Kafkas Univ Vet Fak Derg
21 (5): 673-676, 2015
DOI: 10.9775/kvfd.2015.13026

Kafkas Universitesi Veteriner Fakultesi Dergisi
Journal Home-Page: http://vetdergi.kafkas.edu.tr
Online Submission: http://vetdergikafkas.org

Research Article

Can Melatonin Protect the Endometrium from the Adverse Effects of Caerulein?

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Article Code: KVFD-2015-13026 Received: 13.05.2015 Accepted: 22.06.2015 Published Online: 25.06.2015

Abstract

We investigated the effects of a caerulein-induced acute pancreatitis (AP) on uterus and possible uterine protective effects of melatonin administration. Twenty-eight animals were divided into four groups: (1) control group (n = 7); (2) melatonin group (n = 7); (3) caerulein group (n = 7); (4) melatonin + caerulein group (n = 7). AP was induced by 4 intraperitoneal injection of caerulein given hourly (50 µg/kg) into young female animals. Melatonin (20 mg/kg) was given via intraperitoneal injection 30 min prior to the induction of AP. The rats were sacrificed by decapitation 12 h after the last injection of caerulein and their uterus were taken for histopathological evaluation. Mean body weight and uterine wet weight was recorded. The H-Score method was used to score the degree of histological changes of endo-myometrium edema, hemorrhage, necrosis, leucocyte infiltration, endometrial proliferation and endometrial thickness. There was no significant difference in the mean body weight observed after treatment in each group. The uterine wet weight differences between the control and caerulein given rats were significant ($P<0.01$). The endometrial thickness, edema, hemorrhage, necrosis and leucocyte infiltration of the caerulein group was significantly higher than the control and melatonin groups ($P<0.01$). It was observed that preteratment with melatonin normalized histological abnormalities and significantly reduced uterine wet weight as compared with the caerulein only group. Melatonin application may play an important role in the prophylaxis of uterine endometrium arising from adverse effects of caerulein.

Keywords: *Caerulein, Melatonin, Uterus, Pancreatitis, Rat*

Melatonin Serulein'in Olumsuz Etkilerine Karşı Endometriyumu Koruyabilir mi?

Özet

Serulein ile indüklenmiş akut pankreatiti (AP)'nin uterusa etkisi ve melatonin uygulamasının muhtemel uterus koruyucu etkileri araştırıldı. Yirmi sekiz rat dört gruba ayrıldı: (1) Kontrol grubu (n = 7); (2) melatonin grubu (n = 7); (3) serulein grubu (n = 7); (4) melatonin + serulein grubu (n = 7). AP, genç dişi hayvanlara saatte bir (50 µg/kg) dozunda 4 kez verilen intraperitonal serulein enjeksiyonu ile indüklenmiştir. Melatonin (20 mg/kg) AP indüksiyonundan 30 dakika önce intraperitonal enjeksiyon yolu ile verildi. Ratlar seruleinin son enjeksiyonundan 12 saat sonra sakrifiye edildi ve uterusları histopatolojik değerlendirme için alındı. Ortalama vücut ağırlığı ve uterusun ıslak ağırlıkları kaydedildi. Endo-miyometriyumdaki histolojik değişikliklerin derecesi, ödem, kanama, nekroz, lökosit infiltrasyonu, endometriyal proliferasyon ve endometrial kalınlık skorlaması için H-Puan yöntemi kullanıldı. Her grupta tedaviden sonra ortalama vücut ağırlığında anlamlı bir farkın olmadığı gözlendi. Kontrol ve serulein verilen ratlar arasındaki uterus ıslak ağırlıkları anlamlı derecede farklı ($P<0.01$) olduğu tespit edildi. Serulein grubunda endometriyal kalınlık, ödem, kanama, nekroz ve lökosit infiltrasyonu melatonin ve kontrol grubuna oranla anlamlı derecede daha fazla olduğu görüldü ($P<0.01$). Daha önceden melatonin uygulanan grupta sadece serulein uygulanan gruba oranla histolojik olarak yapısal bozuklukların normale döndüğü ve uterus ıslak ağırlığının anlamlı ölçüde azaldığı gözlendi. Melatonin uygulamasının, uterusun endometriumu üzerine seruleinden kaynaklanan olumsuz etkilerin profilaksisinde önemli bir rol oynayabileceği düşünülmektedir.

