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The effect of generalized seizure activity on ischemia-induced cardiac arrhythmias and myocardial injury with histopathological evaluation in anesthetized rats

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Background/aim: Epileptic seizure leads to sudden unexpected death in epilepsy (SUDEP) among affected patients. The causes of SUDEP are still unclear. The aim of this study was to research the effect of epilepsy on myocardial injury and arrhythmias during experimentally induced acute myocardial ischemia.

Materials and methods: Wistar albino rats were divided into four groups: sham, pentylentetrazole (PTZ) + sham, ischemia, and PTZ + ischemia groups. PTZ (65 mg/kg, ip) was given 2 h before ischemia. Seizure scoring was conducted by evaluating the PTZ-induced behavioral changes in the rats. The left main coronary artery was ligated in anesthetized rats for 30 min. The incidence and the number of ventricular arrhythmias were determined. Histopathological scoring was performed for tissue injury by using a microscope.

Results: Seizure scores were not different among the groups ($P > 0.05$). The incidence and number of ventricular tachycardia (VT) episodes were significantly higher in the PTZ + ischemia group than in the ischemia group ($P < 0.05$). More prominent myocardial damage was observed in the PTZ + ischemia group than in the other groups (histopathological scores: PTZ + ischemia; 2.5 ± 0.5 versus ischemia; 1.2 ± 0.4, $P < 0.05$).

Conclusion: PTZ-induced seizure in rats increased myocardial injury and the incidence and number of VT episodes in myocardial ischemia. These results reveal that seizure in epilepsy patients may increase ventricular arrhythmia and myocardial injury during heart attack.

Key words: Epilepsy, pentylentetrazole, ventricular arrhythmias, myocardial injury, rat

1. Introduction

Epilepsy comprises a group of disorders characterized by seizures and excessive neuronal activity in the brain. The death rate in epilepsy patients is 2 or 3 times higher than that in the general population. Death generally occurs suddenly and unexpectedly (as in SUDEP) following an epileptic seizure (1). While the underlying mechanism of SUDEP is still unclear, it seems to be multifactorial. It has been suggested that SUDEP may be caused by apnea and hypoxia seen in recurrent epileptic seizures, as well as cardiac arrhythmias, and the loss of autonomic function (2–4).

Studies regarding the reasons for SUDEP and the effect of epileptic seizure on myocardial tissue injury and cardiac arrhythmias are mostly based on clinical reports. Tigaran et al. reported that ST-segment depression and elevated cardiac troponine levels are signs of ischemia in patients with drug refractory epilepsy (5). In addition, P-Codrea Tigaran et al. verified myocardial fibrosis in SUDEP patients with their histological evaluations (6). It was also reported that seizure induced ventricular arrhythmias, including ventricular premature contraction (VPC) and ventricular tachycardia (VT), in epilepsy patients (3,7).

Animal models represent a useful tool for mimicking the pathophysiological conditions following an epileptic seizure. In one particular experimental study, epileptic attacks led to ultrastructural changes in rat myocardial tissue (8,9). Metcalf et al. demonstrated that epilepticus status increased the susceptibility of arrhythmias in rats (9). In a recent study, the incidence of ischemia/reperfusion (I/R)-induced ventricular arrhythmias was found to be higher in isolated hearts from rats which had been affected by audiogenic epileptic seizure (10). In contrast with this finding, Tavares et al. reported that pilocarpine-induced epilepsy did not affect the incidence of ventricular arrhythmias in anesthetized rats (11).

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At this point, it must be noted that no study has examined the effect of epilepsy on ventricular arrhythmias and myocardial injury in experimentally induced myocardial ischemia. Therefore, we evaluated the incidence and number of ventricular arrhythmias and tissue injuries in response to myocardial ischemia in anesthetized rats following PTZ-induced convulsive seizure.

2. Materials and methods

2.1. Animals

In this study, we used 45 Sprague Dawley male rats weighing 300–350 g. The animals were obtained from Experimental Animal Production Center, Zonguldak Bülent Ecevit University, Turkey. The animals were kept in a plastic cage in a room with 12-h light–dark cycle, at a temperature of 21 ± 2 °C, and humidity between 40% and 65%. They were fed with rat pellet food and drink tap water ad libitum. All experimental procedures performed in this study were discussed and approved in respect to ethical appropriateness by Zonguldak Bülent Ecevit University, Animal Experiment Local Ethical Committee (protocol no: 210 - 26/1023).

