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Effects of enalapril and losartan alone and in combination with sodium valproate on seizures, memory, and cardiac changes in rats

Dinesh Joshi [a], Jatinder Katyal [a,*], Sudheer Arava [b], Yogendra Kumar Gupta [c]

[a] Neuropharmacology laboratory, Department of Pharmacology, All India Institute of Medical Sciences, New Delhi, India
[b] Department of Pathology, All India Institute of Medical Sciences, New Delhi, India
[c] Translational Health Science and Technology Institute, Faridabad, Haryana, India

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ABSTRACT

Purpose: Cardiac changes accompanying seizures may be responsible for sudden unexpected death in epilepsy (SUDEP), and drugs with antiseizure and favorable cardiovascular profile could be beneficial. The effect of losartan and enalapril alone and in combination with sodium valproate on seizures, cognition, cardiac histopathology, and serum brain-derived neurotropic factor (BDNF) levels were determined.
Methods: Male "Wistar" rats (200–250 g) were administered enalapril (20 mg/kg, intraperitoneally (i.p.)) and losartan (10 mg/kg, i.p.) daily and simultaneously subjected to pentylenetetrazole (PTZ)-kindling (PTZ 30 mg/kg, i.p., every alternate day). Enalapril and losartan were injected 45 & 120 min before seizure stimuli. In another set of experiments, sodium valproate (150 mg/kg, i.p.) alone or in combination with enalapril (20 mg/kg, i.p.) and losartan (10 mg/kg, i.p.) were administered daily during induction of kindling. The effect on seizures and behavior were noted; rats were sacrificed, and blood and hearts were collected for further analysis, i.e., BDNF levels, heart weight–body weight (HWBW) ratio, and cardiac histopathology.
Results: Losartan, but not enalapril, suppressed the seizure score in PTZ kindling. Sodium valproate alone or in combination with losartan or enalapril prevented kindled seizures. Sodium valproate per se caused cognitive impairment, which was prevented on combining with losartan or enalapril. A decrease in HWBW ratio was observed only in enalapril group (p value = 0.02). Kindling led to cardiac ischemic changes, which could be prevented by losartan and sodium valproate. Serum BDNF level was decreased in PTZ (p value = 0.02) and sodium valproate per se group (p value = 0.04), but sodium valproate could reverse the PTZ-induced decrease in serum BDNF level.
Conclusion: The use of losartan with sodium valproate in epilepsy may prevent the cognitive and cardiac sequelae of seizures. The BDNF levels as a marker for cardiovascular risk in persons with epilepsy (PWE) needs to be explored further.

1. Introduction

There has always been an impetus on newer drugs for epilepsies due to the multiple problems ranging from inadequate seizure control, debilitating side effects with the currently available drugs, and a sizable risk of mortality. Epilepsy-related mortality encompasses death in persons with epilepsy (PWE) because of status epilepticus, accidents, drowning, pneumonia, falls, burns, and suicides, on account of antiepileptic drugs (AEDs) and many times sudden unexpected death in epilepsy (SUDEP) [1–4]. The incidence of SUDEP is estimated at ~12 per 10,000 PWE, annually. It is most common in young adults with frequent, pharmacoresistant seizures and can affect children and patients with seemingly well-controlled epilepsy [5].

The mechanisms underlying SUDEP are poorly understood. Natelson et al., reported that the PWE who died suddenly and unexpectedly have cardiac pathologic conditions that may be responsible for their death [6]. The microscopic findings of heart in the SUDEP cases comprised patchy areas of interstitial myocardial fibrosis, myocyte vacuolization, atrophy of myocardiocyte, leucocyte infiltration, and perivascular fibrosis [6,7]. Based on this, it has been suggested that patients with epilepsy may be repeatedly exposed to catecholamine surge following each seizure episode and may harbor a risk for postictal catecholamine surge and catecholamine-induced myocardial dysfunction [8]. Though, the association between seizures, cardiac changes and SUDEP remains elusive, it is likely that seizures and AEDs may adversely affect cardiovascular parameters and can perhaps predispose to SUDEP. Some experimental

* Corresponding author at: Department of Pharmacology, All India Institute of Medical Sciences, New Delhi, India.
E-mail address: jatinderkatyal.aiims@gmail.com (J. Katyal).

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studies have explored the cardiac changes in animals with acute or chronic seizures [9,10], and it has been reported that in rats, death associated with acute seizures involved autonomically mediated asystole supported by hypoxemia [11].

In view of a probable cardiovascular component in mortality seen in many PWE, drugs offering both seizure prevention and favorable cardiovascular profile may be candidate AEDs.

Renin–angiotensin system (RAS) is a major determinant of arterial pressure and volume homeostasis and in the brain angiotensin peptides are also proposed to act as neurotransmitters or neuromodulators [12]; RAS is also one of the many systems activated by seizures, and RAS modulators have shown antiseizure activity [13–15]. Because of this dual activity of RAS, i.e., cardiovascular and antiseizure, in this study, the effect of RAS inhibitors, losartan (angiotensin receptor antagonist) and enalapril (angiotensin converting enzyme (ACE) inhibitor) alone and in combination with commonly used AED sodium valproate (SV) was determined on seizure parameters namely seizure score using chemically pentylenetetrazole (PTZ)-kindled seizures, and since neuroprotection is a desirable characteristic in potential AEDs [16], effect on cognition was also evaluated. In order to evaluate whether seizures and/or AED treatment can cause cardiac damage, the effect on heart weight–body weight (HWBW) ratio, a surrogate marker of heart failure [17], cardiac histopathology to determine any inflammatory, ischemic, necrotic, or fibrotic changes in the heart following seizure/drug treatment and serum brain-derived neurotropic factor (BDNF) levels, which are reportedly affected by both seizures and cardiac pathology [18–20], were also studied.

