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Effect of Acute and Chronic Administration of Losartan, Atorvastatin and Their Combination on Animal Models of Epilepsy.

Sanjeev Kumar[1], Megha M[2], Bharti Chogtu[3]*, and KL Bairy[3].

[1]Department of Pharmacology, Sukh Sagar Medical College & Hospital, Jabalpur, Madhya Pradesh, India.
[2]Department of Pharmacology, Chamarajanagar Institute of Medical Science, Chamarajanagar, Karnataka, India.
[3]Department of Pharmacology, Kasturba Medical College, Manipal University, Manipal, Karnataka, India.

ABSTRACT

To study and compare the antiepileptic effect of losartan, atorvastatin and their combination in maximal electroshock (MES) and pentylenetetrazole (PTZ) induced rat models of epilepsy. The study was divided into acute and chronic stages. In acute study, 60 rats were taken and divided into 30 in each MES & PTZ model. Seizure was induced using maximum electroshock (MES) and pentylenetetrazole (PTZ). In each model, animals were further divided into five groups (n=6). Rats from group I to group V were treated with gum acacia 2% (1ml/kg), phenytoin (25mg/kg) in MES model or sodium valproate (200mg/kg) in PTZ model, Losartan (50mg/kg), Atorvastatin (10mg/kg) and Losartan + Atorvastatin (50mg+10mg/kg) respectively. Chronic study was performed in a similar way as acute study after dosing same drugs and dose for duration of six weeks. Hind limb extension time was noted in MES model whereas latency for myoclonic jerk, seizure and mortality in in PTZ model. In acute study, losartan showed significant anti-seizure activity in both the models while atorvastatin and combination did not show any effect. In chronic treatment losartan showed significant effect in both MES and PTZ model whereas 83.33% of survival rate was observed in atorvastatin group. Losartan has broad spectrum activity against seizure while atorvastatin is efficacious only on chronic treatment. Combination of these two drugs offers no advantage against seizure.

Keywords: losartan, atorvastatin, anti-seizure, MES, PTZ, acute, chronic

Corresponding author

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INTRODUCTION

Epilepsy is a common neurological disorder characterized by recurrent epileptic seizures that affect 1–3% of the world's population [1]. Approx. 70% of seizures are controlled by available antiepileptic drugs [1,2] but because of their toxic profile, narrow therapeutic index and drug resistance there is always need to search for better tolerated antiepileptic drug having novel mechanism of action.

Losartan is an angiotensin receptor blocker which blocks AT_1 receptor at various sites. High AT_1 receptor densities in many regions of central nervous system support a role for neurally derived angiotensin as a neuromodulator [3]. Study shows that losartan by blocking these receptors significantly impairs the triggering and maintenance of epileptic seizures in female wistar audiogenic rats [1]. But there is lack of studies on the effect of losartan on maximum electroshock [MES] and pentylenetetrazole [PTZ] model of epilepsy. Atorvastatin is widely used hypolipidemic having pleotropic effects like improving endothelial function, enhancing stability of plaques, decreasing oxidative stress, inflammation and inhibiting thrombosis has encouraged its study in various other possible pathological conditions [4]. The anticonvulsant activity of statins is most probably because of its antioxidant property [5]. Patients with cardiovascular diseases like hypertension and hyperlipidemia are commonly prescribed losartan and atorvastatin together.

As both these drugs are used in cardiovascular diseases and have potential for anticonvulsant action, the study was planned to evaluate the effect of these drugs individually and in combination. The study aimed to compare the acute and chronic effect of losartan with atorvastatin and losartan + atorvastatin in animal models of epilepsy seizures.

MATERIAL AND METHODS

The study was conducted after clearance from Institutional Animal Ethics Committee (IAEC). All procedures used in this study were reviewed and approved by IAEC.

Animals

Rats (n=6) of both sexes weighing 150 to 250 g were kept separately in polypropylene cages (U.N. Shah Manufacturers, Mumbai) and maintained under standard laboratory conditions in central animal house approved by the Committee for the Purpose of Control and Supervision on Experiments on Animal (CPCSEA). They were maintained at a room temperature and relative humidity of 45-55%. A 12-hour light: dark cycle was followed. They were provided with standard feed and water ad libitum.

Drugs and chemicals

Losartan and atorvastatin (Zydus healthcare), phenytoin (Kare labs pvt ltd), sodium valproate (Torrent Pharmaceuticals) and Pentylenetetrazole (Sigma-aldrich) were used for the study.