2.2. Experimental groups

The study was conducted in four seperate experimental groups (Figure 1).

(I) Sham-operated group: Saline was given 2 h before the sham operation (n = 8),
(II) PTZ + sham group: PTZ was given 2 h before the sham operation (n = 8),
(III) Ischemia group: Saline was given 2 h before the ligation (n = 8),
(IV) PTZ + ischemia group: PTZ was given 2 h before the ligation (n = 8).

2.3. Seizure induction

Pentylentetrazole (PTZ) was purchased from Sigma Chemical Corporation (St. Louis, MO, USA). PTZ was dissolved in sterile physiological saline (NaCl 0.9%). The dose of PTZ used in the present study was based on a previous study performed by Sahin et al., 2009 (12), in which PTZ at a dose of 65 mg/kg/mL ip produced strong seizure activity.

Each animal was individually placed in a plexiglass transparent cylinder (r: 10 cm, h: 50 cm) immediately following the PTZ injection. The behaviors of animals were recorded with a camera (Samsung Galaxy note 3, South Korea) for 1 h and the video recordings were stored in a computer for later analyses (Toshiba Z40-C1411, Japan). 4 animals died from the seizure during this procedure.

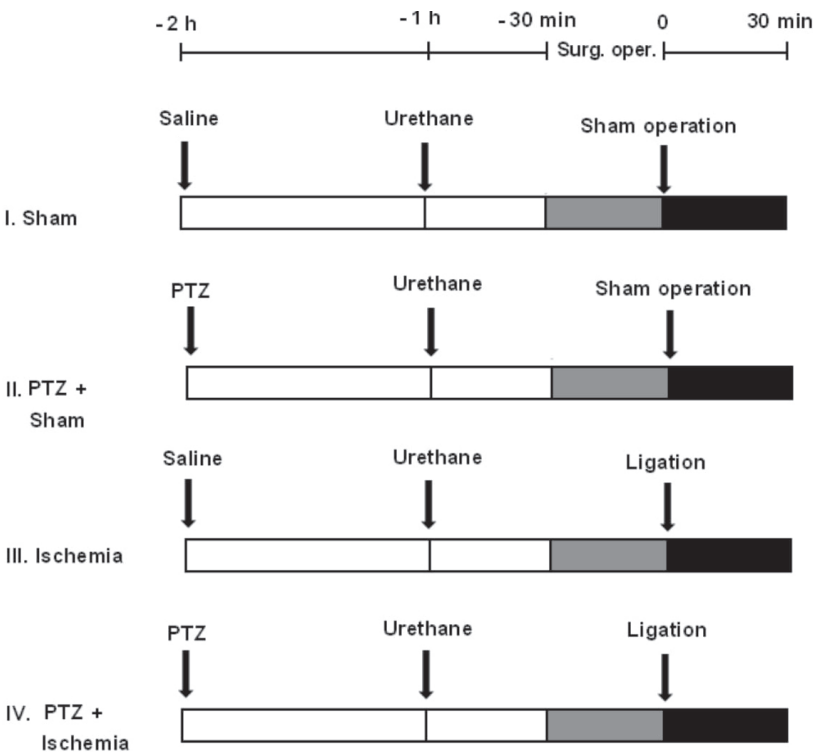

Figure 1. An overview of experimental groups and the study protocol. Arrows indicate the timing of coronary artery ligation and sham operation and the administration of the substances and saline. PTZ: pentylentetrazole, Surg. Oper.: surgical operation.

2.4. Determination of the seizure score

Epileptic seizure activity and seizure-induced behavioral changes were observed following the PTZ injection. The onset, severity, and duration of seizure activity were evaluated and the seizure scoring was done according to the scoring scale described by Velisek et al. (13).

