Neuroendocrinology Letters ISSN 0172–780X

ORIGINAL ARTICLE

The opposite effect of morning or afternoon application of melatonin on collagen accumulation in the sponge-induced granuloma

Jacek Drobnik & Ryszard Dabrowski

Department of Pathophysiology, Medical University of Lodz, Narutowicza 60, Lodz, Poland.

Correspondence to: Jacek Drobnik, Department of Pathophysiology, Medical University of Lodz, Narutowicza 60, Lodz, Poland.
TEL. +48 42 630 61 87
FAX: +48 42 632 23 47
E-MAIL: drob@bgamld.am.lodz.pl

Submitted: *December 21, 1999*
Accepted: *April 10, 2000*

Key words: **connective tissue; healing; photoperiod; the pineal gland; repair**

Neuroendocrinology Letters 2000; ***21****:209–212 pii: NEL210300A04* *Copyright © Neuroendocrinology Letters 2000*

Abstract

OBJECTIVES: Collagen accumulation in the sponge-induced granuloma is under inhibitory influence of melatonin. This effect was observed when the pineal hormone was injected late in the afternoon.
DESIGN: The present study was conducted to compare the afternoon and morning melatonin application and to answer the question whether melatonin effect on the collagen accumulation in wounds is dependent on tissue circadian sensitivity to the pineal hormone.
METHODS: In order to induce granulation tissue development, the Ivalon sponge was implanted subcutaneously in the Wistar rats. Then the animals were divided into 5 groups: intact controls, rats injected with vehicle in the morning or in the late afternoon, as well as animals receiving melatonin (30 μg/100g body wt) in the morning or late afternoon. After 4 weeks, the sponges were removed for the analysis of both soluble and total collagen content. Collagen was analyzed as hydroxyproline content in the tissue. Insoluble collagen was calculated.
RESULTS: The afternoon application of melatonin was seen to decrease the total and insoluble collagen content in the sponge-induced granuloma ($p<0.05$). However, morning administration of the pineal hormone increased collagen capacity in the granulation tissue ($p<0.01$).
CONCLUSION: Therefore, contradictory effect of morning or afternoon melatonin application on the collagen content in the granulation tissue has been shown. This phenomenon is supposed to be due to various circadian sensitivity of tissues to melatonin and may suggest that the mechanism of melatonin regulatory action on collagen accumulation in wounds is complex.

Introduction

Information about the photoperiod is conveyed to tissues by melatonin (MLT). The hormone is released by the pineal gland in the night, but light immediately suppresses its synthesis and release [1]. To explain the mechanism of melatonin message, several hypotheses were formed. Hence, the duration hypothesis suggests that tissue response to the pineal hormone is dependent on the length of melatonin peak. According to the internal coincidence model, action of melatonin could be exerted only when the peak of the pineal hormone coincides with the sensitivity of tissue, which is called the window of sensitivity. The amplitude hypothesis, on the other hand, assumes that the height of melatonin peak is the critical information for tissues [1]

Melatonin is believed to be effective when it is administered in the afternoon [2]. Thus, late afternoon melatonin application inhibited mean mitotic activity of thyroid cells and reversed the stimulatory effect of TSH or pinealectomy on the thyroid gland growth [3]. Decreased weight of testes, sex accessory organs (seminal vesicles and coagulating glands) and uterines were also provoked by afternoon application of the pineal hormone [4]. Afternoon injections of melatonin inhibited also the healing of superficial wounds and decreased collagen content in the sponge-induced granuloma [5, 6, 7].

Melatonin injected in the morning can prevent the antigonadotropin effect of evening application of the pineal hormone [4] but does not affect the testicular growth in rats [8]. Morning injections of melatonin reduced the content of adrenalin and noradrenalin levels in the golden Syrian Hamsters, but afternoon melatonin injections were ineffective [9]. Melatonin administered continuously in pellets does not change the mean mitotic activity of the thyroid gland cells. It abolishes the inhibitory effect of evening melatonin application [3]. All the above discussed papers suggest circadian variability of tissue sensitivity to melatonin. Therefore, the aim of the study was to answer the question whether melatonin action on the collagen accumulation in wounds is dependent on time of its application and circadian sensitivity of tissues. Therefore, we have compared effects of morning or late afternoon melatonin injections on collagen content in the granulation tissue.

Materials and methods

Study design. Thirty four male Wistar rats, weighing 200 g (+ 20g) each, were housed with a free access to commercial food pellets (LSM, Bacutil, Poland), as well as tap water ad libitum. All the animals were kept in light (L)-dark (D) conditions L:D= 12:12. Light was turned on at 07.00. The animals were divided into five groups: Group 1: intact control (CTR); group 2: injected with vehicle in the morning (V_M); group 3: injected with vehicle in the afternoon (V_A); group 4 melatonin administered in the morning (MLT_M); and in group 5: MLT given in the afternoon (MLT_A).