2. Methods

2.1. Experimental animals

All experimental procedures were carried out after being approved by the Institutional Animal Ethics Committee (IAEC), All India Institute of Medical Sciences (AIIMS) (ethics approval no. 867/IAEC/15 and 885/IAEC/15). Male Wistar rats, 200–250 g were used for the study. Animals were obtained from the Central Animal Facility of AIIMS, New Delhi, India. The rats were housed in groups of 3 per cage, in polypropylene cages at ambient temperature (22–25 °C), with 12-h light/dark cycles, and with free access to rat pellet diet (M/s Ashirwad Industries, Chandigarh, India) and tap water. The rats were acclimatized to laboratory conditions 7 days prior to experimentation. All experiments were carried out between 10:00 AM–1:00 PM, and experimental procedures were in accordance with Indian National Science Academy (INSA) guidelines on use of animals in research.

2.2. Drugs and other chemicals

Losartan, enalapril, SV, and PTZ were obtained from Sigma Aldrich, USA. Rat BDNF enzyme-linked immunosorbent assay (ELISA) kits were purchased from Ray Biotech, USA. All other chemicals were sourced locally and were of analytical grade.

Enalapril and losartan were dissolved in normal saline and injected intraperitoneally (i.p.) 45 min and 120 min before seizure stimuli in doses of 20 mg/kg and 10 mg/kg respectively. Sodium valproate at subanticonvulsant dose (150 mg/kg i.p.) was administered 30 min before PTZ administration. The doses used and the pretreatment times for losartan and enalapril were based on previous studies [21].

2.3. Experimental protocol

The PTZ-induced kindling was used as seizure model. The effect of losartan/enalapril alone and in combination with SV in this model was determined. Behavioral, biochemical, and histopathological parameters were also assessed. For detailed protocol refer to Fig. 1.

2.4. Pentylenetetrazole kindling

For induction of kindling, the rats were injected PTZ (30 mg/kg, i.p.) once every alternate day (48 ± 1 h). Rats were then placed in plexiglass cages and observed for 30 min. Seizure activity was scored using a scale of 0 to 5 as standardized in our lab previously [22,23]. The rats showing two stage 4 seizures were considered kindled. A maximum of 30 injections of PTZ was given or until the development of kindling, whichever was earlier. Losartan (10 mg/kg, i.p.) and enalapril (20 mg/kg, i.p.) were administered 120 or 45 min before PTZ, respectively.

2.4.1. Effect of pretreatment with combination of sodium valproate and losartan/enalapril on kindled seizures

In another set of experiments, subanticonvulsant dose of SV (150 mg/kg, i.p.), alone or in combination with losartan (10 mg/kg)/enalapril (20 mg/kg) was administered 30 min before kindling dose of PTZ, and effect on development of kindling was determined.

2.5. Morris water maze test

For assessing memory, MWM (Morris water maze) apparatus (ANY-maze version 4.63) with video tracking was used as described by us earlier [24]. Briefly, a five-day protocol was followed in which four-day training was given, and on the fifth day, latency period was taken. Training was given from all the four quadrants with two-minute cutoff time. On the

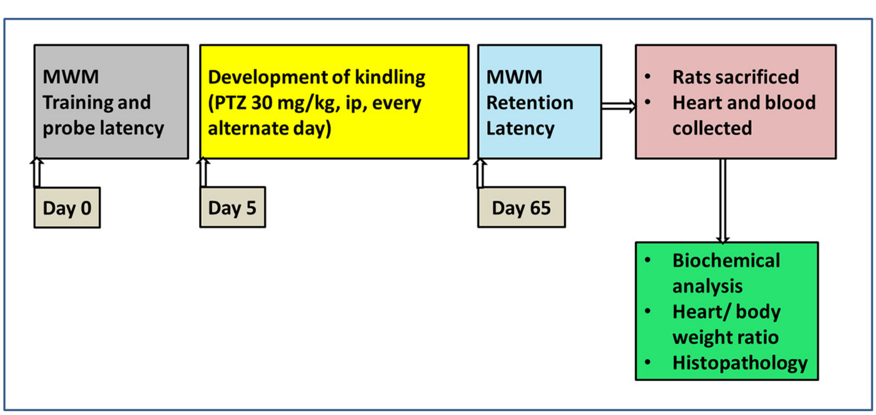

Fig. 1. Experimental protocol for pentylenetetrazole-induced kindling experiments. MWM—Morris water maze, PTZ—Pentylenetetrazole.

fifth day, platform was removed and time to first entry to platform zone, number of entries to platform zone, and time spent in platform zone were noted. Fifth-day data were compared with posttreatment values. The posttreatment trial was conducted 24 h after the 30th PTZ injection.