2.5. Surgical procedure

The animals were anesthetized with urethane at a dose of 1.4 g/kg. They were then placed on a heater plate with rectal thermometer (RTC 9404-A, Commat Ltd, Ankara, Turkey) to keep their body temperature at 37 ± 1 °C. Tracheotomy was performed. The right carotid artery was cannulated for the measurement of the arterial blood pressure (Blood pressure transducer, SS 13 L, Biopac systems, Goleta, CA, USA). Thorachotomy was made by cutting the 2nd and 3rd ribs of the animals. Immediately after the opening of the thoracal cavity, the rats were connected to the small animal ventilators to maintain artificial respiration (60 strokes/min, at a tidal volume of 1.5 mL/100 g; SAR 830, IITC Life Science, Woodland Hills, CA, USA). Pericardial membranes of the hearts were incised. The hearts were gently exteriorized and a silk suture was passed under the left anterior descending coronary artery (LAD) 2 to 3 mm away from the root of the aorta. The hearts were then replaced in its place. The electrodes were inserted under the skin to record the electrocardiogram (ECG) (lead II) (Data acquisition system, MP35, Biopac systems). Their blood pressure and ECG were monitorized and the rats were allowed to stabilize for 5 min before subsequent coronary occlusion. The ligation of LAD was performed for 30 min. The hearts were then gently removed at the end of the 30 min of ligation. After solving the knot in LAD, the hearts were retrogrately perfused with 10 mL of saline solution at 37 °C throughout the aorta to washout the residual blood in the vessel. Following the ligation of LAD once again, 1 mL of 96% of alcohol was given throughout the aorta to delineate the border of the zone at risk. Alcohol only perfused nonischemic myocardial zone and made it white. The zone at risk was not perfused with alcohol and remained in its original tissue color. The zone at risk was separated from the rest of the ventricles by cutting along the border with a fine pair of scissors. The zone at risk and total ventricles were weighed. The percentage of the weight of the zone at risk to that of total ventricles was calculated. The tissue specimens from the zone at risk region were stored in 10% of formaldehyde solution for later histopathological evaluation.

2.6. The exclusion criteria

The coronary artery ligation was not performed on 8 rats having sustained ventricular arrhythmias and the mean arterial blood pressure (MABP) below 70 mmHg during 10 min of stabilization period. ST-segment elevation on ECG and the decline in MABP values (25%–30% in comparison to baseline value) were not seen in nonsuccessful ligation. 5 rats on which successful ligation was not performed were also excluded. Thus, a total of 13 animals were excluded from the experiment.

2.7. Histopathological examination

The heart samples were fixed with 10% formaldehyde and embedded in paraffin and cut into sections of 4–5 μm. The sections were stained with hematoxylin and eosin (H&E) and examined under the light microscope for histological changes by an experienced pathologist who was blinded to the study groups. The tissue damage was scored on a scale of 0–4. Histopathological examination of the heart tissue was based on a staging method described by Goyal et al. (14). The sections were evaluated in respect of myonecrosis, interstisyel inflamatuar cell infiltration, and edema which demonstrates myocardial damage. Cardiac myonecrosis is histologically characterized by swollen, deeply eosinophilic, homogeneous myofibers that lack cross striations. Affected fibers are often vacuolated and fragmented with pyknotic nuclei. A minimum of 10 fields for each section were examined and scored as follows: 0: absence of inflammation, edema, and necrosis; 1: focal areas of inflammation, edema, and necrosis; 2: patchy areas of inflammation, edema, and necrosis; 3: confluent areas of inflammation, edema, and necrosis; and 4: massive areas of inflammation, edema, and necrosis.

2.8. Analyses of ECG and blood pressure recordings

MABP and heart rate (HR) values were determined before and during the ligation and sham operation at 1, 3, 5,10, 15, 25, 30 min (Data acquisition system; MP35, . Biopac Systems). QT and QRS intervals on ECG were also determined before and during the ischemia at regular intervals. Bazzett's formula was used to calculate the corrected QT (QT_c) to eliminate the effect of HR on the QT interval (QT_c = QT/) (15). The type of ventricular arrhythmias were diagnosed as VPC including single, bigeminy and salvo, VT and ventricular fibrillation according to Lambeth Convention (16). The incidence of each type of arrhythmias was determined. The numbers of VPC and VT were counted (Figure 2).

