ORIGINAL ARTICLE

DOI: 10.4274/nkmj.galenos.2023.83703
Nam Kem Med J 2023;11(2):118-126

Effects of Melatonin on Aquaporin Channels in Isoproterenol-induced Myocardial Infarction in Rats*

Sıçanlarda İzoproterenol ile Oluşturulan Miyokardiyal Enfarktüs Modelinde Melatoninin Akuaporin Kanalları Üzerindeki Etkileri*

Nezahat Kübra TÜFEKÇİOĞLU[1], Başak BÜYÜK[2], Metehan UZUN[1]

[1]*Çanakkale Onsekiz Mart University Faculty of Medicine, Department of Physiology, Çanakkale, Turkey*
[2]*İzmir Democracy University Faculty of Medicine, Department of Histology and Embriology, İzmir, Turkey*

ABSTRACT

Aim: Myocardial infarction (MI) commonly results in myocardial edema, but the relationship between aquporin channels (AQP) and the effects of melatonin (MEL) on MI are not well known. Therefore, the aim of the current study was to investigate the effects of MEL on myocardial edema and the change of gene expression level of AQP channels in an experimental MI model.

Materials and Methods: In this study 28 Wistar Albino male rats were used. MI model was established with isoproterenol (85mg/kg). Rats were divided into four groups as: control, isoproterenol (ISO), melatonin (MEL), and isoproterenol+melatonin (ISO+MEL). MEL group was administered 10 mg/kg MEL for 7 days. On day 8, electrocardiographic recordings and blood samples were obtained. Rats were then euthanized and left ventricle tissues were obtained. cTnI and CK-MB levels were examined to assess the success of MI model. AQP1, AQP3, AQP4, AQP7, TNF-α, BAX and Caspase-3 gene expression levels were determined. Histopathological examination was performed on left ventricle samples for the evaluation of edema and mononuclear cellular infiltration.

Results: Histopathological examination and cTnI and CK-MB levels showed that MI model was produced successfully and MEL significantly reduced myocardial edema and decreased AQP1, AQP3, AQP4 and AQP7 gene expression levels.

Conclusion: The results show that MEL decreases myocardial edema by reducing AQP channels, suggesting that it could potentially be used to ameliorate the effects of MI.

Keywords: Melatonin, aquaporin channels, myocardial infarction, edema

*This study was provided from a master of science thesis of first author.

ÖZ

Amaç: Miyokardiyal ödem, aquaporin (AQP) kanalları ve melatonin (MEL) arasındaki ilişki henüz tam olarak bilinmemektedir. Bu çalışmanın amacı deneysel MI modeli oluşturulmuş sıçanlarda MEL uygulamasının miyokardiyal ödem ve AQP kanalları üzerindeki etkilerinin incelenmesidir.

Gereç ve Yöntem: Çalışmada, 28 adet erkek sıçan kullanıldı. Sıçanlar kontrol, izoproterenol (IZO), melatonin (MEL), ve izoproterenol+melatonin (İZO+MEL) olmak üzere dört gruba ayrıldı. MI modeli 85 mg/kg dozunda İZO; (intraperitoneal) verilerek oluşturuldu. MEL gruplarına 10 mg/kg MEL 7 gün süreyle intraperitoneal yolla verildi. Çalışmanın 8. günü elektrokardiyografi kayıtları ve kan örnekleri alındı. Daha sonra sıçanlar ötenazi edilerek sol ventrikül doku örnekleri elde edildi. Kan örneklerinde cTnI ve CK-MB düzeyleri ölçüldü. Sol ventrikül dokusunda AQP1, AQP3, AQP4, AQP7, TNF-α, BAX ve Kaspaz-3 gen ekspresyonu değişimleri belirlendi. Sol ventrikül doku örneklerinin bir kısmı histopatolojik incelemeye tabi tutuldu, ödem ve mononükleer hücre infiltrasyonu açısından değerlendirildi.

Bulgular: MEL uygulamasının İZO tarafından artırılan AQP1, AQP3, AQP4 ve AQP7 gen ekspresyon düzeyleri ile miyokardial ödemi azalttığı belirlendi. Ayrıca MEL'in inflamasyon ve apoptozise karşı kardiyoprotektif etkilerinin olduğu belirlendi.

Sonuç: Bu sonuçlar MEL'in miyokardiyal ödem ve AQP kanalları üzerinde iyileştirici ve düzenleyici bir etki gösterdiğini ortaya koymuştur. Melatonin miyokardiyal ödemin tedavisinde destekleyici bir ajan olarak kullanılma potansiyeline sahiptir.

Anahtar Kelimeler: Melatonin, aquaporin kanalları, miyokardiyal ödem, ödem

*Bu çalışma ilk isim yazarın yüksek lisans tezinden üretilmiştir.

Address for Correspondence: Metehan UZUN MD, Çanakkale Onsekiz Mart University Faculty of Medicine, Department of Physiology, Çanakkale, Turkey
Phone: +90 543 582 86 82E-mail: metehanuzun@comu.edu.tr **ORCID ID:** orcid.org/0000-0003-1406-5473
Received: 27.01.2023 **Accepted**: 08.03.2023

INTRODUCTION

Myocardial infarction (MI) is one of the ischemic heart diseases. Insufficient oxygenated blood flow to the myocardium leads to necrosis and apoptosis inducing myocardial injury in MI[1]. MI also causes edema and it has been reported that ischemic injury extends after longer than 15 min following myocardial edema[2]. Slight increase of myocardial water content has devastating effects on systolic and diastolic functions whereas the 3.5% increase of myocardial edema may cause 30-50% decrease in cardiac output[3,4].

Aquaporins (AQPs), are a family of small integral membrane proteins, facilitating bidirectional flow of water. In mammals, 13 AQPs channels have been identified (AQP 0-12). AQP3, AQP7, AQP9 and AQP10 are called aquaglyceroporins, which permit transcellular passage of glycerol, urea, and other solutes[5]. AQP channels were previously described in the myocardium of rats and cardiac AQPs were associated with myocardial edema after MI and doxorubicin, or cisplatin induced myocardial injury[6-8]. However, the role of AQP channels in myocardial edema have not been fully clarified.

