The preventive and curative effects of melatonin against abdominal aortic aneurysm in rats

Gözde Tekin, MD,[a] Selim İsbir, MD,[a] Göksel Şener, PhD,[b] Özge Çevik, PhD,[c] Şule Çetinel, MD, PhD,[d] Okan Dericioğlu, MD,[a] Sinan Arsan, MD,[a] *and* Adnan Çobanoğlu, MD,[a] *İstanbul and Sivas, Turkey*

ABSTRACT

Objective: Oxygen free radicals are important components involved in the histopathologic tissue alterations observed during abdominal aortic aneurysms (AAAs). This study examined whether melatonin has protective or therapeutic effects against AAAs.

Methods: Sprague-Dawley rats were divided into four groups. A $CaCl_2$ model was used to induce AAA. Starting on the operation day (Mel+AAA+Mel group) or 4 weeks after the operation (AAA+Mel group), the rats received intraperitoneal melatonin (10 mg/kg/day) for 6 and 2 weeks, respectively. The control and AAA groups received vehicle for 2 weeks after the sham operation and AAA induction, respectively. Angiographic measurements were recorded at the beginning, week 4, and week 6 of the study. After decapitation, aorta tissues were taken for the measurement of malondialdehyde, 8-hydroxy-2′-deoxyguanosine, glutathione levels, and myeloperoxidase and caspase-3 activity. Matrix metalloproteinase (MMP)-2, MMP-9, tumor necrosis factor-α, and inducible nitric oxide synthase protein expressions were analyzed by Western blot technique. Aortic tissues were also examined by light microscopy.

Results: $CaCl_2$ caused an inflammatory response and oxidative damage indicated by rises in malondialdehyde and 8-hydroxy-2′-deoxyguanosine levels. Myeloperoxidase and caspase-3 activities were increased, but glutathione levels were reduced. On the one hand, MMP-2, MMP-9, tumor necrosis factor-α, and inducible nitric oxide synthase protein expressions were increased in the vehicle-treated AAA group. On the other hand, melatonin treatment reversed all of these biochemical indices and histopathologic alterations.

Conclusions: According to the data, although melatonin tended to reverse the biochemical parameters given on week 4, the preventive effect is more pronounced when given concomitantly with AAA induction because values were closer to the control levels. (J Vasc Surg 2018;67:1546-55.)

Clinical Relevance: Melatonin prevents abdominal aortic aneurysm formation thorough anti-inflammatory and anti-oxidative effects. The preventive effect of this powerful antioxidant can be attributed to its ability to balance oxidant-antioxidant status, inhibit neutrophil infiltration, and regulate inflammatory mediators, suggesting a future role in the treatment and prevention of abdominal aortic aneurysms.

Owing to the high rupture incidence, abdominal aortic aneurysm (AAAs) is a life-threatening disease.[1] Aneurysm is an irreversible, progressive, and degenerative illness that forms with abnormal expansion of a transverse diameter of ≥1.5 times than normal in any segment of the aorta.[2] AAA diagnosis is problematic because most aneurysms are asymptomatic until rupture. The rupture of aneurysm risk increases with large aortic diameter. Mortality after AAA rupture is ~75% for those who reach the hospital.[3] No therapy has been established for small AAAs.[4]

AAA is not a passive enlargement of vasculature but represents an inflammation and tissue degeneration common to many forms of chronic disease.[5] Matrix metalloproteinases (MMPs), calcium-dependent zinc endopeptidases, are known to contribute to the inflammatory process because increased expression of MMPs has been observed in various inflammatory situations and associated tissue injury.[6] Similarly, pathologic features of AAA include elastin degradation, increased oxidative stress, inflammation, and vascular smooth muscle cell apoptosis.[7]

Medical therapy in AAA patients aims to decrease the widening of the aneurysm diameter and rupture probability. Also, medical therapies, such as β-blockers, angiotensin-converting enzyme inhibitors, and statins, have no effect in decreasing aneurysm diameter but do decrease the probability of rupture.[8-10] In light of the knowledge to date, prevention of aneurysm development should be taken into account in the clinical setting. Because inflammation has been shown in the

From the Department of Cardiovascular Surgery, School of Medicine,[a] and Department of Pharmacology, School of Pharmacy,[b] Marmara University, İstanbul; the Department of Biochemistry, School of Pharmacy, Cumhuriyet University, Sivas[c]; and the Department of Histology and Embryology, School of Medicine, Marmara University, İstanbul.[d]

This project was supported by Marmara University Scientific Research Projects Commission (project no: SAG-C-TUP-12114-0355).

Author conflict of interest: none.

Correspondence: Gözde Tekin, MD, Department of Cardiovascular Surgery, School of Medicine, Marmara University, İstanbul, Turkey (e-mail: gozdekirca@hotmail.com).

The editors and reviewers of this article have no relevant financial relationships to disclose per the JVS policy that requires reviewers to decline review of any manuscript for which they may have a conflict of interest.