2.9. Statistical Analyses

GraphPad Software package (GraphPad Prism version 5, San Diego, CA, USA) was used to analyze the data. Statistical analysis of the survival rate and the incidence of arrhythmias was performed by using Fisher's exact test. All other data were expressed as mean ± SE. The unpaired *t*-test (two-tailed) was used to analyze the number of arrhythmia types, MABP, and HR between ischemia and PTZ + ischemia groups. The unpaired *t*-test (two-tailed) was also used to compare QT, QTc, and QRS values with preocclusion values. One-way ANOVA with Tukey's post hoc test was used to compare QT, QTc, and QRS values before and during ligation among the groups.

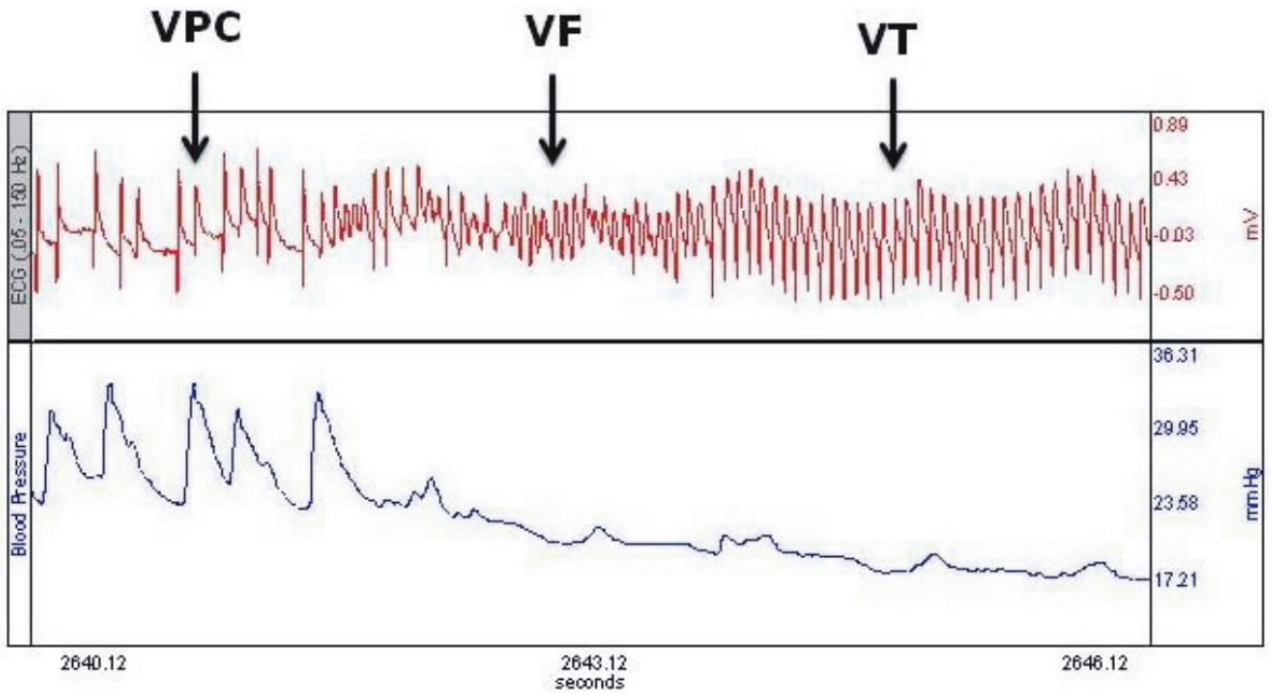

Figure 2. Original ECG and blood pressure tracings from PTZ + ischemia group. VF: ventricular fibrillation; VT: ventricular tachycardia; VPC: ventricular premature contraction.

The nonparametric Mann–Whitney *U* test was used to compare the epileptic seizure score values between ischemia and PTZ + ischemia groups. The nonparametric Kruskal–Wallis test was used to compare the histopathological score values among the groups. Changes in P values less than 0.05 were considered to be significant.