Isoproterenol (ISO; 1-(3,4-dihydroxyphenyl)-2-isopropylamino-ethanol hydrochloride), is a β-adrenergic agonist, and the administration supramaximal dose of ISO provides a rapidly, easy, and non-invasive MI method with the low mortality and high effectiveness. The pathophysiological alterations such as increased oxidative stress, lipid peroxidation, increase of myocardial injury markers [troponin I (TnI), lactate dehydrogenase, creatine kinase (CK-MB)] and pro-inflammatory cytokines [C-reactive protein (CRP), interleukin-6, and tumor necrosis factor-α (TNF-α)], necrosis and apoptosis may occur in ISO induced MI model[7-9].

MEL has cardioprotective effects against environmental chemicals and drugs induced cardiotoxicity[9], cardiac ischemia-reperfusion injury[10], diabetic cardiomyopathy[11] and MI. With those effects, MEL was suggested as a potential therapeutic agent for cardiovascular diseases[12]. However, the effects of MEL on cardiac AQP channels have not been adequately clarified.

Therefore, we aimed to investigate the changes of AQP1, AQP3, AQP4 and AQP7 channels in ISO induced MI model and to investigate the effects of MEL on these channels and preventive effects on myocardial edema and damage.

MATERIALS AND METHODS

Animals

In this study, 28 Wistar albino male rats (2-3 months old) were used. Animals were purchased from Çanakkale Onsekiz Mart University Experimental Research Centre. During the experiment, rats were fed ad libitum. Temperature was maintained at 22±2 °C with a 12 h light/dark cycle. All animal experimental procedures and protocols were approved by Çanakkale Onsekiz Mart University Animal Experiments Local Ethics Committee (decision number 2019/07-04, date: 25.09.2019).

Group Design

Rats were randomly divided into four groups. All experimental procedures were applicated at 08:00-10:00 a.m.

Control group (n=7): The vehicles for MEL and isoproterenol were administrated for 7 days intraperitoneally (ip) in this group.

MEL group (n=7): MEL was administrated with 10 mg/kg/day ip for 7 days.

ISO group (n=7): Isoproterenol was administrated (85 mg/kg) at the 6th and 7th days of the experiment.

Isoproterenol and MEL group (ISO+MEL; n=7): MEL was administrated 10 mg/kg/day ip for 7 days. One hour after MEL administration, ISO was administrated (85 mg/kg) at the 6th and 7th days of the experiment.

Electrocardiographic (ECG) recording was obtained on the 8th day of the experiment.

Preparation and Administration of Drugs

Isoproterenol (Sigma-Aldrich, I6504) was dissolved with 1 mL 0.9% NaCl and freshly prepared before administration during the whole experiment[13].

MEL (N-acetyl-5-methoxytryptamin; product code: M5250-1G) was dissolved in absolute ethanol (absolute GR for analysis, MERCK) and further dilutions were prepared in isotonic saline for final concentration of 2.4% (v/v) ethanol. MEL was daily prepared and administrated freshly[14].

Induction of Myocardial Infarction

The MI was induced with ISO treatment (85 mg/kg, ip) on the 6th and 7th days of the experiment[13].

ECG Recording and Evaluation

ECG recording was obtained at 09:00-09:30 on the 8th day of the experiment after 24 h following the last ISO administration. The extremity derivations DI, DII and DIII, aVR, aVL and aVF were recorded noninvasively (Poly-Spectrum 12 channel ECG-System, Poly-Spectrum-8, Neurosoft, 5, Voronin str., Ivanovo, Russia). Before 10 min of ECG recording, animals were treated

with 30 mg/kg ketamine and 5 mg/kg xylazine for sedation[15]. After evaluation of ECG, morphology of ST segment was examined.

Euthanasia and Tissue Harvesting

At the end of the experiment (8th day), blood samples were obtained from the heart under the anesthesia with ketamine and xylazine . Then, animals were sacrificed. Heart specimen were harvested and left ventricles (LV) were separated. The LV samples were sliced into three pieces for histopathological, genetic analysis and water content examination.

Water Content

LV slices were weighed for wet weight evaluation immediately, then kept for 24 h in an oven at +80 °C. Then, LV slices were used for dry/weight evaluation. Water content of LV samples were calculated as [(wet weight-dry weight)/wet weight] x 100%[16].

Histopathological Evaluation

LVs were fixed in 10% formaldehyde, embedded in paraffin, and processed for staining with hematoxylin&eosin (H&E) for histopathological evaluation.

Biochemical Analysis

cTnI (Catalog≠:MBS2104797) and CK-MB (Catalog≠ :MBS2515061) levels were measured from serum samples using ELISA kits provided according to the manufacturer procedure.

Total RNA Isolation

Total RNA isolation was performed from LV samples. Briefly, 25-30 mg tissue samples were put into 2 mL Eppendorf tubes and incubated for 10 sec in liquid nitrogen, then homogenized. Later, homogenates were manually used for total RNA isolation by PURE Link RNA MiniKit (CatNo.12183018A). Purification and concentration analysis of total RNAs were performed with NanoDrop ND-1000 spectrophotometer. Samples were kept at -80 °C until genetic evaluation. RNAs scored between 1.8 and 2.1 were purified at 260/280 nm and considered appropriate for analysis.

cDNA Isolation

After spectrophotometric evaluation, isolated RNAs were put in 0.2 mL PCR tubes according to RNA concentrations. cDNA isolation was performed manually with a kit. The mixture was put into PCR tubes and evaluation was performed at PCR device (the Applied Biosystems®, 2720 Thermal Cycler 96-Well PCR).

Real-Time PCR (qRT-PCR)

Quantitative real-time PCR (StepOnePlus™ Real-Time PCR System) was performed with DNA samples. TaqMan (TaqManTM Fast Advanced Master Mix, Ampliqon, Lithuania) was used for gene expression levels.

AQP1, AQP3, AQP4, AQP7, TNF-α, Caspase-3 and Bax, gene expression levels were evaluated with qRT-PCR against normalization by β-actin the housekeeping gene.

Taqman primer-probs were used for specific genes in this study given below.