0741-5214

http://dx.doi.org/10.1016/j.jvs.2017.04.028

pathophysiology of AAA, the effects of anti-inflammatory medicines on AAA are being investigated. Current studies are focusing on natural antioxidants.[11]

Melatonin, the major product of the vertebrate pineal gland, functions as a modulator of sleep, sexual behavior, immune function, and circadian rhythms.[12] Melatonin has been shown to have potent receptor-dependent and receptor-independent actions. Receptor-dependent effects are mediated predominantly through the MT_1 and MT_2 G-protein coupled receptors, whereas pleiotropic receptor-independent effects of melatonin involve the reactive oxygen species (ROS) scavenging nature, activation and increased expression of several antioxidant enzymes, and the ability to increase the efficiency of the mitochondrial electron transport chain.[13] Furthermore melatonin reduces blood pressure and has an antiadrenergic action on myocardial contractility, which are mediated by its receptors in the heart and arteries.[14]

In the light of above findings, we aimed to investigate if melatonin could attenuate the development of aneurysms and regress dilated aneurysmal sac in an aneurysmal rat model by periaortic application of $CaCl_2$.

METHODS

The Marmara University Animal Care and Use Committee approved the experimental protocols in this study.

Experimental groups. Sprague-Dawley rats (300-350 g) supplied by the Marmara University Animal Center were housed in an air-conditioned room with 12:12 light/dark cycles, where the temperature (22° ± 2° C) and relative humidity (65%-70%) were kept constant.

Rats were randomly divided into four groups of 10: sham operated control, abdominal aortic aneurysm (AAA), AAA+melatonin (Mel), and Mel+AAA+Mel. The rats in AAA+Mel group and Mel+AAA+Mel group received intraperitoneal melatonin (10 mg/kg/day) 4 weeks after the AAA induction and at the start of AAA induction, respectively. The dose of melatonin is based on our previous studies where melatonin exerted anti-inflammatory and antioxidant effects.[15-17] Melatonin was freshly prepared and given at the same time (1800 hours) every day. The control and AAA group received vehicle (1% alcohol in saline [1 mL/kg]) for 2 weeks after sham operation and AAA induction, respectively. Melatonin was purchased from Sigma-Aldrich (St. Louis, Mo).

AAA induction. A gauze presoaked in 0.5 mol/L $CaCl_2$ was directly applied to the adventitia of the infrarenal abdominal aorta for 15 minutes.[18] The abdominal cavity was washed with warm sterile saline before being closed with 2-0 silk suture.

Measurement of aortic size via fluorescence angiography. Angiography is used in the definitive diagnosis of AAA, so the aim of using angiography was to observe whether the aneurysm had formed. The formation of aneurysm and the diameter changes were evaluated by using the data derived from angiography. Aortic wall thrombus formation was examined histologically. All rats were scanned with florescence angiography at the beginning, at 4 weeks, and at the end of 6 weeks. To measure the infrarenal aorta diameter, rats were placed supine, anesthetized with ketamine (100 mg/kg) and chlorpromazine (30-50 mg/kg) and underwent laparotomy. Intestines were deviated on the right, and the abdominal aorta was isolated with blunt dissection.

After the infrarenal aorta and inferior vena cava were isolated, indocyanine green (0.25 mg/kg) was injected through the inferior vena cava, and then the abdominal aorta was scanned with florescence angiography using the SPY Elite Imaging System (NOVADAQ, Bonita Springs, Fla). The infrarenal aorta diameter was measured, and the dilation ratio (%) was calculated according to the following formula described by Morimoto et al[19]: dilatation ratio (%) = (maximal aneurysm diameter/native aortic diameter) × 100.

Western blot analysis. MMP2, MMP9, tumor necrosis factor (TNF)-α, and inducible nitric oxide synthase (iNOS) protein expression were measured by Western blotting. Samples were homogenized in cell lysis buffer, and protein concentrations were determined using the Bradford method.[20] Samples resolved by 4% to 12% sodium dodecyl sulfate-polyacrylamide gel electrophoresis were transferred to polyvinylidene fluoride membrane, which was blocked with bovine serum albumin. The membrane was incubated overnight with primary antibody (1:500 dilution anti-MMP2 sc-10736, anti-MMP9 sc-10737, anti-iNOS sc-651, anti-TNF- α sc-1351, anti-β-actin sc-47778; Santa Cruz Biotechnology, Heidelberg, Germany) and washed with Tris-buffered saline containing 0.1% Tween-20. The membrane was washed and then incubated with horseradish peroxidase-conjugated secondary antibody for 2 hours. The blot was developed with chemiluminescence reagents and exposed to film. Data were analyzed using ImageJ Programme Optical Density Analysis Software (National Institutes of Health, Bethesda, Md). Signals were normalized with respect to β-actin.

Tissue 8-hydroxy-2′-deoxyguanosine levels measurement. Tissue samples were collected and genomic DNA from tissue was immediately extracted using commercial DNA extraction kit according to the manufacture's protocol (Invitrogen, Frederick, Md). Measurement of tissue 8-hydroxy-2′-deoxyguanosine (8-OHdG) levels was performed by competitive enzyme-linked immunosorbent assay using the OxiSelect Oxidative DNA Damage ELISA kit (Cell Biolabs, Inc, San Diego, Calif), as stated in the manufacturer's instructions.

Measurement of tissue caspase-3 activity. Caspase-3 activity assay was performed using the caspase-3 cellular activity assay kit (Calbiochem, San Diego, Calif),