3. Results

3.1. Epileptic seizure scores

Generalized tonic-clonic seizure activity was observed in all animals in the PTZ + sham and PTZ + ischemia groups that were administered PTZ injections at a dose of 65 mg/kg. Generalized seizures started after clonic jerks that accompanied clonuses of the facial and forelimb muscles (Stage 1, onset); this was followed by clonic activity involving head, neck, and tail extension (Stages 2 and 3), as well as the loss of the righting reflex, which comprises tonic-clonic responses (Stage 4, generalized major seizure); the seizures ended with tonic flexion and extension following prolonged tonic-clonic clonuses (Stage 5). When the severity scores of the PTZ + sham and PTZ + ischemia groups were evaluated statistically, no significant difference was found (4.5 ± 0.2, n = 6 and 4.1 ± 0.1, n = 8 respectively; P = 0.1445).

3.2. Hemodynamic variables

MABP fell immediately following ligation in all animals. The MABP measured following the first minute of ligation was significantly lower than the preischemic values. Neither MABP nor HR was significantly different between the ischemia and PTZ + ischemia groups at all measuring points during ligation (Table 1).

3.3. Ischemia-induced ventricular arrhythmias

In all animals, the ligation of LAD induced ventricular arrhythmias, including predominantly VPC and VT. The incidence and number of VT episodes were significantly higher in the PTZ + ischemia group than in the ischemia group (the numbers of VT episodes were PTZ + ischemia group: 48 ± 30, ischemia group: 1 ± 1; P < 0.05) (Table 2).

3.4. Electrocardiogram evaluation

Further analyses of ECG traces revealed that QT and QTc values were lower in the PTZ + sham and PTZ + ischemia groups than in the ischemia and sham groups before occlusion. QT and QTc values were also lower in the PTZ + ischemia group than in the ischemia group after the first and fifth min of ligation. These values were higher in the ischemia and PTZ + ischemia groups at all measuring points during ligation than the preischemic values (Table 3; Figure 3).

QRS interval values were significantly higher in the PTZ + sham and PTZ + ischemia groups before occlusion. These values were also higher in the ischemia and PTZ + ischemia groups than the preischemic values after the first and fifth minutes of ligation (Table 3; Figure 3).

Table 1. The summary of heart rate (HR) and mean arterial blood pressure (MABP) values during ischemia and reperfusion (results: mean ± SE).

Time	MABP (mm Hg)	
	Ischemia	**PTZ + Ischemia**
Basal	76 ± 2	77 ± 6
1 min	44 ± 3*	60 ± 8*
3 min	45 ± 3	**58 ± 7**
5 min	42 ± 3	56 ± 8
10 min	48 ± 3	56 ± 7
15 min	48 ± 3	55 ± 6
20 min	47 ± 4	53 ± 5
30 min	42 ± 6	48 ± 4
HR		
Basal	364 ± 17	383 ± 13
1 min	366 ± 16	379 ± 14
3 min	363 ± 14	367 ± 15
5 min	352 ± 13	365 ± 18
10 min	338 ± 13	352 ± 16
15 min	333 ± 9	352 ± 16
20 min	344 ± 15	361 ± 13
30 min	370 ±18	374 ± 8

*P < 0.05**:** Compared to preischemic values.
The number of animals in each group: 8.

Table 3. The effects of ischemia and/or PTZ administration on QT, QTc, and QRS intervals (average of 5 beats) before and during coronary artery ligation in anesthetized rats.

QT	**Basal**	**1**	**5**	**20**
Sham	90 ± 6	85 ± 3	84 ± 3	83 ± 6
PTZ + sham	79 ± 4*	81 ± 5	79 ± 4	79 ± 4
Ischemia	94 ± 5	114 ± 4**	116 ± 4**	115 ± 4**
PTZ + ischemia	78 ± 2*	100 ± 4**#	101 ± 7**#	106 ± 7**
QTc				
Sham	7 ± 0.3	7 ± 0.3	7 ± 0.3	7 ± 0.4
PTZ + sham	6 ± 0.1*	7 ± 0.4	7 ± 0.4	7 ± 0.4
Ischemia	7 ± 0.3	9 ± 0.2**	9 ± 0.2**	9 ± 0.3**
PTZ + ischemia	6 ± 0.1*	8 ± 0.3**#	8 ± 0.5**#	8 ± 0.5**
QRS				
Sham	40 ± 7	44 ± 6	44 ± 17	44 ± 6
PTZ + sham	44 ± 6*	44 ± 6	47 ± 6	46 ± 4
Ischemia	36 ± 4	60 ± 3**	51 ± 6**	47 ± 4
PTZ + ischemia	44 ± 6*	64 ± 4**	57 ± 5**	46 ± 4