TaqMan® Gene Expression Assays, 250 rxns AQP1..Rn00562834_m1

TaqMan® Gene Expression Assays, 250 rxns AQP3..Rn00581754_m1

TaqMan® Gene Expression Assays, 250 rxns AQP4..Rn01401327_s1

TaqMan® Gene Expression Assays, 250 rxns AQP7..Rn00569727_m1

TaqMan® Gene Expression Assays, 250 rxns TNF-α..01525859_g1

TaqMan® Gene Expression Assays, 250 rxns Caspase-3.. Rn00563902_m1

TaqMan® Gene Expression Assays, 250 rxns Bax..Rn01480161_g1

TaqMan® Gene Expression Assays, 250 rxns ACTB..Rn00667869_m1

Statistical Analysis

Genetic analysis was performed with qRT-PCR and relative fold changes were estimated with the $2^{-\Delta\Delta Ct}$ formula. The other results were presented as mean±standard error. Data were analyzed by using IBM Statistics Statistical Package for the Social Sciences 20.0. The Kruskal-Wallis test and Mann-Whitney U test were used for the comparison of groups. The significance level was considered as $p<0.05$.

RESULTS

ECG Determination

ST segment elevation was not observed on the 8th day in the control and MEL groups. However, ST segment elevation was determined in all rats of ISO and 2 rats of 7 in the ISO-MEL groups on the 8th day of the experiment. ECG recording obtained from control and ST elevated animals were represented in Figure 1.

LV Water Content Results

LV water content outcomes were presented in Figure 2. The water content levels of the MEL and Control groups were observed to be similar. However, higher water content values were obtained both in the ISO and ISO+MEL ($p<0.01$) groups compared to other groups.

Biochemical Analysis

Plasma CK-MB and cTnI levels were presented in Table 1. There was no significant difference between the groups in terms of CK-MB levels (p>0.05). Higher and statistically significant cTnI levels were observed in the ISO and ISO+MEL group compared to the control group (p<0.01).

Genetic Analysis

Inflammation and Apoptosis Related Molecules

The change of inflammation (TNF-α) and apoptosis (Caspase-3 and BAX) related molecules gene expression levels were submitted in Figure 3. The higher and significantly increasing values were observed in the ISO group for all mentioned molecules compared to the control (p<0.05 or p<0.01). MEL reduced TNF-α, Caspase-3 and BAX gene expression levels.

AQP Channels

Higher AQP1, AQP3, AQP4 and AQP7 levels were observed in the ISO group compared to the control (p<0.01), MEL (p<0.05) and ISO+MEL groups (p<0.01), as represented in Figure 4. MEL treatment significantly decreased all AQP channels gene expression levels.

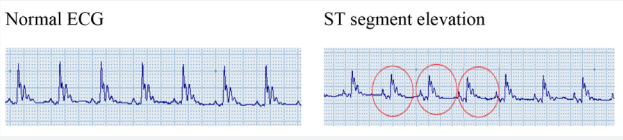

Figure 1. Normal and ST segment elevated ECG samples obtained from rats (1 mV=20 mm, 100 mm/sec).

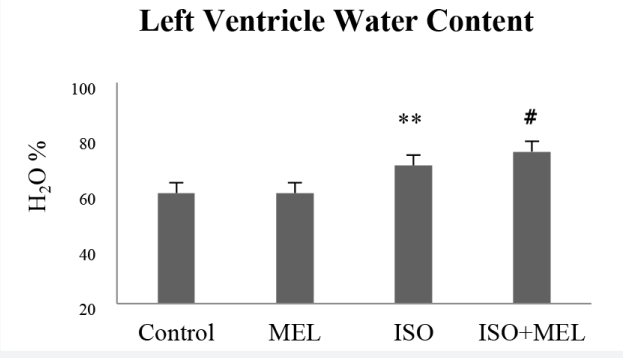

Figure 2. The change of left ventricle water content in all groups. **Statistically significant differences compared to control group, p<0.01; #: Statistically meaningful differences compared to Control and MEL groups, p<0.01. Data are expressed as mean±SE.

Table 1. Plasma levels of cTnI and CK-MB of experimental groups (compared to the control group p<0.01)**

Groups	cTnI (pg/mL)	CK-MB (pg/mL)
Control	240±20	175±36
MEL	237±24	175±18
ISO	249±21**	178±34
ISO-MEL	255±17**	170±30

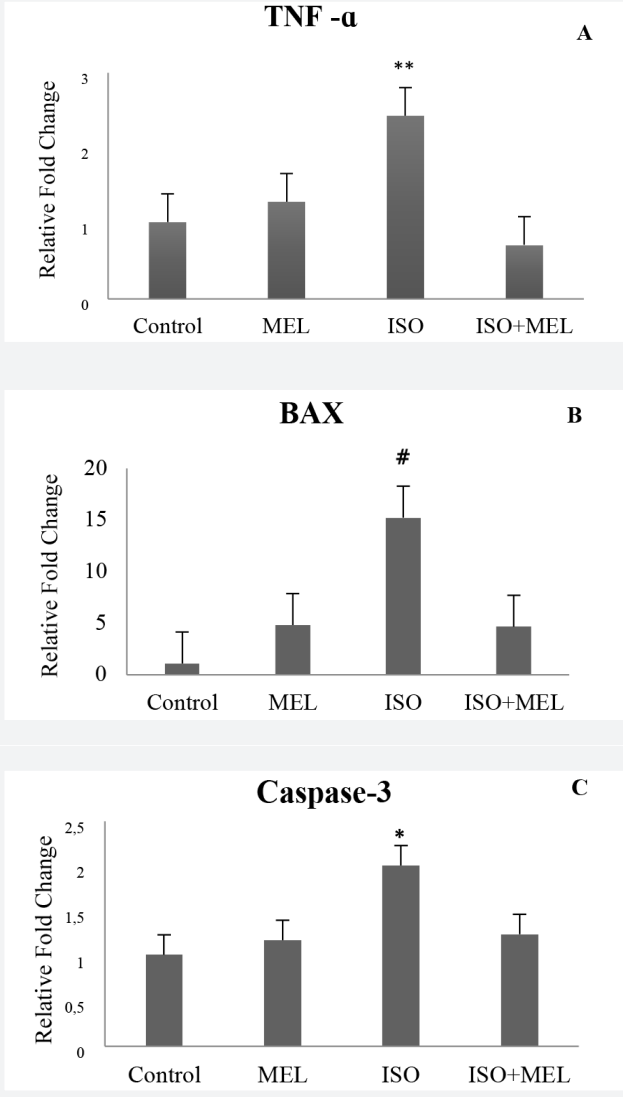

Figure 3. Representative fold changes of TNF-α **(A)**, BAX **(B)** and caspase-3 **(C)** gene expression levels. *: Statistically differences compared to Control group (p <0.05); **: Statistically differences compared to Control and ISO+MEL groups (p<0.01); #: Statistically meaningful differences compared other groups (p<0.01). Data are expressed as mean±SE.