Anahtar sözcükler: *Serulein, Melatonin, Uterus, Pankreatit, Rat*

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INTRODUCTION

The nuclear factor kappa beta (NF-kB) pathway was indicated to be active in the normal endometrium of healthy women [1-3]. NF-kB has been shown to interact with the progesterone receptor and inhibit its action at endometrium [4]. Evidence supports that a physiologic amount of inflammation is necessary for endometrial receptivity and early pregnancy implantation. However, pathologic inflammation was found to interfere with the endometrial receptivity and early pregnancy implantation. AP is an inflammatory disease with wide clinical variations, which may present sepsis, multiple organ failure, and even death [5]. To date, most investigators believe that caerulein-induced rat pancreatitis is caused by the unregulated activation of NF-κB within the many organs including pancreas, gut, stomach and distand organs such as uterus and ovary leads to the local inflammation [6]. Moreover, NF-κB is activated during early stages of pancreatitis and regulates many genes that may control inflammatory activities. Most recently, a study showed that the level of NF-κB activation correlates with the severity of AP [7].

Melatonin (*N*-acetyl-5-methoxytryptamine) is the major secretion product of the pineal gland. Melatonin is a direct neutralizer of free radicals, an indirect antioxidant. It plays a role in neuroendocrine regulation, the increase of immunity, neutralization of free radicals, reduction of angiogenesis and increase of apoptosis; in studies on animals and humans, it was demonstrated that melatonin has important antineoplastic properties [8] and inversely correlated with the tumor proliferation index in patients with endometrial pathologies [9]. Previous study Turkoz et al.[10] demonstrated that melatonin protects the ovaries against oxidative damage associated with reperfusion following an ischemic insult.

This study investigates the mechanisms by which melatonin treatment in rats with caerulein-induced rat pancreatitis influences a number of factors such as: uterine weight, endometrial thickness, endometrial proliferation, endo-myometrial edema, hemorrhage, necrosis and leucocyte infiltration

MATERIAL and METHODS

Studies were performed on female Wistar rats weighing 280-350 g. Animals were housed in cages under standard conditions at room temperature on a 12 h light : 12 h dark cycle with commercial pellet chow. Rats were deprived of food 17 h prior to the start of the experiment, but drinking water was available *ad libitum*. All experiments were approved by the Ethics Committee of Inonu University Experimental Animals Production and Investigation Centre. Twenty-eight animals were divided into four groups: (1) control group (n=7); (2) melatonin group (n=7); (3) caerulein group (n=7); (4) melatonin + caerulein group (n =7). AP was induced by 4 intraperitoneal injection of caerulein given hourly (50 µg/kg/dose total of 200 µg/kg; Sigma-Aldrich Co., Taufkirchen, Germany) into animals. A total dose of caerulein (200 µg) was given at 2-h intervals, each injection was containing 50% of the doses [11]. Animal in the melatonin group was treated with 20 mg/kg body weight melatonin. Melatonin was given via intra-peritoneal (ip) injection 30 min prior to the induction of AP. Because melatonin was dissolved in absolute ethanol and further dilutions were made in saline, with a 1% final concentration of ethanol, animals in the control group received i.p. injections of 0.9% saline at 2-h intervals. Animal in the caerulein+melatonin group received same dose cerulein and melatonin. It has not been stated in any comments by the manufacturers whether caerulein has any effect on the estrous cycle. Therefore, a daily vaginal smear was monitored during the treatment period and after the permanence of the estrous cycle was confirmed. The rats were sacrificed by decapitation 12 h after the last injection of caerulein. The uterus was carefully removed by severing the attachments to the ovaries, and vagina for histo-morphological evaluation. After the uterus was rinsed and weighed (wet), pieces of it were excised from the body portion, fixed in 10% formalin and prepared for routine paraffin embedding. Paraffin-embedded specimens were cut into 5-µl sections and stained with hematoxylin eosin (H&E). Sections were examined and photographed using a Nikon Optiphot-2 light microscope and Nikon DS-L3 Image Analysis System (Nikon Corporation, Tokyo, Japan). The semi-quantitative method was used to score the degree of histological change of edema, hemorrhage, necrosis, leucocyte infiltration, endometrial proliferation and endometrial thickness for all groups.

