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Original Investigation

The Preventive Effect of Mexiletine on Cerebral Ischemic Injury Following Experimental Middle Cerebral Artery Occlusion

Orta Serebral Oklüzyon Modeli ile Oluşturulan Serebral İskemi Hasarında Meksiletinin Koruyucu Etkisi

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ABSTRACT

AIM: Previous studies demonstrated that mexiletine has some important features in the prevention of ischemic brain injury such as sodium and calcium canal blockage and free radical occurrence. Our aim was to investigate the effects of mexiletine on ischemic brain injury.

MATERIAL and METHODS: Experiments were performed on 30 adult male Sprague-Dawley rats (285-425 g). Left middle cerebral artery occlusion following microcraniectomy and simultaneous bilateral carotid artery occlusion were performed. Three different treatments were included in this study: (a) "naïve" control group (no drug applied; n = 10); (b) "sham surgery" control group (only saline was applied; n = 10); and a (c) "treatment group (n = 10) where mexiletine was applied. After 24 h from ischemic insult, all rats were decapitated and prepared for immunocytochemical and histopathological analyses. Cerebral infarct volumes were calculated and compared using ANOVA and a Post- Hoc Bonferroni test in each group statistically.

RESULTS: The results showed statistically significant differences between the treatment (81.98 ± 12.58 mm?), control (121.57 ± 11.41 mm?) and sham (116.08 ± 12.36 mm?) groups ($p < 0,0001$), respectively.

CONCLUSION: Mexiletine should be considered as an alternative medication for prevention and treatment of ischemic brain injury due to its multipotent effects.

KEYWORDS: Cerebral ischemia, Mexiletine, Middle cerebral artery occlusion

ÖZ

AMAÇ: Yapılmış çalışmalarda meksiletinin sodyum ve kalsiyum kanal blokajı ve serbest radikal oluşumunu engelleyerek iskemik beyin hasarında koruyucu etkisinin olduğu gösterilmiştir. Bu çalışmada beyinin iskemik hasarında meksiletinin etkisi araştırılmıştır.

YÖNTEM ve GEREÇ: Çalışmada 30 yetişkin erkek Sprague-Dawley türü sıçan kullanıldı (285-425 g). Mikrokraniyotomi sonrası orta serebral arter oklüzyonu ve simultane bilateral karotid arter oklüzyonu uygulandı. Üç ayrı uygulama yapıldı: (a) "naïve" kontrol grubu (ilaç uygulanmadı; n = 10); (b) "sham grubu" kontrol grubu (sadece salin solüsyonu; n = 10); ve (c) "tedavi grubu (n = 10) meksiletin uygulandı. İskemiden 24 saat sonra tüm sıçanlar dekapite edilerek immünohistokimyasal ve histopatolojik inceme için hazırlandı. Serebral infarkt hacimleri hesaplandı ve sonuçlar ANOVA ve Post- Hoc Bonferroni test ile istatistiksel olarak değerlendirildi.

BULGULAR: Sonuçlar karşılaştırıldığında tedavi grubu ile (81,98 ± 12,58 mm?), kontrol (121,57 ± 11,41 mm?) ve sham (116,08 ± 12,36 mm?) grupları arasında sırasıyla anlamlı farklılık saptandı ($p < 0,0001$).

SONUÇ: Meksiletin iskemik beyin hasarında hem koruyucu hem de tedavi edici etkisi nedeniyle iyi bir alternatif olarak kabul edilmelidir.

ANAHTAR SÖZCÜKLER: Serebral isemia, Meksiletin, Orta serebral arter oklüzyonu

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INTRODUCTION

Ischemic cerebrovascular diseases are important causes of significant morbidity and mortality. Cerebral ischemia cannot be explained with a single mechanism and it is related to a complex chain of events having various etiological factors and is linked to many pathophysiological factors.(1,6,26,28) By reduction of glucose and oxygen to the tissues, acidic end-products are increased and intracellular pH decreases. The released toxic substances damage glutamate channels and Na-Ca exchange pumps. Calcium entering into cells increase and ion channels and membrane receptors begin to malfunction.(30,31) Additionally, free radicals are released and these together with lipid peroxidation play an important role in the etiopathogenesis of cell damage.(42) These free radicals have toxic effects on cerebral vascular and surrounding cerebral parenchyma leading to destruction of cell lipid, protein and nucleic acid structures.(7) Weakening of antioxidant mechanisms as a result of the oxidative stress also contributes to the expansion of the destruction.(11)