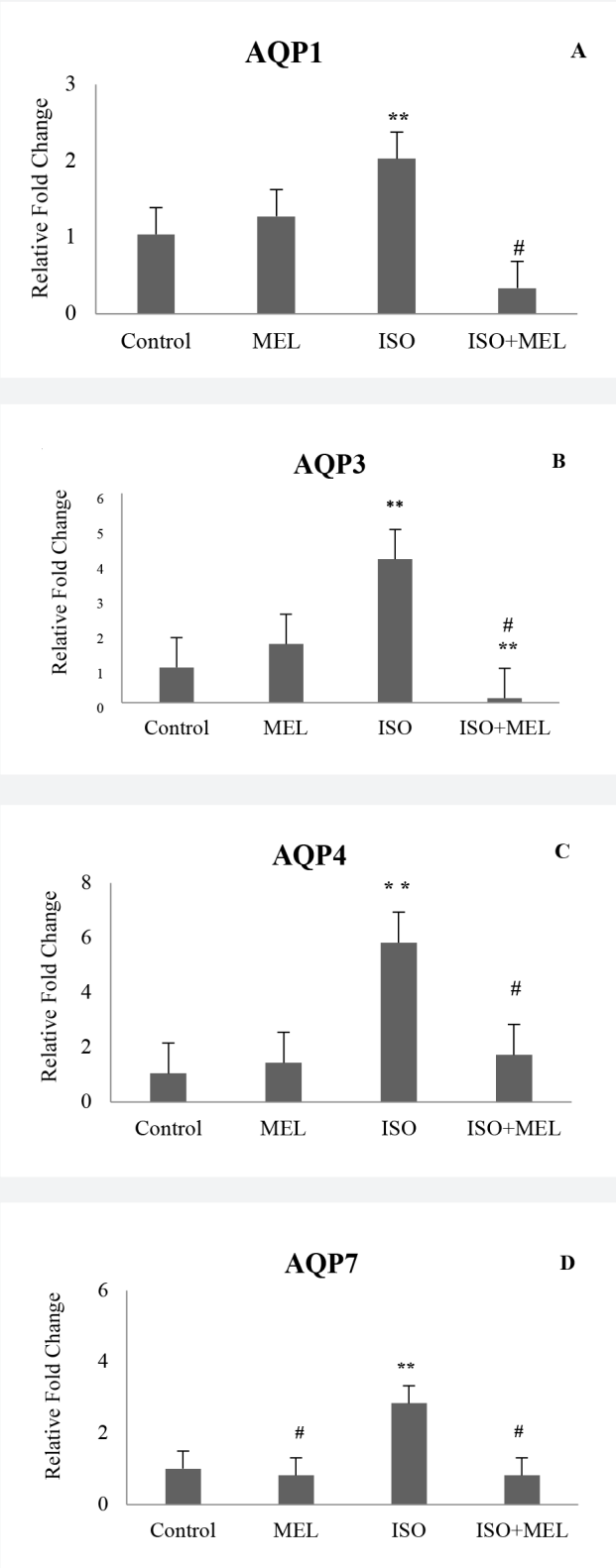

Figure 4. Representative fold changes of AQP1 **(A)**, AQP3 **(B)**, AQP4 **(C)**, AQP7 (D) channels. **: Statistically differences compared to Control group, p<0.01; #: Statistically meaningful differences compared to ISO group, p<0.01. Data are expressed as mean±SE.

Histopathological Results

H&E staining results were represented in Figure 5. Our results indicated that there was no edematous area in the control and melatonin groups (Figure 5A, 5B and 5E). However, several edematous areas were obtained in the ISO group (p<0.01, Figure 5C and 5E). MEL treatment significantly decreased edema score compared to ISO (p<0.01, Figure 5E). These results indicated that MEL had an ameliorative effect on ISO induced myocardial edema at histopathological level. Mononuclear cellular infiltration examination of LV was performed by H&E staining and results were also presented in Figure 5F. There was no mononuclear cell infiltration in the control group while there was a quite low mononuclear cell infiltration in the MEL group (Figure 5A, 5B). Our results indicated that there was a significant increase of mononuclear cell infiltration in the ISO group (p<0.01, Figure 5C and 5F). However, MEL administration had a slight and insignificant decreasing effect on mononuclear cellular infiltration score in the ISO+MEL group (Figure 5F). These results pointed out that MEL had an ameliorative effect on mononuclear cellular infiltration but this effect was not as strong as on myocardial edema.

DISCUSSION

This is the first study evaluated the changes of AQP1, AQP3, AQP4 and AQP7 gene expression levels in myocardium after ISO induced MI model in rats and the effects of MEL on AQP channels.

ISO is a widely used agent for inducing experimental MI model in rats. ISO induced MI model provides an advantage for the investigation of cardioprotective agents[17]. ST segment elevation is considered a prominent indicator of ISO induced MI[18,19]. In our study, we observed ST segment elevation in all 7 rats in the ISO group (Figure 1). Increases of both cTnI and CK-MB[20,21] or only CK-MB levels[22] were reported after ISO induced MI in rats. Also, it has been suggested that increased CK-MB can usually be detected in about 4-8 hours after the onset of MI; however, the cTnI is a more specific marker for the determination of MI[23]. In our study, cTnI levels were found significantly higher in the ISO-treated group compared to the control group (p<0.01; Table 1). We could not obtain significant changes in blood CK-MB levels. Histopathological changes such as irregular manner of myocardial cells, inflammatory cell infiltration (mononuclear cellular infiltration), and deterioration in myocardial fibers were observed in the myocardium after ISO-induced MI[18,24]. In accordance with previous studies, we determined edema and cellular infiltration in the myocardium after ISO administration. In our study, melatonin administration reduced inflammatory cell infiltration, but this result was statistically insignificant. This outcome indicated that the protective effect of melatonin on inflammatory cell infiltration might not strongly come out in the first 24 hours after MI.