Study design

A total of 120 Wistar rats were used for the study. The entire study was done in two stages; Stage-I: acute study & Stage-II: chronic study.

Acute study was done on 60 rats with two models- maximum electroshock (MES) seizure and pentylenetetrazole (PTZ) induced seizure model. Each model had five groups (n=6).

For chronic study animals were administered drugs for six weeks and the anti-seizure activity was assessed.

Acute study

A total of 60 equal numbers of male and female rats were randomly allotted to two models in which each model consisted of five groups.

Maximal electroshock (MES) induced seizure model

This is one of the most widely and best studied animal model of seizures. Animals were pre-screened to check for their ability to develop full tonic extension by giving the shock 24 hours before the day of experiment and all the rats showed full tonic extension. Group I to group V were treated with gum acacia 2% (1ml/kg), phenytoin (25mg/kg), losartan (50mg/kg), atorvastatin (10mg/kg) and losartan + atorvastatin (50mg+10mg/kg) respectively. All the drug dosages were selected as per previous studies [1, 6]. Rats were treated with drugs and after one hour seizures were induced by an electro-convulsiometer (Technoelectronics Ltd., Lucknow, India) as described by Toman et al with a current of 150 mA, 50Hz delivered through the ear clip electrode for 0.2 sec [7]. Duration of hind limb tonic extension (HLTE) was recorded and percentage protection of seizure was calculated. The abolition or reduction in the duration of tonic extension was considered as the index for antiepileptic activity. The tonic component was judged as abolished if the hind limb extension did not exceed 90 degree angle with the plane of the body [8].

Pentylenetetrazole (PTZ) induced seizure model

Pentylenetetrazole induces myoclonic jerks and generalized tonic seizures in rats. It is used to identify drugs useful in absence seizures. This model also indicates the possible mechanism of action of new drugs acting through GABA. In this model group I to group V were treated with gum acacia 2% (1ml/kg), sodium valproate (200mg/kg), losartan (50mg/kg), atorvastatin (10mg/kg) and losartan + atorvastatin (50mg+10mg/kg) respectively. All the drug dosage was selected as per the previous studies [1, 6]. Pentylenetetrazole 60mg/kg body weight was injected intraperitoneally one hour after the administration of test drugs. Each animal was placed in individual cage and observed for 30 minutess. Onset of myoclonic jerk and generalized tonic seizures were observed. Animals were further observed upto 72hr for mortality [8].

Chronic study

A total of 60 rats were randomly allotted to two models in which each model consist of five groups. Animals were treated orally once a day by gavage for six weeks between 15:00 and 17:00 hours. Experimental procedure, drugs and end points at the end of six weeks were the same as it was in acute study for both MES and PTZ induced seizure model.

Statistical analysis

All data were expressed as mean ± standard error of mean (SEM). Statistical difference between various groups were analyzed by one-way analysis of variance test (ANOVA), followed by post-hoc Tukey's test using SPSS version 16.0. A P value ≤ 0.05 was considered as statistically significant.

RESULTS

Acute study

MES induce tonic seizure

Table 1: Seizure protection rate and hind limb extension in the acute MES model.

Groups	Seizure protection rate (%)	HLTE (seconds)
Control (2% gum acacia)	0%	10.83 ± 0.30
Phenytoin (25mg/kg)	33.33%	2.50 ± 0.80***
Losartan (50mg/kg)	0%	6.33 ± 0.33**
Atorvastatin (10mg/kg)	16.6%	7.33 ± 1.68
Losartan + Atorvastatin (50mg+10mg/kg)	0%	9.83 ± 0.47

HLTE, Hind limb tonic extension.
***P ≤ 0.001, **P ≤ 0.01 as compared to control

All the rats in control group showed hind limb tonic extension (HLTE) with latency of 1.83 ± 0.16 seconds. There was significant (p ≤ 0.02) increase in latency for HLTE in losartan and atorvastatin (ATV) treated groups. When compared to control, phenytoin (p≤0.001) and losartan (p≤ 0.01) significantly reduced the total duration of HLTE in their respective groups. The ATV group and combination group did not show any additive or beneficial effect on latency or duration of HLTE in comparison to control. The phenytoin and ATV treated group showed 33.33% and 16.66% seizure protection respectively. (Table 1)

Pentylenetetrazole induced seizures

Latency for the onset of first clonus

The control group showed an average of ~54 seconds for the onset of first clonus. As compared to control, sodium valproate and losartan significantly (p ≤ 0.001) increased the latency whereas ATV and combination increased latency which was not significant. (Table 2)

Latency for the onset of generalized seizures

Sodium valproate and losartan treated groups showed significant (p ≤ 0.001) increase in the latency for the onset of generalized tonic clonic seizure as compared to control. There was also a non-significant increase in latency in ATV group and the combined group as compared to control group.

