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

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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 pretreatment 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

Özet

Serülein ile indüklenmiş akut pankreatiti (AP)’nin uterusa etkisi ve melatonin uygulamasının muhtemel uterus kuruyucu etkileri araştırıldı. Yirmi sekiz rat dört gruba ayrıldı: (1) Kontrol grubu (n = 7); (2) melatonin grubu (n = 7); (3) serülein grubu (n = 7); (4) melatonin + serülein grubu (n = 7). AP, genç dişi hayvanlara saatte bir (50 μg/kg) dozunda 4 kez verilen intraperitoneal serülein enjeksiyonu ile indüklenmiştir. Melatonin (20 mg/kg) AP indüksiyonundan 30 dakika önce intraperitoneal enjeksiyon yolu ile verildi. Ratlardan serüleinin son enjeksiyonundan 12 saat sonra sakrifiye edildi ve uterusleri histopatolojik değerlendirmeye alınmıştı. Ortalama vücut ağırlığı ve uterusun ıslak ağırlıkları kaydedildi. Endo-miyometriumun endometrial edema, kanama, nekroz ve leucocyte infiltration of the caerulein group was significantly higher than the control and melatonin groups (P<0.01). It was observed that pretreatment 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.

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INTRODUCTION

The nuclear factor kappa beta (NF-κB) pathway was indicated to be active in the normal endometrium of healthy women [1-3]. NF-κB 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 distant 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 intraperitoneal (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
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 of 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.

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 caerulein 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.

REFERENCES