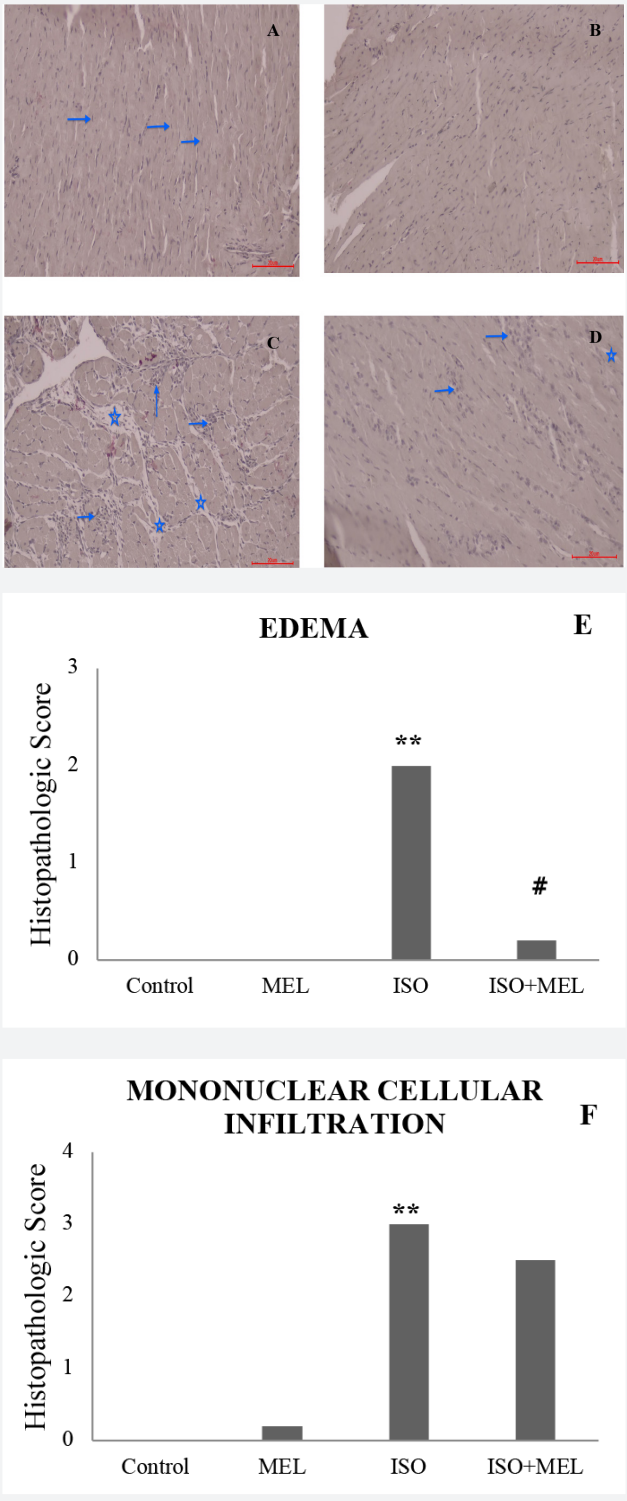

Figure 5. Representative images of H&E staining and histopathological scores of edema and mononuclear cellular infiltration. Image of H&E-stained myocardium of Control **(A)**, MEL **(B)**, ISO **(C)**, and ISO-MEL groups **(D)**. Blue arrows highlighting mononuclear infiltration and stars show edema. Edema **(E)**, and mononuclear cellular infiltration scores in all groups **(F)**. **: Statistically differences compared to Control and MEL groups, p<0.01; #: Statistically differences compared to ISO group, p <0.01. Data are expressed as mean±SE.

The cardioprotective effects of MEL are well known with antioxidant, anti-inflammatory and endothelial protective properties[23]. In our study, the cardioprotective effects of MEL were determined with ECG, biochemical, and histopathological evaluation. ST segment elevation was observed in all 7 rats in the ISO group; however, ST elevation was obtained only in two rats in the MEL group (Figure 1). Histopathological evaluation indicated that MEL administration reduced cardiac edema (Figure 5B, 5E). However, the results for the heart water content were not fully compatible with edema score. We observed high edema score in the ISO group, but we did not obtain higher water content in this group. Thus, we suggested that there could be other effective factors on water content without edema score. MEL treatment continued after two days of MI inducement, suggesting that MEL has also a protective effect on ISO induced MI.

Apoptosis has an important role in the pathogenesis of various heart diseases including MI[25]. The role of BAX and Caspase-3 in cardiomyocyte apoptotic pathway is well known[26]. It is reported that increased BAX and Caspase-3 gene expression levels were determined in MI[27]. In our study, the highest levels of Caspase-3 and BAX levels were investigated in the ISO group. MEL administration reduced the increased levels of Caspase-3 and BAX levels. MEL is a well-known anti-apoptotic agent in other organs[28], also in the heart[8,29]. It was investigated in our study that MEL ameliorated ISO induced apoptosis.

Ischemia/reperfusion may cause fluid accumulation through both interstitial and intracellular compartments and produce edema and cardiomyocyte damage[30]. It has been reported that increase of sarcolemmal breakdown, stimulation of phospholipases activity and dysfunction of Na+-K+ ATPase might trigger myocardial edema and cardiomyocyte damage[31]. Eventually, an increase of cardiac edema significantly reduces the systolic and diastolic functions of the heart[2]. Although several AQPs are determined in the myocardium and AQPs play a pivotal role in body water balance, the physiological role of cardiac AQPs in the heart remains unknown. In the present study, we investigated AQP1, AQP3, AQP4 and AQP7 gene expression levels in LVs after ISO induced MI and effects of MEL on these AQP channels.

Previous studies clarified the relationship between the AQP1 channel and myocardial edema. High AQP1 levels directly increased water permeability and caused myocardial edema[31]. Yan et al.[32] observed a significant increase both in AQP1 gene expression level and myocardial edema in goats undergoing cardiopulmonary bypass. In a similar study, an increase of myocardial edema and cGMP related AQP1 were observed in LV after the cardiopulmonary bypass in sheep. On the other hand, the administration of ODQ (a specific inhibitor of soluble guanylate cyclase) caused a decrease both in myocardial

edema and AQP1 level[33]. It was previously determined that the effects of AQP1 on ischemia/reperfusion induced myocardial edema and high levels of AQP1 increased LV water content in rats[31]. In our study, we obtained higher AQP1 and LV water content in the ISO induced MI group compared to the control group ($p<0.01$, Figure 4A). MEL significantly decreased AQP1 in the ISO+MEL group ($p<0.01$). MEL administration did not significantly decrease LV water content compared to the ISO group; however, we observed significantly lower edema score in the MEL administrated group at histopathological level. Although MEL and AQP1 interaction was investigated in the kidney[34] and medulla spinalis[35], there is scarcity about the effects of MEL on myocardial AQP1. Furthermore, there are limited studies that reported both cardiac AQP1 existence and function of myocardial AQP1. Best of our knowledge, our study was the first research representing the interaction between MEL and AQP1 in MI.