Statistical analysis: Data distribution was tested using the Kolmogorov-Smirnov test. Comparison among the groups was performed using the Kruskal-Wallis analysis of variance and post-hoc Mann-Whitney U tests for continuous variables. Data was presented as mean and standard deviation (SD) for continuous variables. The statistical software package SPSS 21.0 (SPSS Inc., Chicago, IL, USA) was used for data analysis.

RESULTS

There was no significant difference in the mean body weight after treatment in each group. The uterine wet weight differences between the control and caerulein given rats were significant ($P<0.01$, *Table 1*). The uterine wet weight of the caerulein group was higher than the control and melatonin group and the differences were significant ($P<0.01$). Pretreatment with melatonin demonstrated a significantly reduced uterine wet weight as compared with the caerulein only group. Evaluation of uterine histology revealed remarkable changes in endometrial thickness, edema, hemorrhage, necrosis and leucocyte infiltration in

Table 1. *The comparation of mean body weight, uterine wet weight and histological structural defects*
Tablo 1. *Islak uterus ağırlığı, ortalama vücut ağırlığı ve histolojik yapısal bozuklukların karşılaştırılması*

Parameters	Control	Melatonin	Caerulein	Melatonin+Caerulein
Mean body (g)	304.4±4.41	301.4±5.43	311.4±57.2	298.1±2.25
Uterus (g)	0.810±1.32*	0.796±2.23*	1.103±0.015	0.753±4.65*
Edema*	1.53±1.45	1.41±1.78	3.93±5.75	1.45±6.28
Hemorrhage*	1.64±4.65	1.51±0.18	3.26±3.54	1.54±8.32
Necrosis*	2.26±6.12	2.45±4.06	3.87±4.11	2.08±5.44
Leucocyte infiltration*	2.94±5.44	2.71±0.43	3.77±0.61	2.31±5.31
Endometrial proliferation	Normal	Normal	Normal	Normal
Endometrial thickness (mm)	0.124±0.11	0.128±0.45	0.144±0.31	0.126±0.01