2.6. Heart weight–body weight ratio

Animals were weighed on the day of sacrifice. Under pentobarbitone (50 mg/kg, i.p.) anesthesia, pumping heart was removed and cleaned in normal saline. The blood vessels were removed, and the hearts were washed with normal saline and dried by gently blotting on tissue paper. The hearts were weighed, and HWBW ratio was obtained using the following formula.

$$[\text{Heart weight (g)}/\text{body weight (g)}] * 1000$$

2.7. Histopathology

For histopathological analysis, heart sections were fixed in 10% buffered formalin and processed through routine automated histokinetic processor. Paraffin blocks were prepared, and 3- to 5-μm sections were cut with the help of microtome (SHANDON AS 325) and stained with hematoxylin and eosin (H & E) stain using standard technique. All the H & E stained slides were evaluated for histopathological changes like inflammation, necrosis, ischemia, and fibrosis. Samples showing evidence of any change were subjected to Masson's trichrome (MT) stain for cardiac fibrosis. The findings were evaluated by a cardiac pathologist blinded to experimental groups.

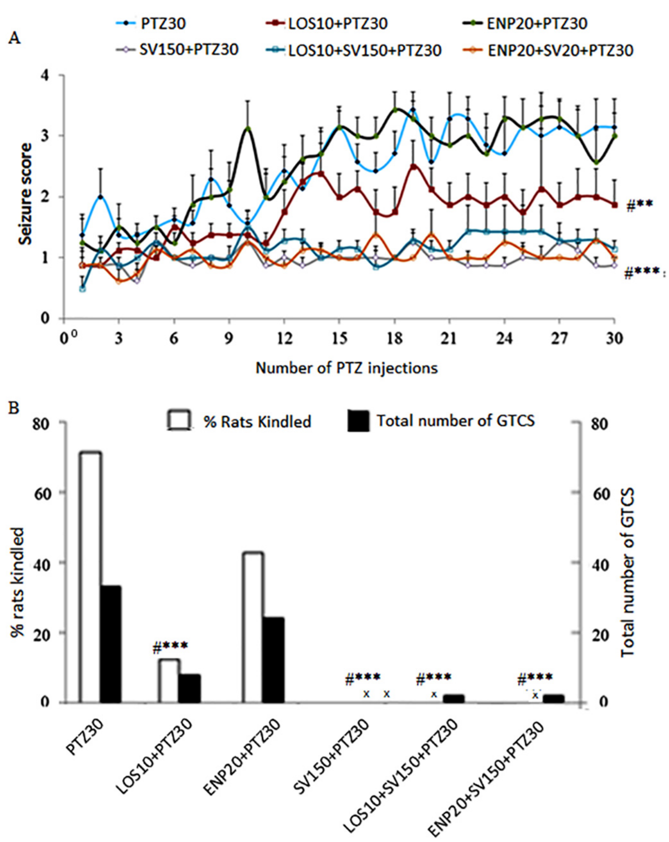

Fig. 2. Effect of losartan (10 mg/kg, i.p.)/enalapril (20 mg/kg, i.p.) pretreatment alone and in combination with sodium valproate (150 mg/kg, i.p.) on pentylenetetrazole (30 mg/kg, i.p.) induced kindling. (A) Mean seizure scores during development of kindling and (B) Effect of losartan/enalapril and combination of losartan/enalapril with sodium valproate on percentage of animals kindled and total number of generalized tonic–clonic seizures (GTCS) observed during PTZ induced kindling. Results are presented as Mean ± SEM. ***p value ≤ 0.001, **p value ≤ 0.01; #—as compared with PTZ30 group. X- Absence of kindling development or seizure activity. PTZ—Pentylenetetrazole, LOS—Losartan, ENP—Enalapril, SV—Sodium valproate.

2.8. Estimation of serum brain-derived neurotropic factor (BDNF)

Serum BDNF was estimated using rat BDNF ELISA kit (RayBiotech, USA) following the manufacturer's instructions. The ELISA plate was read at 450 nm using ELISA reader.

2.9. Statistical analysis

Results are expressed as mean ± SEM (Standard Error of Mean). Statistical analysis of the data was done using Statistical Package for the Social Sciences (SPSS) software version 22. Bartlett's test was used to determine homogeneity of variances. One way analysis of variance (ANOVA) with Bonferroni's posthoc test was used to compare significance between multiple groups. In the MWM test, baseline data were compared using one way ANOVA with Bonferroni's posthoc test and differences between pre- and posttreatment groups were determined using paired *t*-test (two-tailed). The α-level was set at 0.05.

3. Results

3.1. Effect of losartan and enalapril on chronic pentylenetetrazole (PTZ)-kindled seizures

In vehicle-treated group, repeated administration of subconvulsant dose of PTZ (30 mg/kg) every alternate day (for 60 days, 30 injections) resulted in progressive increase in convulsive activity. Losartan pretreatment suppressed the seizure score as compared with PTZ alone while enalapril was ineffective. Sodium valproate alone or combination of losartan/enalapril with SV prevented kindled seizures (Fig. 2A).

The total number of animals kindled after 30 injections were 71.42% (n = 7) in PTZ alone group, 12.5% (n = 8) in losartan-pretreated, and 42.85% (n = 7) in enalapril-pretreated groups. Total number of generalized tonic–clonic seizures (GTCS) observed was maximum in PTZ group (33 GTCS) followed by enalapril pretreatment (24 GTCS). None of the rats were kindled in groups that were administered SV alone or in combination with losartan/enalapril (Fig. 2B).