Basal: before ligation and PTZ administration. 1: 1st min of ischemia, 5: 5th min of ischemia, and 20: 20th min of ischemia. PTZ: pentylentetrazole. *P < 0.05: compared to sham and ischemia groups, **P < 0.05: compared to preischemic values, #P < 0.05: compared to ischemia group. Values represent mean ± standart error (SE) of n = 8 animals/groups.

Table 2. The incidence and number of arrhythmia types during 30 min of ischemia.

Group	N	Arrhythmia incidence (*n* /%)			The number of arrhythmia types	
		VF	**VT**	**VPC**	**VT**	**VPC**
Ischemia	8	0/0		8/100	1 ± 1	164 ± 47
PTZ + Ischemia	8	1/13	5/63*	8/100	48 ± 30†	186 ± 82
P value			0.0256		0.0000082	

VF: ventricular fibrillation; VT : ventricular tachycardia;
VPC: extrasystoles, salvos, bigeminy.
N: The number of animals before and at the end of ischemia
n: The number of animals that experienced arrhythmias
*P < 0.05, †P < 0.00001**:** compared to the ischemia group.

3.5. Histopathological observations

Histopathological scores, demonstrating myocardial damage of the groups, are summarized in Table 4. Upon histopathological examination of the rats' myocardium, it was observed that in the sham group, there existed minimal myocardial damage in the focal areas or no inflammation; edema and necrosis were also present (Figure 4A). Myocardial damage was prominent in the PTZ + sham and ischemia groups when compared to the sham group, with focal or patchy areas of inflammation, edema, and necrosis (Figures 4B and 4C). More prominent myocardial damage was observed in the PTZ + ischemia

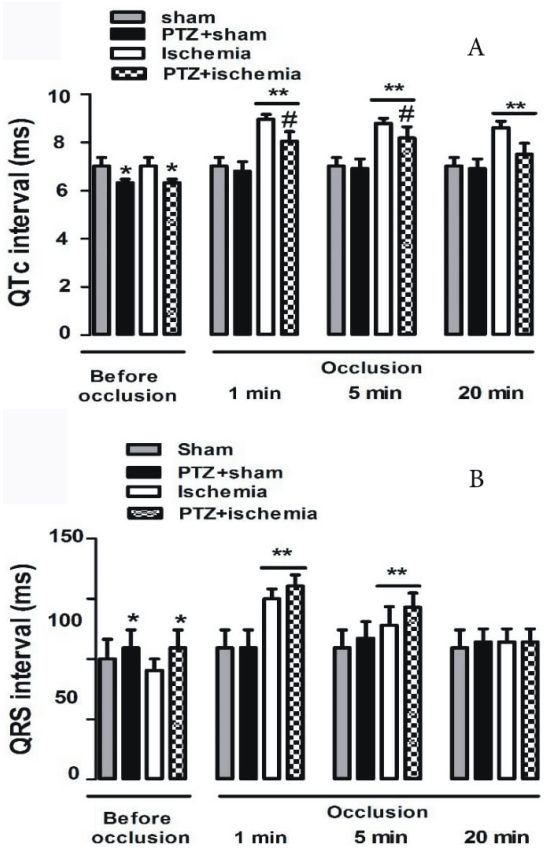

Figure 3. The effects of ischemia and/or PTZ administration on QTc (A) and QRS (B) intervals (average of 5 beats) before and during coronary artery ligation in anesthetized rats. *P < 0.05: compared to sham and ischemia groups, **P < 0.05: compared to preischemic values, #P < 0.05: compared to ischemia group. Values represent mean ± standart error (SE) of n = 8 animals/groups. PTZ: pentylentetrazole.

group than in the other groups, with patchy or confluent areas of inflammation, edema, and necrosis (Figure 4D).