AQP3 is known as a member of aquaglyceroporin family and was determined at the kidney, skin, digestive tract, and salivary gland in rats[36-39]. Nevertheless, there are controversial and limited studies focusing on cardiac AQP3[2]. Also, the role of AQP3 in cardiovascular diseases and MEL-AQP3 interaction in the myocardium remains unknown. Most recently, our group has previously observed AQP3 expression in LV and MEL has a decreasing effect on AQP3 expression in cisplatin induced cardiac injury of rats[8]. To the best of our knowledge, our study is the first research investigating MEL and AQP3 relationship in ISO induced MI. AQP3 transports H_2O_2 to intracellular compartment and H_2O_2 acts as a secondary messenger on several pathways including inflammation, cell development and migration[40]. However, an increase of AQP3 expression leads to transferring of large amounts of H_2O_2 into the cell and higher intracellular H_2O_2 stimulates cellular damage[41,42]. It has been determined that quercetin, a powerful antioxidant, has cellular protective effect against H_2O_2 toxicity through the downregulation of AQP3 channels in the intestinal tract. Another study reported that the increase of AQP3 expression in the kidney was related to oxidative effects of fructose in metabolic syndrome[43]. In our study, we obtained significantly higher AQP3, TNF-α and Caspase-3 expression levels and widespread myocardial edema in the ISO administrated group compared to other ($p<0.01$). We also determined powerful inhibitory effect of MEL on AQP3 and TNF-α expression and reduced myocardial edema. Furthermore, MEL moderately decreased Caspase-3 and mononuclear cell infiltration. These outcomes indicate that MEL affects the AQP3 channel by reducing inflammation and apoptosis.

The AQP4 is the most investigated AQP channels in respect with myocardium and in relation to MI. AQP4 is an important AQP channel for the heart and has widespread distribution in the myocardium, such as cardiac muscle, intercalated discs, endothelial cells, sarcolemma, and serosa[44]. Zhang et al.[7] observed an increase in AQP4 within 1 week after MI. In another study, it was reported that there was an increase in AQP4 at the 15th minute and reached the highest level at the 45th minute during the acute period of MI. These results indicated that AQP4 was a crucial component of myocardial edema. There was a relationship between AQP4 and infarct size, which was also reported[45]. Both studies mentioned above were conducted on mice. One of other studies[46] determined that AQP4 expression increased in a cardiopulmonary resuscitation model of rats. They revealed that there was a relationship between the PI3K/Akt pathway and AQP4, and this pathway had a reducing effect on myocardial damage. The increase of AQP4 was also reported in cardiopulmonary resuscitation and considered as an important criterion of myocardial damage[47]. Novel studies reported the relationship between MEL and AQP4 in the medulla spinalis[48,49] and brain[50]. However, AQP4 and its relationship with MEL in the heart remains unknown. We observed that MEL had a decreasing effect on myocardial edema and AQP4. These are the first findings indicating that MEL has ameliorative effects on ISO induced myocardial edema and increase of AQP4.

There are more researches on AQP7 channel in the myocardium compared to AQP1, AQP3 and AQP[42,30]. AQP7 is permeated glycerol as well as water and subcategorized as aquaglyceroporins[51]. Hibuse et al.[52] revealed that AQP7 deficiency in the heart significantly reduced glycerol consumption and ATP content. However, they suggested that there was no relationship between AQP7 and myocardial edema. Higher AQP7 levels were observed in rats consuming high protein diet[53]. It is generally accepted that AQP7 channels are effective for metabolic adaptation of the heart. Glycerol is one of the main myocardium energy sources transported through AQP7[54]. We observed a significant increase of AQP7 gene expression ($p<0.01$) and ameliorative effects of MEL on AQP7 ($p<0.01$, Figure 4D). We focused on the change of LV AQP channels and we did not investigate the metabolic condition of the myocardium. Therefore, we could not explain the inducible factor of changing in AQP7 channel in ISO induced MI.

Study Limitations

In our study, we applied a chemical method for inducing MI.

CONCLUSION

In this study, we demonstrated that MEL administration had protective effects against the increase in AQP1, AQP3, AQP4 and AQP7 channels. MEL also decreased myocardial edema in ISO induced MI model. This is the first study investigated the protective effects of MEL on the level of myocardial edema and the changes of AQP1, AQP3, AQP4 and AQP7 channels after

MI. Besides the other cardioprotective effects, according to our results, MEL was also suggested as a potential adjuvant agent, which decreases cardiac edema and regulates the cardiac AQP channels.

Ethics

Ethics Committee Approval: All animal experimental procedures and protocols were approved by Çanakkale Onsekiz Mart University Animal Experiments Local Ethics Committee (decision number 2019/07-04, date: 25.09.2019).

Informed Consent: Animal experiment.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Concept: N.K.T., M.U., Design: N.K.T., M.U., Data Collection or Processing: N.K.T., B,B., M.U., Analysis or Interpretation: N.K.T., B,B., M.U., Literature Search: N.K.T., B,B., M.U., Writing: N.K.T., B,B., M.U.

Conflict of Interest: No conflict of interest was declared by the authors.

Financial Disclosure: This work was funded by the Scientific Research Coordination Unit (BAPD) of Çanakkale Onsekiz Mart University (project number: TYL-2019-3081).