3.2. Effect of losartan/enalapril alone and in combination with sodium valproate on spatial memory using Morris water maze

Fig. 3A gives the effect of different treatments on MWM parameters, and the representative track plots are given in Fig. 3B. There was no statistically significant difference in baseline data among groups. No cognitive impairment was seen in any of the parameter tested on MWM in PTZ alone group and losartan/enalapril pretreatment groups. Sodium valproate pretreatment resulted in cognitive impairment as evidenced by decreased path efficiency (p value = 0.003; F value = 4.426), decreased number of entries to platform zone (PZ) (p = 0.03; F value = 2.688), decrease in time spent in PZ (p value = 0.005; F value = 4.270), increased latency to first entry to PZ (p value = 0.002; F value = 75.11), and increased distance traveled until first entry to PZ (p value = 0.03; F value = 58.77). Pentylenetetrazole alone (p value = 0.0004; F value = 1.110) and in combination with losartan (p value = 0.009; F value = 2.349) increased the time spent in 4th zone. All other parameters showed no significant change when SV was combined with either losartan or enalapril.

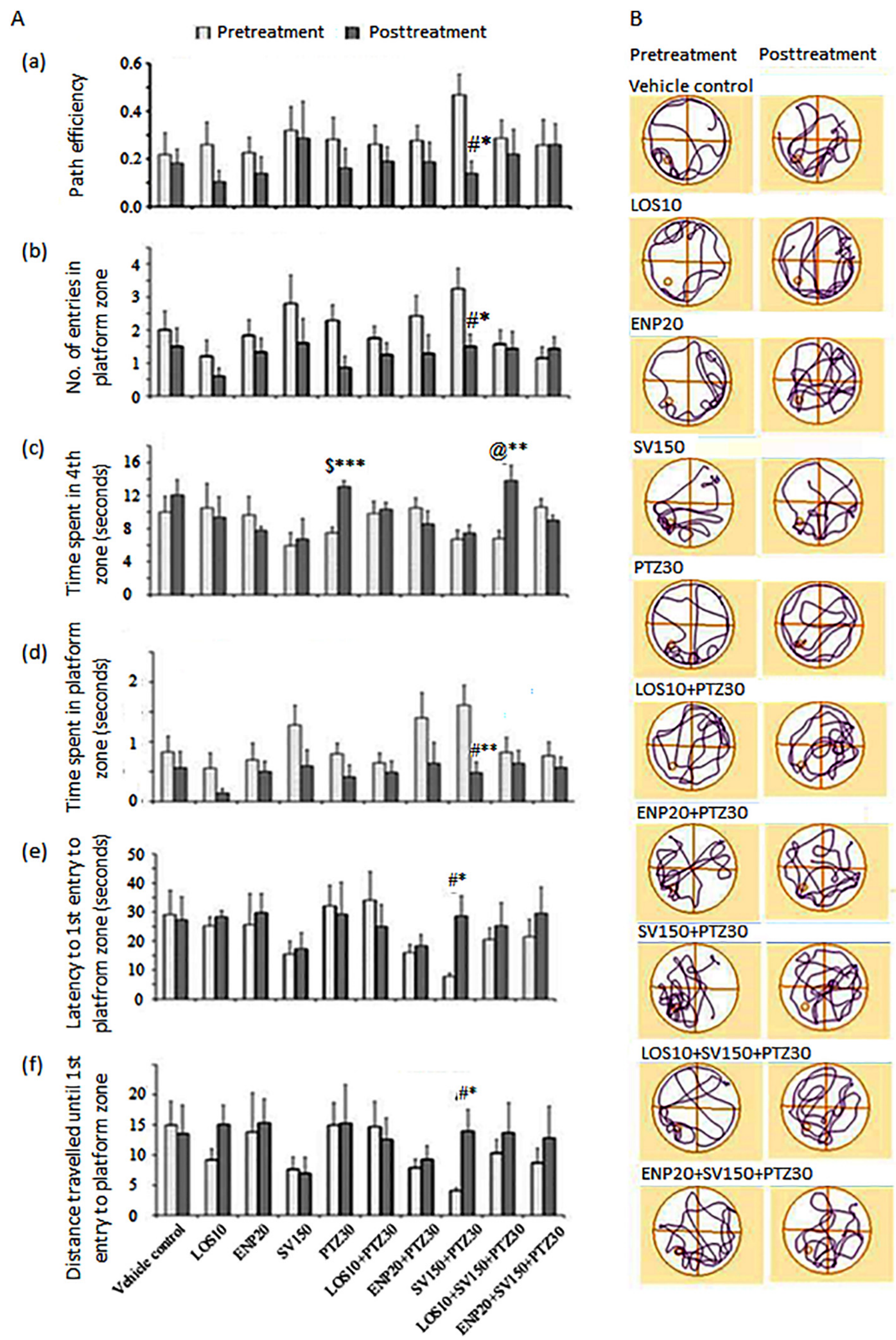

Fig. 3. Effect of pretreatment with losartan/enalapril alone and in combination with sodium valproate before PTZ (30 mg/kg, i.p., every alternate day) in Morris water maze test. (A) Effect on various parameters recorded (a) path efficiency; (b) number of entries in platform zone; (c) time spent in zone 4; (d) time spent in platform zone (PZ); (e) latency to first entry to the platform zone (PZ); (f) distance traveled until entry in platform zone. Results are presented as Mean ± SEM. ***p value ≤ 0.001, **p value ≤ 0.01, *p value ≤ 0.05; #—as compared with SV + PTZ pretreatment group; $—as compared to pretreatment group; @—as compared to pretreatment group. (B) Representative track plots of different groups. PTZ—Pentylenetetrazole 30 mg/kg, LOS—Losartan 10 mg/kg, ENP—Enalapril 20 mg/kg, SV—Sodium valproate 150 mg/kg.