4. Discussion

The present study revealed that PTZ-induced generalized tonic-clonic seizure augmented myocardial structural damage during myocardial ischemia in rats. This finding is consistent with the clinical data reported by Tigaran et al. and Park et al. (17,18). Tigaran et al. found that patients with refractory focal epilepsy had seizure-related ST-segment depression, suggesting that cardiac ischemia might occur during seizure (17). Park et al. reported elevated troponin I as an indicator of myocardial infarction in a patient after an episode of generalized tonic-clonic seizure (18). Similarly, in a recent experimental study on rats, Read et al. found that kainic acid-induced status epilepticus caused hypercontraction band necrosis, inflammatory cell infiltration, and edema in ventricular mycocardium

Table 4. The histopathological scores.

Group	N	Scores
Sham	6	0.1 ± 0.4
PTZ	6	1.2 ± 0.4†
Ischemia	6	1.2 ± 0.4†
PTZ + ischemia	6	2.5 ± 0.5*†

N: The number of animals
Data are expressed as the mean ± standard error.
*P < 0.05: compared to ischemia group
†P < 0.05: compared to sham group

(8). The present study also revealed, for the first time, that epileptic seizure increased myocardial damage in rats affected by cardiac acute myocardial ischemia. These findings show that epileptic seizure may not only cause structural damage in the myocardial tissue of epileptic patients but also could augment the extent of myocardial tissue injury during possible myocardial infarction. Therefore, these results reveal that epileptic seizure may alone be a profound risk factor for myocardial enfarctus during the condition of possible myocardial ischemia.

In our study, ECG analyses revealed that QRS intervals were prolonged in rats with PTZ-induced seizure. This finding is in agreement with our pathological analyses, which showed fibrosis of myocardial tissue in rats with tonic-clonic seizure. The fibrosis of the myocardium may have caused the slowing of impulse conduction in the ventricular myocardium, which could have widened the QRS complex in the rats of these groups. The QRS intervals increased during the ischemic period compared with the preischemic values that we would expect; the slow conduction in the ischemic myocardium could be the reason for this prolongation. Similarly, QT and QTc values were also found to be longer in the ischemic period than the preischemic values. This is because the slowed conduction in the rats could be partly responsible for QT interval prolongation (19).

All epileptic attacks develop through different mechanisms; however, they all share common features, including increased autonomic dysregulation and increased sympathetic activation (20–23). In the present study, the increased myocardial tissue injury in the rats which exhibited tonic-clonic seizure activity may have been caused by the possible massive sympathetic discharge during the seizure. Likewise, several studies have reported that excessive sympathetic activation can cause myocardial tissue injury through beta-adrenergic activation (24,25). In addition, the excess amount of beta-adrenergic activation may also lead to cellular Ca^{++} overload, which could contribute to myocardial tissue injury (10,26).