REFERENCES

1. Krijnen PA, Nijmeijer R, Meijer CJ, Visser CA, Hack CE, Niessen HW. Apoptosis in myocardial ischaemia and infarction. J Clin Pathol. 2002;55:801-11.
2. Egan JR, Butler TL, Au CG, Tan YM, North KN, Winlaw DS. Myocardial water handling and the role of aquaporins. Biochim Biophys Acta. 2006;1758:1043-52.
3. Laine GA, Allen SJ. Left ventricular myocardial edema. Lymph flow, interstitial fibrosis, and cardiac function. Circ Res. 1991;68:1713-21.
4. Spotnitz HM. Effects of edema on systolic and diastolic function in vivo. J Card Surg. 1995;10(4 Suppl):454-9.
5. Delporte C, Soyfoo M. Aquaporins: Unexpected actors in autoimmune diseases. Autoimmun Rev. 2022;21:103131.
6. Tan C, Zeng J, Wu G, Zheng L, Huang M, Huang X. Xinshuitong Capsule extract attenuates doxorubicin-induced myocardial edema via regulation of cardiac aquaporins in the chronic heart failure rats. Biomed Pharmacother. 2021;144:112261.
7. Zhang HZ, Kim MH, Lim JH, Bae HR. Time-dependent expression patterns of cardiac aquaporins following myocardial infarction. J Korean Med Sci. 2013;28:402-8.
8. Koral L, Ovali MA, Tufekcioglu NK, Karakilic E, Adali Y, Uzun M. The role of AQP3 and AQP4 channels in cisplatin-induced cardiovascular edema and the protective effect of melatonin. Mol Biol Rep. 2021;48:7457-65.
9. Zare S, Heydari FS, Hayes AW, Reiter RJ, Zirak MR, Karimi G. Melatonin attenuates chemical-induced cardiotoxicity. Hum Exp Toxicol. 2021;40:383-94.
10. Randhawa PK, Gupta MK. Melatonin as a protective agent in cardiac ischemia-reperfusion injury: Vision/Illusion? Eur J Pharmacol. 2020;885:173506.
11. Song YJ, Zhong CB, Wu W. Cardioprotective effects of melatonin: Focusing on its roles against diabetic cardiomyopathy. Biomed Pharmacother. 2020;128:110260.
12. Pourhanifeh MH, Dehdashtian E, Hosseinzadeh A, Sezavar SH, Mehrzadi S. Clinical Application of Melatonin in the Treatment of Cardiovascular Diseases: Current Evidence and New Insights into the Cardioprotective and Cardiotherapeutic Properties. Cardiovasc Drugs Ther. 2022;36:131-55.
13. Murugesan M, Revathi R, Manju V. Cardioprotective effect of fenugreek on isoproterenol-induced myocardial infarction in rats. Indian J Pharmacol. 2011;43:516-9.
14. Uzun M, Gencer M, Turkon H, Oztopuz RO, Demir U, Ovali MA. Effects of Melatonin on Blood Pressure, Oxidative Stress and Placental Expressions of TNFα, IL-6, VEGF and sFlt-1 in RUPP Rat Model of Preeclampsia. Arch Med Res. 2017;48:592-8.
15. Ovali MA, Uzun M. The effects of melatonin administration on KCNQ and KCNH2 gene expressions and QTc interval in pinealectomised rats. Cell Mol Biol (Noisy-le-grand). 2017;63:45-50.
16. Weis S, Shintani S, Weber A, Kirchmair R, Wood M, Cravens A, et al. Src blockade stabilizes a Flk/cadherin complex, reducing edema and tissue injury following myocardial infarction. J Clin Invest. 2004;113:885-94.
17. Wong ZW, Thanikachalam PV, Ramamurthy S. Molecular understanding of the protective role of natural products on isoproterenol-induced myocardial infarction: A review. Biomed Pharmacother. 2017;94:1145-66.
18. Kannan MM, Quine SD. Ellagic acid ameliorates isoproterenol induced oxidative stress: Evidence from electrocardiological, biochemical and histological study. Eur J Pharmacol. 2011;659:45-52.
19. Patel V, Upaganlawar A, Zalawadia R, Balaraman R. Cardioprotective effect of melatonin against isoproterenol induced myocardial infarction in rats: A biochemical, electrocardiographic and histoarchitectural evaluation. Eur J Pharmacol. 2010;644:160-8.
20. Acikel M, Buyukokuroglu ME, Aksoy H, Erdogan F, Erol MK. Protective effects of melatonin against myocardial injury induced by isoproterenol in rats. J Pineal Res. 2003;35:75-9.
21. Govindasami S, Uddandrao VVS, Raveendran N, Sasikumar V. Therapeutic Potential of Biochanin-A Against Isoproterenol-Induced Myocardial Infarction in Rats. Cardiovasc Hematol Agents Med Chem. 2020;18:31-6.
22. Prince PS. A biochemical, electrocardiographic, electrophoretic, histopathological and in vitro study on the protective effects of (-) epicatechin in isoproterenol-induced myocardial infarcted rats. Eur J Pharmacol. 2011;671:95-101.
23. Simko F, Bednarova KR, Krajcirovicova K, Hrenak J, Celec P, Kamodyova N, et al. Melatonin reduces cardiac remodeling and improves survival in rats with isoproterenol-induced heart failure. J Pineal Res. 2014;57:177-84.
24. Zhou R, Xu Q, Zheng P, Yan L, Zheng J, Dai G. Cardioprotective effect of fluvastatin on isoproterenol-induced myocardial infarction in rat. Eur J Pharmacol. 2008;586:244-50.
25. Zidar N, Dolenc-Strazar Z, Jeruc J, Stajer D. Immunohistochemical expression of activated caspase-3 in human myocardial infarction. Virchows Arch. 2006;448:75-9.
26. Nakamura T, Ueda Y, Juan Y, Katsuda S, Takahashi H, Koh E. Fas-mediated apoptosis in adriamycin-induced cardiomyopathy in rats: In vivo study. Circulation. 2000;102:572-8.
27. Jin Q, Li R, Hu N, Xin T, Zhu P, Hu S, et al. DUSP1 alleviates cardiac ischemia/reperfusion injury by suppressing the Mff-required mitochondrial fission and Bnip3-related mitophagy via the JNK pathways. Redox Biol. 2018;14:576-87.
28. Doğanlar ZB, Güçlü H, Öztopuz Ö, Türkön H, Dogan A, Uzun M, et al. The Role of Melatonin in Oxidative Stress, DNA Damage, Apoptosis and Angiogenesis in Fetal Eye under Preeclampsia and Melatonin Deficiency Stress. Curr Eye Res. 2019;44:1157-69.
29. Doğanlar O, Doğanlar ZB, Ovali MA, Güçlü O, Demir U, Doğan A, et al. Melatonin regulates oxidative stress and apoptosis in fetal hearts of pinealectomised RUPP rats. Hypertens Pregnancy. 2020;39:429-43.
30. Butler TL, Au CG, Yang B, Egan JR, Tan YM, Hardeman EC, et al. Cardiac aquaporin expression in humans, rats, and mice. Am J Physiol Heart Circ Physiol. 2006;291:705-13.