3.3. Effect of losartan or enalapril alone and in combination with sodium valproate on body weight and heart weight–body weight ratio in PTZ kindling

No significant difference in body weight was observed in any of the group compared to the vehicle control at the end of the experiment (Fig. 4A–C).

Treatment with losartan/SV/enalapril per se or in combination with PTZ had no effect on HWBW ratio. A decrease in HWBW ratio was observed when enalapril was combined with SV (p value = 0.02; F (6,41) = 2.966) (Fig. 4D).

3.4. Effect on heart histopathology

The representative images are given in Fig. 5A. Chronic administration of PTZ caused early ischemic changes in the cardiac myocytes showing interstitial edema and thin wavy cardiac myofiber (in 2 out of 4 samples). Chronic administration of enalapril/SV alone resulted in ischemic damage in the myocardium as seen by necrosis and fibrosis (3 out of 3 in both groups). Pretreatment with losartan/SV prevented seizure-induced ischemic changes. A mild focal inflammation without ischemic changes and fibrosis was seen in losartan group (2 out of 3

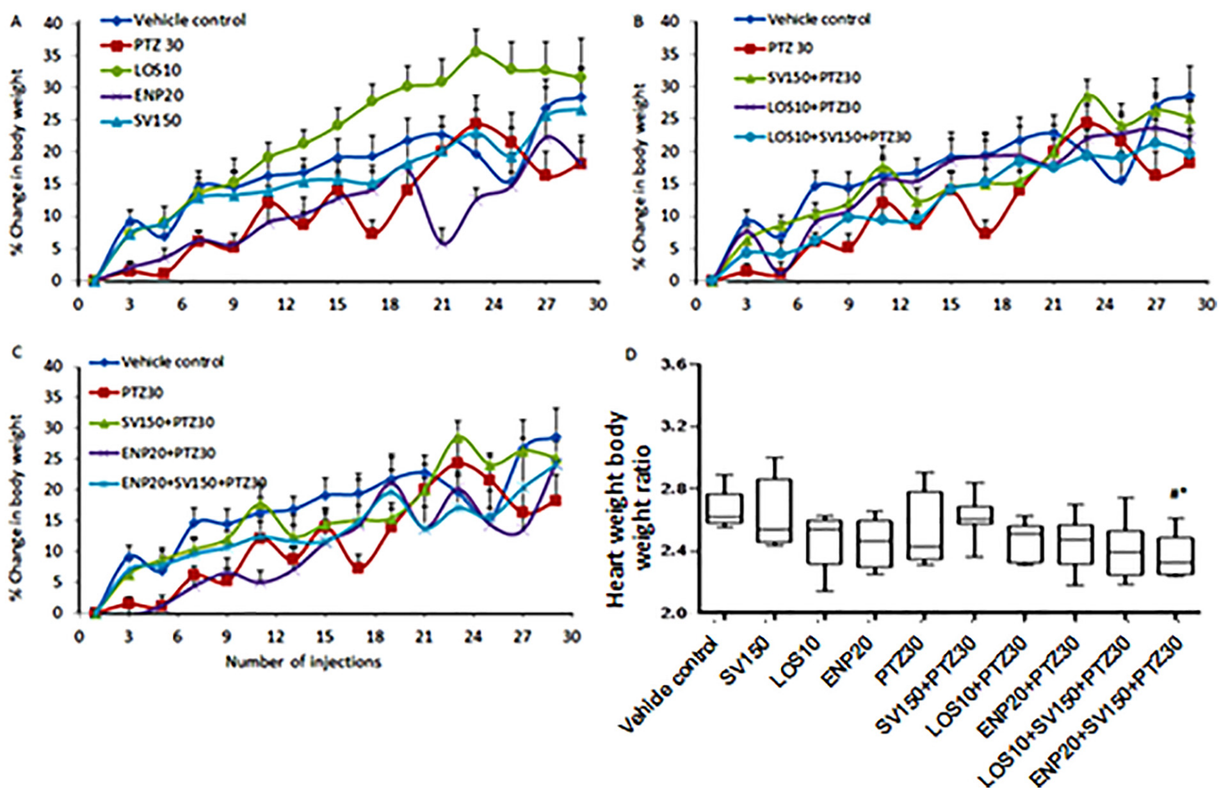

Fig. 4. Effect of pentylenetetrazole-induced kindling on percent change in body weight and heart weight–body weight ratio. (A) Effect of seizures per se and sodium valproate (SV), losartan (LOS), or enalapril (ENP) treatment per se on percent change in body weight, (B) Effect of combination of losartan (10 mg/kg, i.p.) with or without sodium valproate (150 mg/kg, i.p.) on percent change in body weight. (C) Effect of enalapril (20 mg/kg, i.p.) with or without sodium valproate (150 mg/kg, i.p.) on percent change in body weight. Results are presented as mean ± SEM. (D) Effect on heart weight–body weight ratio for different groups. Heart weight–body weight ratio is presented as box whisker plot. The box represents the interquartile range, the line within the box the median. The ends of the 'whiskers' show maximum and minimum. LOS—losartan, ENA—enalapril, SV—sodium valproate, *p value ≤ 0.05, #—as compared with vehicle control.

samples) and with SV (3 out of 4). Pretreatment with enalapril in PTZ-treated group enhanced the myocytes damage (in 2 out of 3 samples).