Due to the important role of free radicals and intracellular calcium concentration in the pathogenesis of ischemic injury many antioxidants and calcium channel blockers have been tested for prevention of ischemic brain injury in the literature(33,37)

Regarding mexiletine is a class 1b antiarrhythmic drug widely used in treatment of ventricular arrhythmias and other muscular disorders linked to abnormal excitability of skeletal muscle fibers. We therefore investigated the preventive role of mexiletine, which is a calcium channel blocker, ATP (adenosine triphosphate) sensitive potassium channel activator and sodium channel blocker, and its antioxidant properties in ischemic brain injury.(30,31)

MATERIALS and METHODS

Experimental Protocol

All procedures were conducted in accordance with the strict policies of the Ethics and Animal Care and Use Committee of Baskent University. A total of 30 adult male Sprague-Dawley rats weighing between 285-425 grams (mean 338 gr) were used in the study. No female rats were used as many studies show estrogen as having a neuroprotective effect.(2)

Experimental Groups

The rats were divided into three groups each having ten rats. Group 1 (n=10) animals were subjected to ischemia only. Group 2 (n=10) animals were subjected to ischemia and treated with sham (normal saline) 1 mg/kg by intraperitoneal. Group 3 (n=10) animals were subjected to ischemia and treated with 60 mg/kg mexiletine (Boehringer Ingelheim International GmbH Ingelheim am Rein, Germany) by intraperitoneal (ip) injection.

Feeding was ceased for all rats one hour in advance of the procedure. Anesthesia was achieved with 60 mg/kg ketamine and 9 mg/kg xylazine applied intraperitoneally. Anesthesia was adjusted in order to achieve complete pain relief during the study and continuing spontaneous breathing. Body temperature was maintained normothermic (37 ± 0.5) and followed by rectal thermometer during the surgeries. The femoral artery of the rats was cannulated and arterial blood samples (PaCO2 and PaO2) were taken from each animal for blood gas analysis before and after ischemia. Postoperatively rats were kept in normal room temperature (20-22° C).

Surgical Procedure

At the operating table after anesthesia, local antisepsis was achieved with polyvinyl pirolidon iyodine. A midline neck incision was made using a surgical microscope. Both carotid arteries were identified and suspended with 4/0 silk sutures. A temporal craniectomy with the zygoma preserved was made on the left side of the skull using an electric drill. Dura was opened and the left middle cerebral artery (MCA) was identified and coagulated with bipolar. Simultaneously, both carotid arteries were closed temporarily with microclips. Rats were left in cerebral ischemia for 60 minutes and then the microclips in both carotid arteries were opened. All skin incisions were sutured with 3/0 silk sutures. Following the procedures, rats receiving mexiletine or normal saline were put in their cages for postoperative care. An intracardiac perfusion was made at the postoperative 24th hour. High-dose intraperitoneal anesthesia (60 mg/kg ketamine + xylazine 9 mg/kg) was applied first for perfusion. The thoracic wall of the rats was opened under anesthesia with an incision beginning from the processus xiphoideus and advancing from both sides of the costas on the parasternal line, lifted and stabilized cranially. The aperture and pericardium were cut. 5000 U sodium heparin was dissolved in 1 ml 0.9% physiological serum and administered to the circulation via the left ventricle. An incision was then made to the atrium, and blood was let to flow out. The

left ventricle was entered by cannula from the apex while the contraction of the heart was continuing. 0.9% physiological serum solution (approximately 100-150 ml) was given through the cannula until the blood was completely removed from the body. Approximately 5-10 minutes after the beginning of the perfusion, all blood was drained from the bodies of the rats and the same volume of 10% formol solution was given to the left ventricle. Involuntary contractions were observed in the extremities and facial muscles of all rats due to formol perfusion. Fixation was continued until the whole body hardened, and especially internal organs (liver, lung, etc) were pale. The rats were decapitated after the perfusion. The brains were removed and kept in 10% formol solution for 1-2 days. They were then embedded in paraffin tissue blocks in the rostro-caudal direction.