Mortality

There was no mortality with sodium valproate and losartan treated group. There was 83.33% with control and 50% with ATV and combination observed after 72 hrs.

Table 2: Latency for the onset of first clonus and generalized tonic clonic seizure with mortality (%) in acute study.

Groups	Time for onset of first clonus (sec)	Time for onset of seizure (sec)	Mortality (%)
Control (2% gum acacia)	53.66 ± 3.00	61.50 ± 3.00	83.33%
Sodium valproate (200mg/kg)	124.00 ± 6.34***	300.00 ± 22.00***	0%
Losartan (50mg/kg)	117.00 ± 5.19***	289.00 ± 17.97***	0%
Atorvastatin (10mg/kg)	56.50 ± 2.70	100.00 ± 8.00	50%
Losartan + Atorvastatin (50mg+10mg/kg)	50.16 ± 3.28	57.50 ± 3.47	50%

***P ≤ 0.001, **P ≤ 0.01 as compared to control

Chronic study

MES induce tonic seizure

Animals in control group showed an average of ~11 seconds hind limb tonic extension. Phenytoin (p≤ 0.001) and losartan (p ≤ 0.01) treated group significantly reduced the duration of total HLTE on chronic treatment. An insignificant effect was also observed with ATV treated group whereas no effect was observed on combination group. Seizure protection rate was 16.66% in phenytoin treated group. (Table 3)

Table 3: Seizure protection rate and hind limb extension in the chronic MES model.

Groups	Seizure protection rate (%)	HLTE in seconds
Control (2% gum acacia)	0%	10.50 ± 0.34
Phenytoin (25mg/kg)	16.6%	5.33 ± 1.20***
Losartan (50mg/kg)	0%	7.17 ± 0.48**
Atorvastatin (10mg/kg)	16.6%	8.00 ± 0.36
Losartan + Atorvastatin (50mg+10mg/kg)	0%	8.33 ± 0.42

HLTE, Hind limb tonic extension

***P ≤ 0.001, **P ≤ 0.01 as compared to control

Pentylenetetrazole induced seizures

Latency for the onset of first clonus

Sodium valproate (p≤ 0.01) and losartan (p≤ 0.045) treated group showed significant increase in the latency for the onset of first clonus whereas other groups did not show any significant effect.

Latency for the onset of generalized seizures

Significant effect (P≤ 0.001) was observed with sodium valproate treated group and losartan (p≤ 0.05) treated group. An insignificant effect was also observed with ATV treated group. (Table 4)

Mortality

No mortality was observed with sodium valproate treated group. Only 16.6% mortality was seen with ATV group. Combination group had 33.33% mortality whereas losartan treated group shows 66.66% mortality compare to control which had 100% mortality.

Table 4: Latency for the onset of first clonus and generalized tonic clonic seizure with mortality (%) in chronic study.

Groups	Time for onset of first clonus (sec)	Time for onset of seizure (sec)	Mortality (%)
Control (2% gum acacia)	52.16 ± 03.94	59.66 ± 03.79	100%
Sodium valproate (200mg/kg)	111.00 ± 05.65**	252.00 ± 17.00***	0%
Losartan (50mg/kg)	70.83 ± 05.39*	94.83 ± 05.20*	66.66%
Atorvastatin (10mg/kg)	60.00 ± 04.24	70.00 ± 04.94	16.6%
Losartan+ Atorvastatin (50mg+10mg/kg)	53.00 ± 01.85	62.50 ± 02.76	50%

***P ≤ 0.001, **P ≤ 0.01, * P≤0.05 as compared to control

DISCUSSION

The growing literature data suggests role of angiotensin peptides in brain excitability including susceptibility for seizures. Evidence also indicates that statins have protective effects in several neurological diseases including epilepsy. The present study compared the effects of angiotensin receptor blocker, HMGCoA reductase inhibitor and their combination in acute and chronic administration.