Combination of losartan and SV but not enalapril and SV prevented the ischemic changes in the myocardium in PTZ-treated group as indicated by no significant inflammation or fibrosis. Isoproterenol run as a control for cardiac damage also showed marked ischemic damage in the myocardium.

3.5. Effect of losartan or enalapril alone and in combination with sodium valproate on serum BDNF levels in PTZ kindling model

Fig. 5B gives the effect of different treatments on serum BDNF levels. Serum BDNF level was decreased in PTZ (p value $= 0.02$; $F(4,19) = 5.964$) and SV group (p value $= 0.04$; $F(4,19) = 5.964$), but SV pretreatment reversed the PTZ-induced decreased serum BDNF level. The BDNF levels for the losartan group and two samples in the enalapril group were below limit of detection.

4. Discussion

It is now very clear that seizure control is but only one aspect in management of PWE, and there can be one or more comorbidities secondary to seizures, which may need to be addressed simultaneously. While cognitive issues are well-recognized, cardiovascular problems may also be a matter of major concern.

The renin–angiotensin system (RAS) is a major determinant of arterial pressure and volume homeostasis in mammals through the action of the vasoactive peptide angiotensin II (Ang II) on Ang II type 1 receptors (AT1Rs). Although individual components of RAS system have not been detected in the brain, existence of a brain RAS is unequivocal. This brain RAS is implicated in regulation of cardiovascular and endocrine functions, body fluid, salt balance, cell growth, and some types of behavior like thirst, sexual behavior, anxiety, memory, etc. The different angiotensin peptides are also proposed to act as neurotransmitters or neuromodulators [12]. The RAS is one of the many systems activated by seizures. Chronic seizures upregulate the brain RAS, and drugs modulating RAS are known to be effective in controlling seizures in animal studies [13–15].

There are two pharmacological approaches to modulate the effect of RAS — (1) Use of ACE inhibitors to block formation of Ang II, (2) Blocking the AT1 receptor by angiotensin receptor blocker (ARB). Because alternative ACE-independent pathways of Ang II formation exist, the use of ARB could be more specific in targeting Ang II mediated effects. However, blockade of AT1 receptor results in a compensatory increase in Ang II, which may activate AT2 receptor mediated signaling. To target RAS, we used both the approaches, i.e., ACE inhibitor enalapril as well as AT1 receptor blocker losartan. The doses of these drugs used were based on literature survey.

The model used in this study is chemical kindling. Kindling is a process of repetitive stimulation of brain by electrical or by chemical means in subconvulsant dose, which will ultimately decrease threshold of seizure and increase the seizure severity. Kindling is a chronic model for Temporal Lobe Epilepsy (TLE), and it clinically relates with partial seizures and secondarily generalized seizures [25,26]. Losartan suppressed the seizure severity throughout the kindling process while enalapril pretreatment did not do so. Enalapril has been shown to decrease seizure severity and attenuate tonic–clonic seizures in Wistar rats [14]. It also potentiated the protective effect of SV and lamotrigine in mouse maximal electroshock seizure model [15,27]. However, conflicting results have also been reported. In audiogenic seizures in DBA/2 mice, all ACE inhibitors except enalapril decreased severity of seizures and augmented protection of AEDs including valproate [28]. The difference