Wound model. Under ether anesthesia a medial incision 3 cm long was made in the dorsal surface of the lumbar region. Then pieces (1cm x 1cm x 0.5cm) of formaldehyde sterilized Ivalon sponges were inserted into subcutaneous tissue. The wounds were closed with four silk sutures [10]. After 28 days the sponges were removed for the analysis of collagen.

Applied drugs. The animals were subcutaneously injected, either with ethyl alcohol 2% (v/v) in physiological saline at the dose of 0.1ml/100g body wt. as the vehicle (V), or melatonin (MLT) at the dose of 30μg/100g body wt, dissolved in the vehicle. The morning injections were given between 08.30 and 09.00 a.m. and the afternoon administration was done between 05.30 and 06.00 p.m. for 28 days.

Determination of collagen. The total collagen was estimated according to the method described by Woessner [11]. For the assessment of soluble collagen, the macerated tissue was suspended in ten times its weight of 0.45M NaCl (pH 7.4) and kept for 48 hours at 2°C with penicillin (10,000 units/sample) to reduce bacterial growth. The mixture was centrifuged and evaporated to dryness. Then, samples were assayed together with samples containing total collagen. For the evaluation of total collagen, macerated tissue was extracted with ether-acetone and vacuum-dried at 90°C. Samples of the total collagen were assayed for hydroxyproline by hydrolysis with 6N HCl (3ml/10mg of dry tissue) at 110°C for 24h. After hydrolysis, all the hydrolizates were evaporated to dryness in a water bath and the precipitates were dissolved in 3ml of redistilled water. The samples, neutralized by 1N NaOH, were diluted to 10 ml with redistilled water. From the tubes, 0.2 ml samples were taken for further analysis and diluted with redistilled water to 2 ml of the final volume. Hydroxyproline was oxidized to pyrrole by 1.25 ml of chloramine T in a citrate buffer (pH=6.0), then shaken for 5 min and incubated for 20 minutes at 20°C. In order to remove the excess of chloramine T, 1.25 ml of 3.15 M perchloric acid was added. After 5 minutes, the samples were treated with 1 ml of 20% p-dimethylaminobenzaldehyde and incubated in a 60°C water bath for 20 minutes. The optical density was measured at 560 nm on a spectrophotometer. Salt insoluble collagen was calculated.

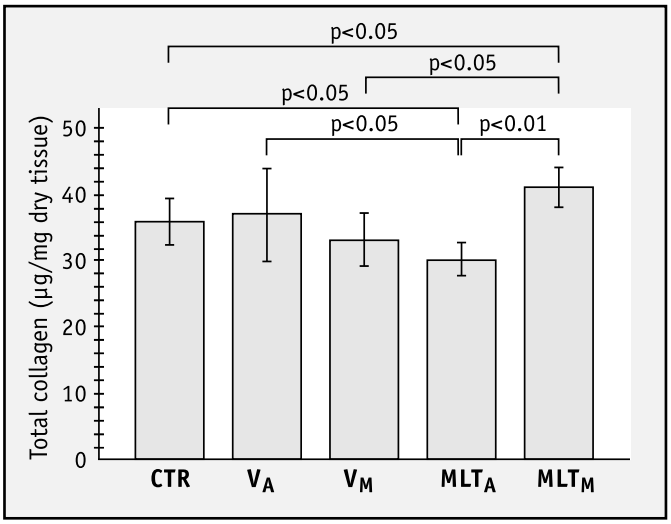

Fig. 1. Total collagen content in the sponge-induced granuloma of controls (CTR), rats treated with vehicle in the morning (V_M) or in the afternoon (V_A) as well as injected with melatonin in a dose of 30µg/100g body wt. in the morning (MLT_M) or in the afternoon (MLT_A). Each bar represents the mean ±SD.

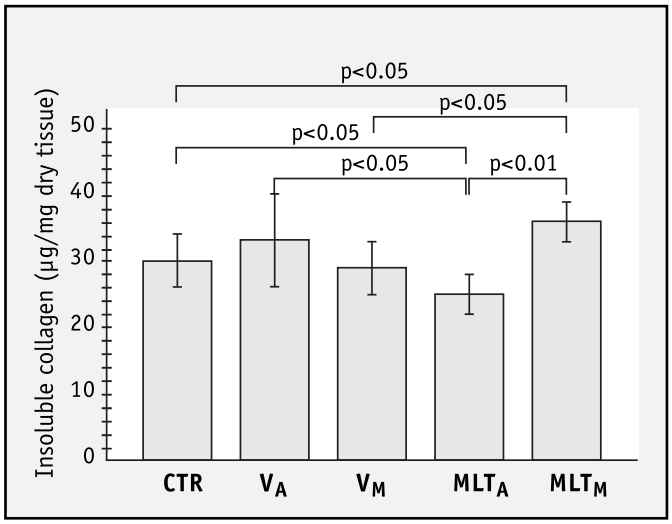

Fig. 2. Insoluble collagen content in the sponge-induced granuloma of controls (CTR), rats treated with vehicle in the morning (V_M) or in the afternoon (V_A) as well as injected with melatonin in a dose of 30µg/100g body wt. in the morning (MLT_M) or in the afternoon (MLT_A). Each bar represents the mean ±SD.

