, Adjene JO. Histological studies of the effects of oral administration of artesunate on the superior colliculus of adult wistar rats. Internet J Trop Med 2008;4:1-9
11. Perazzo FF, Carvalho JC, Carvalho JE, Rehder VL. Central properties of the essential oil and the crude ethanol extract from aerial parts of Artemisia annua L. Pharmacol Res 2003;48:497-502.
12. Salah SM, Jager AK. Two flavonoids from Artemisia herba-alba Asso with *in vitro* GABAA-benzodiazepine receptor activity. J Ethnopharmacol 2005;99:145-6.
13. Ghosh MN. Fundamentals of experimental pharmacology. Calcutta: Scientific book agency; 1984. p. 154-5.
14. Swinyard EA. Laboratory evaluation of antiepileptic drugs. Review of laboratory methods. Epilepsia 1969;10:107-19.
15. Brewer TG, Grate SJ, Peggins JO, Weina PJ, Petras JM, Levine BS, *et al*. Fatal neurotoxicity of arteether and artemether. Am J Trop Med Hyg 1994;51:251-9.
16. Karbwang J, Na-Bangchang K, Thanavibul A, Bunnag D, Chongsuphajaisiddhi T, Harinasuta T. Comparison of oral artesunate and quinine plus tetracycline in acute uncomplicated falciparum malaria. Bull World Health Organ 1994;72:233-8.
17. Dondorp AM, Fanello CI, Hendriksen IC, Gomes E, Seni A, Chhaganlal KD, *et al***.** Artesunate versus quinine in the treatment of severe falciparum malaria in African children (AQUAMAT): An open-label, randomised trial. Lancet 2010;376:1647-57.
18. Duraisami R, Srinivasan D, Subramanian P. Anticonvulsant activity of bioflavonoid gossypin. J Bangladesh Pharmacol Soc 2009;4:51-4.
19. Silambujanaki P, Chitra V, Kumari S, Sankari M, Raju D, Bala Tejo Chandra Ch. Anti-convulsant activity of methanolic extract of *Butea monosperma* leaves. Res J Pharm Biol Che Sci 2010;1:431-5.
20. Loscher W, Schmidt D. Which animal models should be used in the search for new antiepileptic drugs? A proposal based on experimental and clinical considerations. Epilepsy Res 1988;2: 145-18.
21. Lehmann J, Hutchison AJ, McPherson SE, Mondadori C, Schmutz M, Sinton CM, *et al*. CGS 19755, a selective and competitive N-methyl-D-aspartate-type excitatory amino acid receptor antagonist. J Pharmacol Exp Ther 1988;246:65-75.
22. Kulkarni SK, Mehta AK, Ticku MK. Comparison of anticonvulsant effect of ethanol against NMDA-, kainic acid- and picrotoxin-induced convulsions in rats. Life Sci 1990;46:481-7.

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