Histopathological Evaluation

After removing the cerebellum, 2 mm series of zones were obtained in the coronary plane from caudal to rostral. An average of six slices were obtained for each brain in this way. These slices were then fixated with 4% paraformaldehyde buffered with phosphate. 20μm coronary sections obtained from paraffin blocks by microtome were painted with hematoxylin-eosin (H&E). Cerebral infarction area measurements of the sections were calculated with an analysis system of images loaded to computer (Image J, version 1366, NIH, Bethesda, Maryland). Using a "grid" supplement designed for Image J software (http://rsb.info.nih.gov/ij/), a point-on measurement ruler was placed on microscope images and cerebral infarction volumes were calculated with the Cavalieri principle, which is a stereological volume calculation method. The Cavalieri principle is a calculation method designed to measure the volume of a structure divided by equally spaced parallel slices. Cross-sectional areas of the structure surfaces turned towards the same direction are calculated and multiplied by the average thickness of the sections, and the total volume of the structure can be obtained objectively. The precision and accuracy of the Cavalieri volume calculation method has been shown to be the same as advanced image analysis systems.(4) Thus, this method is practical, easy to apply, and is significant in terms of cost and time.

Statistical Analysis

All data are expressed as mean ± standard deviation. A one-way analysis of variance (ANOVA) test was used in all groups to show whether the data was homogeneous or not. The differences between the groups were assessed with the Post-Hoc Bonferroni test. ANOVA and Post-Hoc Tukey B tests were used for comparison of the artery blood gases taken immediately before and after ischemia.

RESULTS

Mean arterial blood pressure, PaCO2, PaO2, pH, and body temperatures were within normal physiological ranges for all groups both before the ischemia and after the ischemia (Figure 1-4). No deaths or seizure occurred from surgical procedures within 24 hours. Sections stained with H & E were examined within the following groups. Infarction volumes in three groups of a focal cerebral ischemia-reperfusion model were calculated with standard deviations and compared between the groups (Figure 5, 6 A,B,C). Initially, three groups were evaluated by the ANOVA test (Figure 5). Results of the statistical examination were significant ($p < 0.0001$). In Group 1, only focal cerebral ischemia was created on ten rats. According to the Post-Hoc Bonferroni test there were no significant differences compared to the group receiving normal saline ($p=1.000$), but the difference compared to the