Statistical analysis. The Kruskal-Wallis' test was used for statistical analysis. Statistical differences were evaluated by the U Mann-Whitney's test.

Results

Vehicle injections in the morning (V_M) or in the afternoon (V_A) do not influence total soluble and insoluble collagen content in the sponge-induced granuloma. Moreover, collagen content in the granulation tissue of the rats injected with vehicle (V_M, V_A) is identical with the controls (CTR). Afternoon administration of melatonin (MLT_A) decreased ($p<0.05$) total and insoluble collagen content in the granulomas, as compared with intact controls (CTR) and vehicle treated rats (V_A). Morning injections of the same dose of the pineal hormone (MLT_M) act oppositely to its afternoon application ($p<0.01$) and increase both the total and insoluble collagen content in the wound, as compared with the controls (CTR) or vehicle treated animals (V_M; $p<0.05$). The experimental conditions did not influence the soluble collagen capacity in the granuloma (data not shown).

Discussion

The study shows the inhibitory effect of afternoon melatonin applications on both total and insoluble collagen content in the sponge-induced granuloma. Indeed, this fact confirms our earlier publications which indicated that melatonin applied in the afternoon, at the dose of 30μg/100g body wt., exerted the most effective inhibitory effect on the collagen capacity in sponge induced granuloma [5, 7]. Moreover, that dose of melatonin was the lowest one which markedly inhibited the contraction and healing of the superficial wounds, being the only dose which inhibited the accumulation of both the total and insoluble collagen, as well as reversed the effect of pinealectomy on the collagen level in the sponge-induced granuloma [5]. Continuous darkness, which increased the background level of the pineal hormone [12], decreased also the collagen content in the granulation tissue [6]. Morning application of melatonin, oppositely to afternoon injections, increased both total and insoluble collagen content in the sponge-induced granuloma. Contradictory effects of melatonin given in the morning or afternoon were also reported by others in different investigated processes [3, 4, 8, 9]. The opposite action of the morning and afternoon injections of melatonin on collagen content in wounds shows that the sensitivity of a tissue to melatonin changes during the day and night and may determine the final effect of the pineal hormone.

The effect of the pineal hormone could be exerted by binding to the receptors on the cellular membrane. Receptor density changes diurnally. Thus, the highest density of melatonin binding sites in hamster hypothalamus [13] and in suprachiasmaticus nucleus of rats [14] was found in the late afternoon. Contrary to that, the lowest density of melatonin binding sites

was detected in the early morning [14]. Melatonin is also able to act via receptor independent pathway. Hence, it can bind to calmodulin or nuclear protein as well as may work as a free radical scavenger [14]. Intracellular localization and distribution of melatonin in Swiss 3T3 mouse fibroblasts (cells responsible for collagen synthesis) were characterized by an experiment with fluorescein-labeled immunoglobulin [15]. The addition of melatonin to the cultured fibroblasts retarded the progression of the cells throughout the cell cycle [15]. The above described complex mechanism of melatonin action is useful in understanding the contradictory effect of melatonin administered in different times of the photoperiod. The final effect of the pineal hormone on the collagen level in the wound seems to be the result of various mechanisms diurnally changeable. Thus, the effects dependent on the melatonin receptors are supposed to be visible mainly in rats injected with the pineal hormone at the end of the light phase of the photoperiod.

Melatonin treatment in the morning or in the afternoon exerted parallel changes in total and insoluble collagen, but the soluble collagen was not influenced. This fact is supported by our earlier study, which showed that soluble collagen is beyond the pineal gland regulation [5]. Thus, the present and earlier results indicate that melatonin, independently of the time of its application, does not modify either cross linking or pattern of the collagen, which determine the solubility of the protein [16].

Opposite effects of melatonin administration showed that its action is dependent on the application time. This means that tissue sensitivity varies in early and late phases of the photoperiod and is important in the expression of the final effect of melatonin on collagen accumulation in the sponge-induced granuloma.

Acknowlegment

The authors are grateful to Mrs Alicja Witczak for her excellent technical assistance.

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