The present study demonstrated that acute and chronic dose of losartan was significantly effective in both the models in controlling seizure indicating its broad spectrum activity against seizures like valproate. AT_1 receptors located at pre- and post- synaptic sites are able to modulate GABAergic glutamatergic transmission [9]. AT_1 receptor blockers like losartan cross blood brain barrier and block angiotensin II that interferes with modulation of inhibitory and excitatory CNS neurotransmitters. Losartan infusion prevented kainite induced seizure activity in spontaneously hypertensive rats [10]. Losartan and telmisartan when given in combination with valproate did not alter the total brain concentration of valproate and hence there is no pharmacokinetic interaction. However both drugs potentiated the anticonvulsant action of valproate [11]. In our study losartan was effective in both MES and PTZ model indicating that this drug might be acting through Na^+ cannel and GABA which can be further explored. Possible mechanisms of losartan like adenosine or calcium channel blocking activity can also be hypothesized. In acute study, 100% survival in losartan treated group in PTZ model shows its potency against absence seizures. Chronic use of losartan has shown significant effect in MES and PTZ model indicating that losartan on long term can show antiepileptic effect in generalized tonic clonic seizure as well as in absence seizure [12].Similar results have been seen in our study. However, there was a decrease in effectiveness of losartan regarding mortality (66.66%) on chronic use in PTZ model which indicates there may be an alteration of AT_1 receptor densities in the CNS on chronic treatment.

Lovastatin and simvastatin show anticonvulsant activity against tonic phase of audiogenic seizures whereas fluvastatin and atorvastatin possess a much weaker efficacy and low potency [13]. Simvastatin and lovastatin are lipophilic agents that can cross the blood–brain barrier [14] and showed marked

neuroprotective activity against kainic acid induced seizures in mice [15]. In a study, single injection of atorvastatin (10 mg/kg, 30 min after kainate administration) did not alter seizure and wet dog shake scores, suggesting that acute atorvastatin does not reduce kainate-induced seizures [9]. Atorvastatin did not affect the duration of status epilepticus or the development of epilepsy evoked by electrical stimulation [16]. Atorvastatin has less effect in temporal lobe epilepsy, where the innate immune response is more prominent [16]. These results are in accordance with our findings as atorvastatin on acute administration did not show antiepileptic effects in both the models. However other studies have shown that acute dose of fluvastatin enhanced the anticonvulsant action of carbamazepine and valproate in MES model and increased the concentration of carbamazepine by 61% [17].Chronic treatment of ATV also did not show any significant effect in our study except on survival rate. A recent study on the contrary showed that chronic atorvastatin increased seizure threshold in PTZ induced seizures [17]. Chronic co-administration of nitric oxide synthase (NOS) inhibitor l-NAME(L-arginine methyl ester dihydrochloride) with ATV inhibited ATV induced anticonvulsant effect implying that nitric oxide (NO) signaling through NOS is involved in anticonvulsant effect of ATV [18]. Anti-seizure activity of ATV is mediated through nitric oxide pathway and it is independent of changes in plasma or cerebral cortex cholesterol levels or in BBB permeability [15, 19]. It has also been demonstrated that lovastatin exacerbates atypical absence seizures with minimal effects on brain sterols content but inhibits neurosteroids derived from cholesterol [20]. Protective effect of statins could be due to direct antagonism on glutamate receptors [21]. Survival rate of 83.33% in PTZ model in our study indicates neuroprotective and anti-seizure activity of statins which is in accordance with previous studies [6, 9]. Use of ATV has shown to decrease the hospitalization rate in epilepsy [22]. The variations in the results to atorvastatin can be due to methodological differences that may account for it, including the statin used, the type and duration of treatment, the nature of the convulsant stimulus used, and the experimental design itself [15].

A remarkable additive/synergistic effect exists between low-doses of a statin (atorvastatin) and an ARB (losartan), resulting in cardiovascular protection [23]. But the same synergism could not be found in our study in epilepsy models. On the contrary statins nullified the effect of losartan; which may be attributed to its pharmacokinetic interaction as we have used higher doses [24]. Losartan alone has shown a significant effect in both models. As there are various animal models available these drugs need to be further explored in preventing epileptogenesis and in pharmacoresistance [4].The data in this study is experimental and further long term studies will be required before extrapolating these effects in humans.

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