31. Wong GT, Li R, Jiang LL, Irwin MG. Remifentanil post-conditioning attenuates cardiac ischemia-reperfusion injury via kappa or delta opioid receptor activation. Acta Anaesthesiol Scand. 2010;54:510-8.
32. Yan Y, Huang J, Ding F, Mei J, Zhu J, Liu H, et al. Aquaporin 1 plays an important role in myocardial edema caused by cardiopulmonary bypass surgery in goat. Int J Mol Med. 2013;31:637-43.
33. Ding FB, Yan YM, Bao CR, Huang JB, Mei J, Liu H, et al. The role of aquaporin 1 activated by cGMP in myocardial edema caused by cardiopulmonary bypass in sheep. Cell Physiol Biochem. 2013;32:1320-30.
34. Li Z, Wang Y, Sun N, Liu X, Song E, Zhang Z, et al. Melatonin therapy protects against renal injury before and after release of bilateral ureteral obstruction in rats. Life Sci. 2019;229:104-15.
35. Nesic O, Lee J, Unabia GC, Johnson K, Ye Z, Vergara L, et al. Aquaporin 1 - a novel player in spinal cord injury. J Neurochem. 2008;105:628-40.
36. Gouda ZA, Khalifa MEA, Shalaby SM, Hussein S. Mechanistic effect of human umbilical cord blood derived mesenchymal stem cells on the submandibular salivary gland in ovariectomized rats. Biochem Cell Biol. 2018;96:57-67.
37. Camilleri M, Carlson P, Chedid V, Vijayvargiya P, Burton D, Busciglio I. Aquaporin Expression in Colonic Mucosal Biopsies From Irritable Bowel Syndro. 2019;10:e00019.
38. Chung S, Kim S, Son M, Kim M, Koh ES, Shin SJ, et al. Empagliflozin Contributes to Polyuria via Regulation of Sodium Transporters and Water Channels in Diabetic Rat Kidneys. Front Physiol. 2019;10:271.
39. Kamar SS, Abdel-Kader DH, Rashed LA. Beneficial effect of Curcumin Nanoparticles-Hydrogel on excisional skin wound healing in type-I diabetic rat: Histological and immunohistochemical studies. Ann Anat. 2019;222:94-102.
40. Miller EW, Dickinson BC, Chang CJ. Aquaporin-3 mediates hydrogen peroxide uptake to regulate downstream intracellular signaling. Proc Natl Acad Sci U S A. 2010;107:15681-6.
41. Thiagarajah JR, Zhao D, Verkman AS. Impaired enterocyte proliferation in aquaporin-3 deficiency in mouse models of colitis. Gut. 2007;56:1529-35.
42. Dong Y, Hou Q, Lei J, Wolf PG, Ayansola H, Zhang B. Quercetin Alleviates Intestinal Oxidative Damage Induced by H2O2 via Modulation of GSH: In Vitro Screening and In Vivo Evaluation in a Colitis Model of Mice. ACS Omega. 2020;5:8334-46.
43. Ewida SF, Al-Sharaky DR. Implication of Renal Aquaporin-3 in Fructose-Induced Metabolic Syndrome and Melatonin Protection. J Clin Diagn Res. 2016;10:6-11.
44. Tie L, Wang D, Shi Y, Li X. Aquaporins in Cardiovascular System. Adv Exp Med Biol. 2017;969:105-13.
45. Warth A, Simon P, Capper D, Goeppert B, Tabatabai G, Herzog H, et al. Expression pattern of the water channel aquaporin-4 in human gliomas is associated with blood-brain barrier disturbance but not with patient survival. J Neurosci Res. 2007;85:1336-46.
46. Song D, Liu X, Diao Y, Sun Y, Gao G, Zhang T, et al. Hydrogen rich solution against myocardial injury and aquaporin expression via the PI3K/Akt signaling pathway during cardiopulmonary bypass in rats. Mol Med Rep. 2018;18:1925-38.
47. He W, Liu Y, Geng H, Li Y. The regulation effect of ulinastatin on the expression of SSAT2 and AQP4 in myocardial tissue of rats after cardiopulmonary resuscitation. Int J Clin Exp Pathol. 2015;8:10792-9.
48. Li C, Chen X, Qiao S, Liu X, Liu C, Zhu D, et al. Melatonin lowers edema after spinal cord injury. Neural Regen Res. 2014;9:2205-10.
49. Liu J, Clough SJ, Hutchinson AJ, Adamah-Biassi EB, Popovska-Gorevski M, Dubocovich ML. MT1 and MT2 Melatonin Receptors: A Therapeutic Perspective. Annu Rev Pharmacol Toxicol. 2016;56:361-83.
50. Shahrokhi N, Khaksari M, AsadiKaram G, Soltani Z, Shahrokhi N. Role of melatonin receptors in the effect of estrogen on brain edema, intracranial pressure and expression of aquaporin 4 after traumatic brain injury. Iran J Basic Med Sci. 2018;21:301-8.
51. Palabiyik O, Karaca A, Taştekin E, Yamasan BE, Tokuç B, Sipahi T, et al. The Effect of a High-Protein Diet and Exercise on Cardiac AQP7 and GLUT4 Gene Expression. Biochem Genet. 2016;54:731-45.
52. Hibuse T, Maeda N, Nakatsuji H, Tochino Y, Fujita K, Kihara S, et al. The heart requires glycerol as an energy substrate through aquaporin 7, a glycerol facilitator. Cardiovasc Res. 2009;83:34-41.
53. Gladka M, El Azzouzi H, De Windt LJ, da Costa Martins PA. Aquaporin 7: the glycerol aquaeductus in the heart. Cardiovasc Res. 2009;83:3-4.
54. Falato M, Chan R, Chen LY. Aquaglyceroporin AQP7's affinity for its substrate glycerol: Have we reached convergence in the computed values of glycerol-aquaglyceroporin affinity? RSC Adv. 2022;12:3128-35.