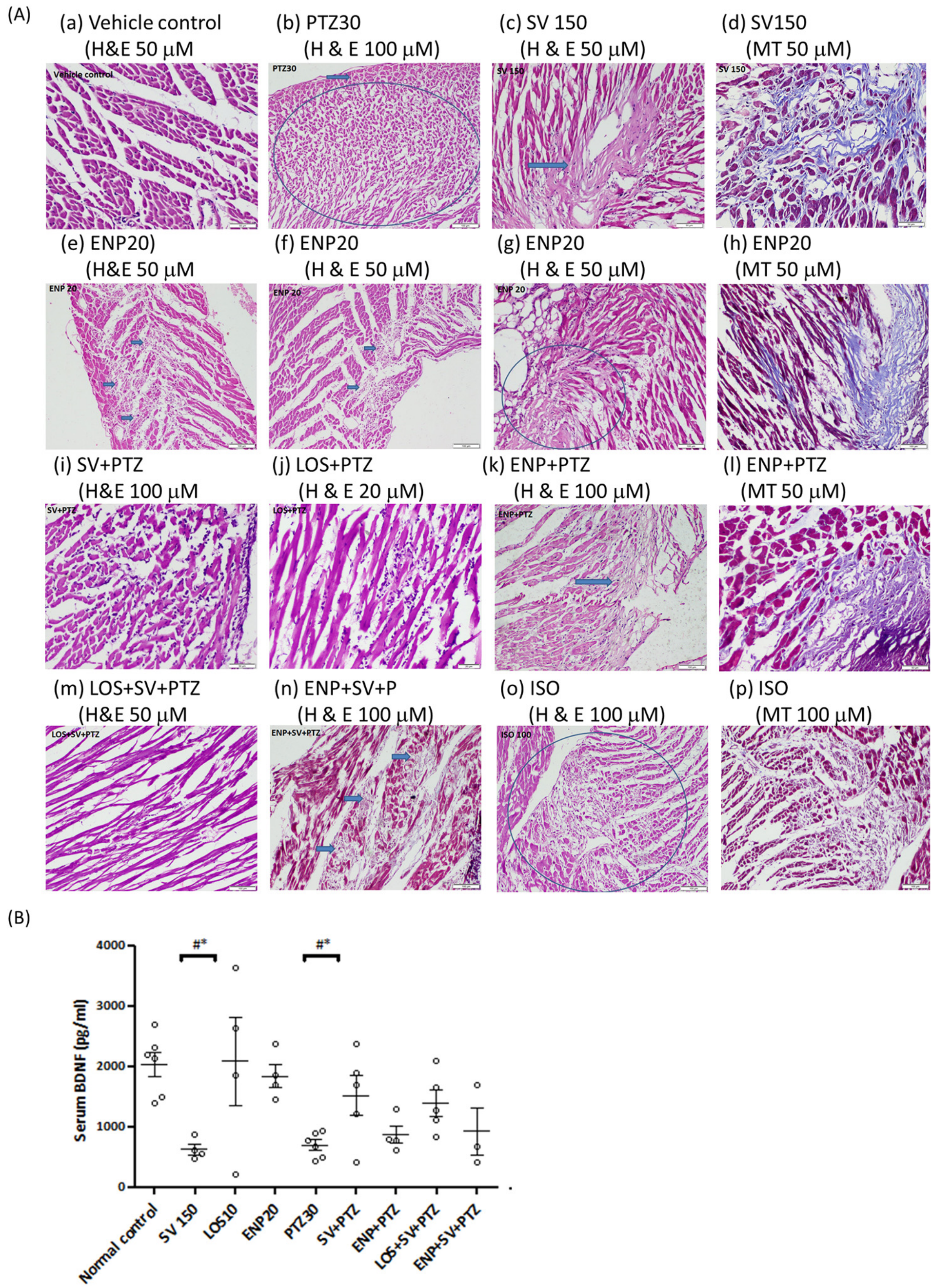

Fig. 5. (A) Effect of pentylenetetrazole (PTZ 30 mg/kg, i.p., every alternate day) per se and in combination with losartan (LOS 10 mg/kg, i.p.)/enalapril (ENP 20 mg/kg, ip)/sodium valproate (SV 150 mg/kg, i.p.) and positive control isoproterenol (ISO 100 mg/kg) on cardiac changes using hematoxylin and eosin (H & E) and Masson's Trichrome (MT) staining. (a) Vehicle control (b) PTZ30, arrow shows normal myocyte; circled area is large areas of early ischemic changes in the cardiac myocytes showing interstitial edema and thin wavy cardiac myofiber (c and d) SV150, arrow indicates patchy perivascular and interstitial fibrosis (e) ENP, arrow indicates large patchy area of ischemic damage in the myocardium (f) ENP, arrow indicates area of sub endocardial ischemic necrosis (g) ENP, circled area of replacement fibrosis (h) ENP, arrow indicates fibrosis (i) SV + PTZ (j) LOS + PTZ (k) ENP + PTZ, arrow indicates replacement fibrosis with absence of significant inflammation (l) ENP + PTZ, arrow indicates fibrosis (m) LOS + SV + PTZ (n) ENP + SV + PTZ, arrow indicates patchy areas of ischemic myocytes damage without significant fibrosis (o) ISO, circled area indicates marked ischemic damage in the myocardium (p) ISO, arrow indicates fibrosis. Isoproterenol 100 mg/kg, i.p. was administered twice at 0 and 24 h and rats sacrificed at 48 h.(B) Effect of pentylenetetrazole-induced kindled seizures on serum brain-derived neurotropic factor (BDNF) levels. Results are presented as mean ± SEM, and individual values are also plotted. LOS—losartan, ENP—enalapril, SV—sodium valproate, **p value≤0.01, *p value ≤ 0.05, #—as compared with vehicle control.

in results can perhaps be accounted for by the nature of the experimental model used, i.e., acute or chronic. This however indicates that AT1 receptor blocker losartan may be better than ACE inhibitor enalapril in preventing epileptogenesis. The difference in protection could be due to difference in the degree of block of RAS. After inhibition of ACE, non-ACE pathways for the production of Ang II become dominant whereas blockade of AT1 receptor for Ang II results in complete blockade of RAS. It is noteworthy that coadministration with SV did not compromise the protection offered by this AED. From our study, it can be inferred that protection of SV will not be affected if RAS modulators, losartan or enalapril are given to patients with epilepsy on SV.