Notes: *The data were expressed as mean ± SD; * P<0.05 compared with caerulein*

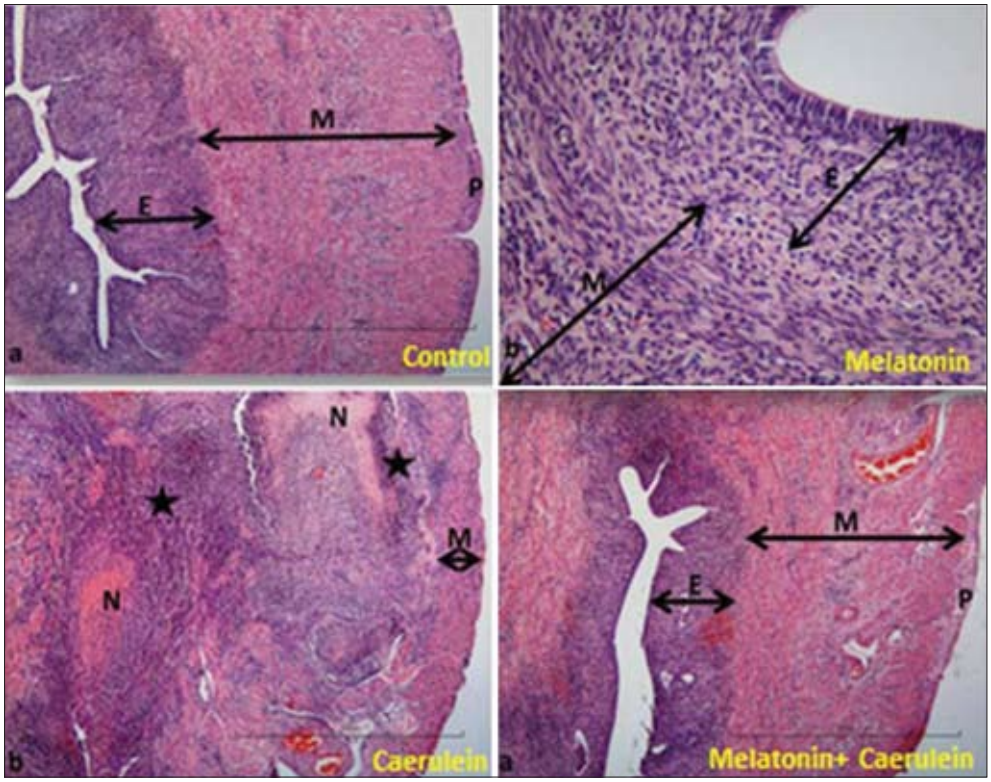

Fig 1. Histopathological appearance of uterus in all groups. Caerulein related diffuse necrosis *(black stars)* was prevented melatonin pretreatment. **N-** Necrosis and hyalinization; **M-** Muscle; **E-** Endometrium; **P-** Periton, (H&E X10)

Şekil 1. Tüm gruplarda uterusun histopatolojik görünümü. Seruleine bağlı diffüz nekroz alanları melatonin uygulaması ile önlendi. Kısaltmalar: **N-** Nekroz ve hiyalinizasyon; **M-** Kas; **E-** Endometrium; **P-** Periton (H&E X10)

the caerulein treated animals. The endometrial thickness, edema, hemorrhage, necrosis and leucocyte infiltration of the caerulein group was higher than the control and melatonin group and the differences were significant ($P<0.05$, *Fig. 1*). Morphologically, polymorphonuclear neutrophil infiltration and vascular dilatation were obvious in the caerulein given animals myometrium, and the changes also completely reversed by melatonin. Preteratment with melatonin normalized histological structural defects. Degree of endometrial proliferation was not significantly different among the groups. The addition of melatonin to caerulein treatment is associated with a decrease in endometrial thickness and prevents the edema, hemorrhage, necrosis and leucocyte infiltration in endometrium and myometrium.

DISCUSSION

Beginning with the evidence on the role of caerulein in systemic inflammation we considered that melatonin fulfills all the requirements for it to be considered as an antiinflammator drug which interact with the estrogen signaling pathways such. In the present study, morphologically, polymorphonuclear neutrophil infiltration were obvious in the caerulein given animals myometrium, and the changes also completely reversed by melatonin. Recent study demonstrated that mice developed more severe acute pancreatitis after caerulein hyperstimulation, which was explained by a decrease of apoptosis and a higher baseline proinflammatory state indicated by constitutively higher expression of inflammatory cytokines [12,13].

In our study, uterine wet weight of the caerulein group was higher than the control and melatonin group. Pretreatment with melatonin demonstrated a significantly reduced uterine wet weight. This may be results the combination of melatonin with caerulein injections increases fibrosis and loss of parenchyma. Another explanation is that melatonin decreases the endo-myometrial edema and causes reduction in uterine weight. Overuses of melatonin, which is an endogenous anti-inflammatory and antioxidative protein may attenuate uterine fibrosis. The hyperstimulation with caerulein increases edema, inflammation and necrosis ranges between 5 to 10 µg/kg/h in rats. Maximal tissue injury occurs after 12 h of continuous infusion but changes can be monitored already 15 minutes after the start of the caerulein infusion. One of the earliest consequences of hyperstimulation is the formation of edema. This edema is probably the result of several factors: increased vascular permeability, increased hydrostatic pressure from the constriction of small vessels. These events lead to a systemic inflammatory response syndrome, which includes extrapancreatic tissues including reproductive organs [12,13].

Addition of melatonin to caerulein in rats led to a decrease of endometrial thickness, necrosis and hemorrhage severe impairment of the endo-myometrial junction. Caerulein injections caused prominent histological damage with increased hemorrhage, endometrial thickness and necrosis was observed. This adverse effect of caurelein turned upside down with melatonin pretreatment. Control animals that were treated with saline or melatonin alone did not show any signs of inflammation. Melatonin had a significant anti-proliferative effect on Ishikawa cells (with estrogen receptors) at different cellular densities and different incubation times.

In this study, we used a caerulein-induced rat acute pancreatitis model to investigate the protective effects of melatonin on endometrium. Our results demonstrated that melatonin inhibited the production of proinflammatory cytokines and reversed abnormal histopathological changes of endometrium in caerulein-induced rat acute pancreatitis. In addition, rats which received melatonin treatment showed improved myometrial morphology.

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