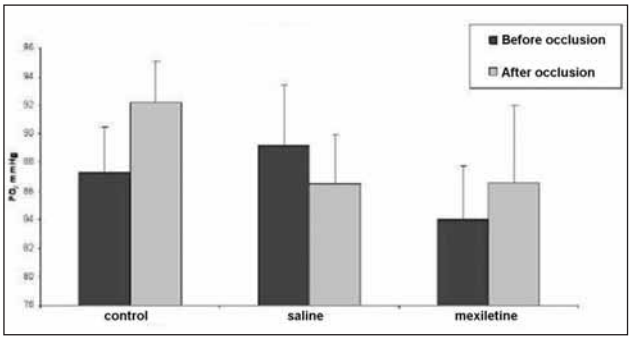

Figure 1: *PO2 values of the groups before and after the operation.*

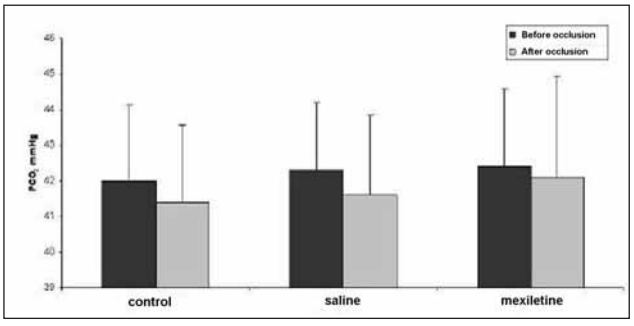

Figure 2: *PCO2 values of the groups before and after the operation.*

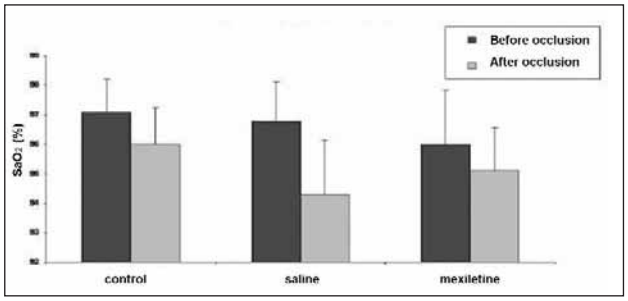

Figure 3: *SaO2 values of the groups before and after the operation.*

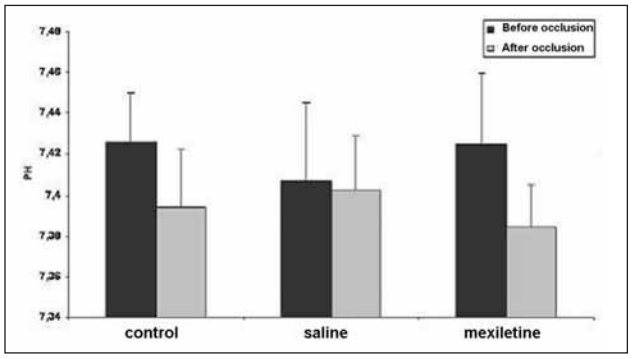

Figure 4: *pH values of the groups before and after the operation.*

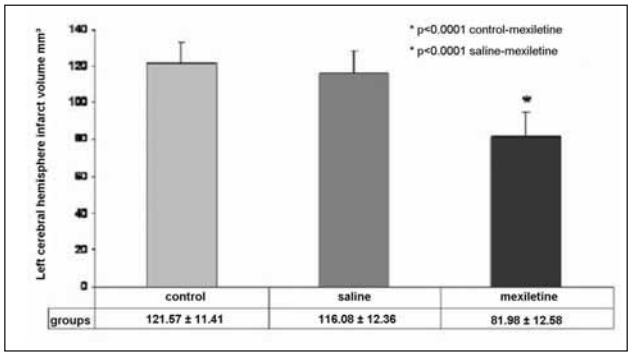

Figure 5: *Mean cerebral infarction volume of the groups and their comparisons.*

group treated with mexiletine was significant (p <0.0001). Average cerebral infarction volume was 121.57 ± 11.41 mm? in this group (Figure 5). In Group 2, rats were given 1 ml/kg normal saline ip after ischemia. According to the Post-Hoc Bonferroni test there was no significant difference when compared with the group that had only ischemia (p = 1.000). There was a significant difference when compared with the group treated with mexiletine (p<0.0001). The average cerebral infarction volume in this group was 116.08 ± 12.36 mm? (Figure 5). In Group 3, ten rats were given mexiletine 60 mg/kg ip after ischemia. According to the Post-Hoc Bonferroni test, there were significant differences when compared to the other two groups (the group which had ischemia only and the group that was given normal saline) (p <0.0001). The average cerebral infarction volume was 81.98 ± 12.58 mm? in this group (Figure 5).

DISCUSSION

The brain, despite comprising only 2-3% of total human body weight, consumes 25% of the available glucose and 20% of the systemic oxygen. Approximately half of the central nervous systems energy supplies are utilized to drive a Na-K ATPase ion pump that maintains ionic balance in glial cells and also repolarizes neurons after an action potential. Due to the recent advances in the understanding of the pathogenesis of ischemic brain injury, there is increased realization of the important roles of free radicals, and intracellular sodium and calcium concentration (Figure 7).(6,23,40) Additionally, many antioxidants and sodium and calcium channel blockers have been tested in the literature.(12,22,33, 34,37) MCA occlusion serves as a good model to create ischemic brain damage and responds very sensitively to different neuroprotective drugs.(3,12,16,19,22,24,

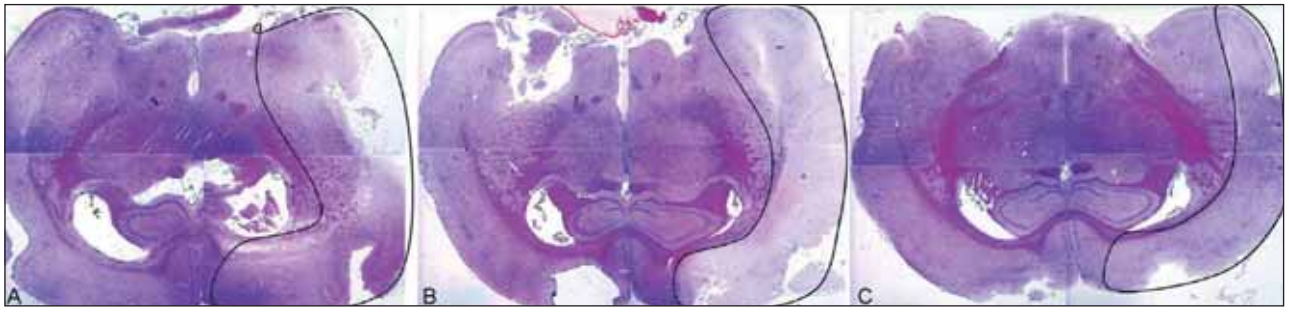

Figure 6: *Representative slices of the animals subjected to ischemia; without any treatment with broad ischemia volume (**A**), treated with sham with broad ischemia volume (**B**) and treated with mexiletine with significantly lower ischemia volume (**C**) (all samples' ischemia areas are surrounded with a black line).*