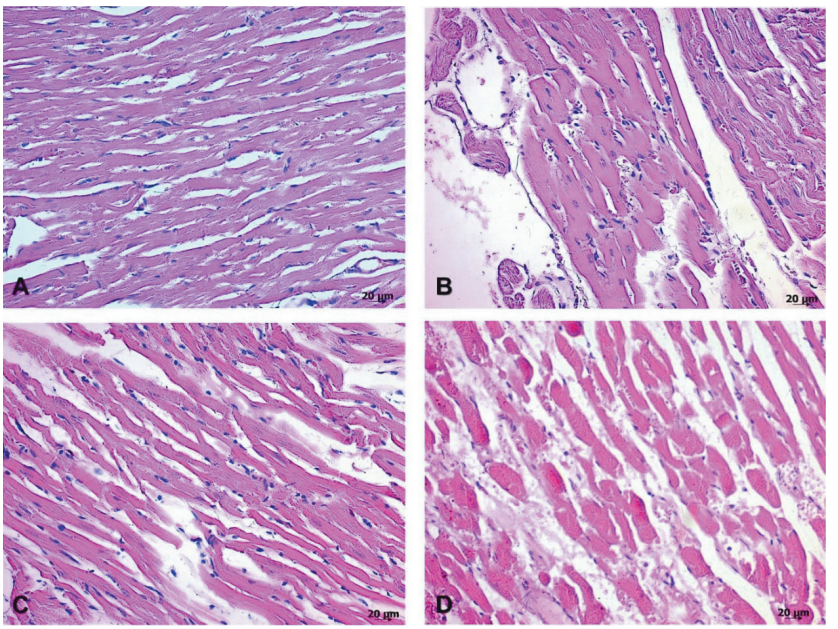

Figure 4. Histopathological changes in the rat myocardium in groups with hematoxylin and eosin staining (A) sham group, displaying minimal myocardial damage with minimal inflammation, edema, and necrosis; (B) PTZ + sham group, prominent evidence of myocardial damage with patchy areas of inflammation, edema, and necrosis; (C) ischemia group, myocardial damage similar to PTZ group, with patchy areas of inflammation, edema, and necrosis; (D) PTZ + ischemia group, evident myocardial damage with confluent areas of inflammation, edema, and necrosis.

In the present study, PTZ-induced seizure increased the incidence and number of VT episodes during myocardial ischemia. This result is in line with the clinical data (27,28). In consistence with the present results, Damasceno et al. reported that epileptic seizure induced by both maximal electroshock and acoustic stimulation increased the incidence and duration of I/R-induced ventricular arrhythmias in isolated rat hearts (10,29). In contrast with this finding, Tavares et al. reported in their in vivo study that pilocarpine-induced seizure activity did not affect the incidence of ventricular arrhythmias during ischemia and reperfusion periods (11). The discrepancy in these results may be clarified by referring to the difference in epilepsy models and the time period between the onset of epileptic agent administration and the ischemia and reperfusion protocol. In the present study, the ischemia protocol was performed 2 h following the PTZ administration. However, Tavares et al. performed the ischemia and reperfusion protocol 122 days following pilocarpine hydrochloride administration (11).

In our study, the high incidence and duration of VT may have resulted from excess sympathetic discharge due to high seizure activity. Likewise, studies have reported that high concentration of plasma and tissue catecholamine levels is an important arrhythmogenic stimulus (30). Epinephrine may cause the generation of ventricular arrhythmias through the mechanism of early afterdepolarization (31). Structural alteration in myocardial tissue may also contribute to the generation of ventricular arrhythmias due to the reentrant mechanism during ischemia (32).

In the present study, QT and QTc intervals were found to be shorter in rats with epilepsy. Epilepsy-induced gene mutation in potassium and Ca^{++} channels might have caused the shortening of the QT and QTc intervals in rats with epilepsy (33). QT and QTc shortening in rats with epilepsy may be another reason for the high incidence and duration of VT. This is because it may have prepared the appropriate conditions for reentrant excitation, thus leading to the generation of VT (34,35).

In a recent study, Damasceno et al. provided evidence to show that audiogenic epileptic seizure increased the peak amplitude of myocyte intracellular Ca^{++} due to enhanced sympathetic activity (10). Myocardial Ca^{++} overload contributes to myocardial tissue injury in various cellular mechanisms and may cause the generation of ventricular arrhythmias through the mechanism of delayed afterdepolarization (36–38). In the present study, the increased beta-adrenergic activation due to the possible excess adrenergic discharge may have caused myocardial cell Ca^{++} overload. In addition, Myocardial Ca^{++} overload in PTZ-induced generalized-seizure rats may have increased the occurrence of arrhythmias.

In summary, the present results reveal that PTZ-induced generalized tonic-clonic seizure may be an important risk factor for both myocardial tissue injury and ventricular arrhythmias in rats affected by acute myocardial ischemia. Epileptic seizure activity may also be an important risk factor for SUDEP in epilepsy patients through the generation of life-threatening ventricular arrhythmias and tissue injury during possible heart attack.

Limitation of the study

In the present study, histopathological examinations clearly reveal more prominent myocardial damage in the PTZ + ischemia group than in the other groups. However, the histopathological data could have been supported by the biochemical and immunohistochemical findings to strengthen the results.

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