Since cognitive effects are recognized as a major consideration in patients with epilepsy, the MWM paradigm for spatial memory was used. Kindling development using PTZ was not associated with any deficit rather an increase in time spent in 4th zone was observed. This is in agreement with our previous study wherein an improved performance MWM was seen in the vehicle-treated group [24]. Sodium valproate prevented development of kindling but impaired memory as evidenced by significant changes in multiple parameters in the MWM paradigm. Both the angiotensin modulators prevented this impairment when combined with SV. This would imply that not only does the seizure protection remain unaffected when these two classes of drugs are coadministered, but there also is a benefit in cognitive activity.

Very few studies have attempted to correlate the cardiovascular and antiseizure effects of ACE inhibitors and AT1 receptor antagonists. In this study, we primarily focused on chronic irreversible changes like cardiac myocyte damage, cardiac fibrosis, mainly contributing to adverse events/death but not acute reversible changes like heart rate and blood pressure. The histopathological findings corroborated ischemic changes in cardiac myocytes in response to kindling that could be reversed if seizures are prevented either with SV or on administering losartan but not enalapril. Naggar et al. have also demonstrated that cardiac changes accompany epilepsy, and these can lead to significant seizure associated cardiac performance decrease [29]. In another study using spontaneously hypertensive rats, AT1 receptor antagonism delayed the onset of seizures and alleviated their frequency and duration during and after discontinuation of treatment [30]. Our study is notably different from these studies with respect to species, convulsant challenge used, and duration. We also determined the effect on HWBW ratios, which remained unaffected except in enalapril-treated kindling group. The HWBW ratio was determined as a marker for cardiac hypertrophy. An increase in this ratio is considered as an independent and powerful predictor of heart failure and mortality [17].

Another parameter evaluated in this study was the effect on serum BDNF levels. Circulating BDNF derives from both peripheral and cerebral sources, and a correlation of circulating BDNF with brain levels as well as brain phenomena has been suggested by animal and human studies [31]. We determined BDNF levels in serum. It is reported that the average serum BDNF level is about 200-fold higher that of plasma [32]. Different workers have reported association of BDNF with multiple pathologies, i.e., the central nervous system (CNS) as well as the cardiovascular system (CVS), and higher, lower, or similar levels of circulating BDNF have all been described. A relationship between BDNF, seizures, and epileptogenesis is seen in many studies [18]. Hong et al., found an association of low serum BDNF level with disease severity in people with epilepsy and suggested BDNF as a helpful marker for epilepsy severity [33]. Upregulation of BDNF mRNA and immunoreactivity in different brain areas occurs in many experimental models of seizures while exogenous BDNF demonstrates a protective effect in seizures [19]. In rats, BDNF is also reported to be cardioprotective via a CNS mediated pathway [20, 34]. Furthermore, BDNF enhances angiomyogenesis, contributes to survival of endothelial cells during cardiovascular development, and provides protection against cardiac remodeling after myocardial infarction [35]. Low serum or plasma BDNF levels are associated with higher risk of future coronary and stroke events, and in recent study, plasma BDNF levels were significantly lower in patients with angina, heart failure, and could be related to mortality [36]. Interestingly, a connection between BDNF and RAS modulation has also been reported with RAS inhibitors upregulating expression of BDNF in the hippocampus [37]. Intracerebroventicular (i.c.v.) injection of BDNF, upregulates expression of AT1 receptor mRNA and increases the heart rate, blood pressure, sympathetic tone, and weight loss, which is blocked by losartan administration [38]. These studies prompted us to explore the relationship between serum BDNF levels, seizures, cardiac changes, and RAS modulation. As reported previously [19,33], in this study, also a decrease in BDNF level was observed following kindling, which was reversed in groups where kindled seizures were suppressed, i.e., pretreatment with SV or combination of SV and losartan or enalapril. Cardiac sequelae were also seen in some kindled rats with low serum BDNF. Sodium valproate per se was associated with a significant decrease in BDNF levels and ischemic changes in the myocardium. While the reversal of BDNF levels in the SV-pretreated kindling group may be on account of suppression of seizures, the fact that SV had also induced a decrease in BDNF levels and still BDNF levels were not affected when used before PTZ challenge is difficult to comment on. Since it has been reported that BDNF cardioprotection in rats is centrally mediated [20,34], it is likely that change in BDNF levels and cardiac sequelae with SV is centrally mediated as well, and hence, an alteration in central homeostasis following PTZ administration in some manner prevented the SV-induced alteration of BDNF levels. An intriguing finding was losartan suppressed kindled seizures improved cardiac and cognitive sequelae, but the BDNF levels were below limit of detection in some rats. A decreased level though consistent with previous studies does not fit in with our hypothesis. While methodological issues though unlikely cannot be ruled out, a possible explanation may be losartan's antiplatelet effect. It has been suggested that a reduction in circulating BDNF may reflect changes at the level of the CNS and/or depend on defective platelet release, and drugs influencing platelet activity should be factored in [39]. Chlopicki et al. indicated that losartan had an antiplatelet effect by blocking thromboxane A2 receptors [40]. Losartan also reduced platelet activation and aggregation in another study [41].

5. Conclusion

In conclusion, it is apparent that neither enalapril nor losartan is likely to vitiate seizure control with SV rather both the drugs prevent adverse cognitive sequelae because of valproate. However, use of enalapril in seizures may be detrimental from a cardiovascular perspective. The BDNF levels can serve as a marker for cardiac risk after seizure, but further studies with other seizure models and AEDs are warranted.

Acknowledgments

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Disclosure

None of the authors has any conflict of interest to declare.

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