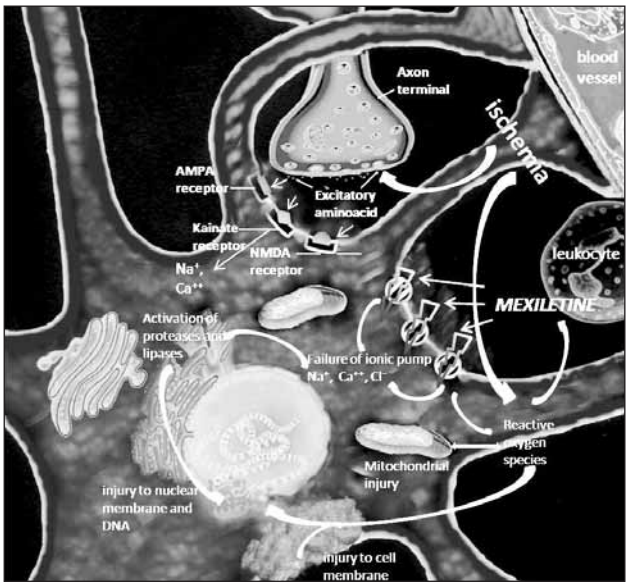

Figure 7: *Summary of the process of cerebral ischemia and the potential effects.*

25,29,34,38,43,44,45) The method described by Hiramatsu et al. is a rat model of focal neocortical ischemia and it is the most frequently applied experimental model for assessing cerebroprotective properties of drugs which are candidates for clinical trial.(17) In our study, we also preferred to use the rat model of MCA occlusion to examine the effect of the potent antioxidant mexiletine on cerebral injury due to focal ischemia.

The protective effect of mexiletine in anoxic ischemic damage depends on the inhibition of Na+ - Ca ++ exchange and its antioxidant properties. Mexiletine also blocks transfer to the voltage and receptor-dependent Ca++ channels. It prevents Ca++ release from intracellular depots (Figure 7).(9,27,36,39) Chang et al. and Kimberley et al. had previously shown that mexiletine is protective against cerebral ischemia in both grey and white matter.(8,20) In addition, mexiletine was also shown to be effective in treatment of vasospasm following experimental subarachnoid hemorrhage and traumatic spinal cord injury.(5,18) It was shown to prevent lipid peroxidation and reduce cerebral vasospasm in rabbits, blocking ion channels.(21,22,41) Mexiletine was shown to block KCl (potassium chloride)-induced great vessel contraction strongly with in-vivo studies and it was shown in-vitro to prevent lipid peroxidation potently in a study using the Fe-Asc-H2O2 system.(10,14,15,35) Stys et al. showed that mexiletine concentrations reached levels high enough to afford significant protection after intraperitoneal administration in their study by in situ examination of the central nervous system (CNS) tissue. They reported mexiletine as a use-dependent Na+ channel blocker that is capable of CNS penetration and that can offer neuroprotection from ischemic tissue.(36)

Considering the factors that play a role in the pathophysiology of cerebral ischemia, an agent needs to affect many factors in order to prevent cerebral ischemic injury. Therefore, we thought mexiletine might be effective in cerebral ischemic injury due to its multipotent activity. In our study, we created three groups by using a total of 30 rats. In Group 1, we just applied a focal cerebral ischemia-reperfusion model without using any drugs. In Group 2, normal saline (physiological serum) was given i.p and in Group 3: mexiletine was given 60 mg/kg i.p. Drug dose was determined by reviewing previous studies in the literature.(22) Rats were sacrificed at the 24th hour and the brains were removed whole as suggested by the literature(16,25)

Coronary sections obtained from these brains were stained with H&E. Cerebral infarction volumes were calculated by the Cavalieri method by using the necessary software. The calculations that were made by the Stereological Cavalieri volume calculation method are shown to be comparable to the calculations that are made by advanced image analysis systems in terms of certainty and accuracy.(13) In addition, in a study made using intra-arterial nimodipine cerebral infarct volumes created by the focal cerebral ischemia reperfusion model were calculated according to the Cavalieri principle.(32)

In conclusion, our study suggested that mexiletine significantly reduces the volume of cerebral infarction volumes due to ischemia. This effect of mexiletine might have occurred due to Na+ channel blockade, Ca++ channel blockade and/or through antioxidant effects. We think that the protective effects of mexiletine in cerebral ischemic injury are multifactorial.

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