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Dose-Dependent Anti-Inflammatory Activity of Melatonin in Experimental Animal Model of Chronic Inflammation

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Abstract: Melatonin is a natural hormone-like compound mainly produced by the pineal gland. It possesses a number of important biologic activities including oncostatic, anti-oxidant and immunostimulatory actions; however, the dose-response relationship was not well characterized. The present study was designed to evaluate the dose-response relationship for the anti-inflammatory activity of melatonin in the experimental animal model of formalin-induced chronic inflammation in rats. Fifty-four Sprague-Dawley rats were allocated into 9 subgroups, 6 rats in each one. These subgroups represent saline-treated, piroxicam (5mg/kg), dexamethasone (1mg/kg) and melatonin (0.25, 0.5, 1.0, 2.0, 5 and 10mg/kg) treated subgroups. All drugs were administered intraperitonealy (IP) 30 min before inducing inflammation by injecting 0.1 ml of 2% formalin into the subplanter surface of the right hind paw of ether-anesthetized rats and continued for 7 consecutive days. The results of the study showed that all treatment groups (except melatonin 0.25 mg/kg) significantly reduced paw thickness ($P<0.05$) compared to saline treated group after 7 days of treatment. Melatonin 5 mg/kg group produced a highest level of anti-inflammatory activity, which is comparable to that of piroxicam 5mg/kg group. In conclusion, melatonin exerts a well defined anti-inflammatory activity in animal model of chronic inflammation that is comparable in certain circumstances to that produced by standard drugs in this respect.

Key words: Melatonin · Chronic inflammation · Dose-response

INTRODUCTION

Inflammation is a beneficial host response to external changes or cellular injury that leads to the activation of a complex array of inflammatory mediators, finalizing and restoring tissue structure and function. Although it is a beneficial response, prolonged inflammation can be detrimental to the host, contributing to the pathogenesis of many disease states [1]. Chronic inflammation is a long lasting type changes that may persist for weeks, months or even years and brought on by acute inflammation or may be the result of an autoimmune disease [2]. It is a pathological condition characterized by concurrent active inflammation, tissue destruction and attempts at repair. During these processes, acute inflammatory cells are replaced by a chronic type one such as macrophages under appropriate activation, infiltrating into the inflamed tissue and existing macrophages exit the tissue via lymphatic system [3]. Melatonin is a hormone synthesized and released by the pineal gland, which reaches the highest levels during the night and is involved in the regulation of circadian rhythms and seasonal changes in vertebrate physiology [4]. Many evidence indicated that melatonin shows an outstanding functional versatility, by exhibiting antioxidant [5], oncostatic [6], antiaging [7] and immunomodulatory properties [8]. Moreover, melatonin also has shown to play an important role in immuno-pathological conditions such as endotoxic shock [9-11]. The present study was designed to evaluate the dose-response relationship of the anti-inflammatory effect of melatonin in experimental animal model of chronic inflammation.

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MATERIALS AND METHODS

Sprague-Dawley rats weighing 180-220 g of both sexes were purchased from the National Centre for Drug Research and Quality Control, Baghdad. They were kept in the animal house of the department of Pharmacology and Toxicology, College of Pharmacy, University of Baghdad at 25±2°C, relative humidity 60-70% and light:dark cycle of 12:12 h for 1 wk before starting experiments. Animals were housed in groups of 6 in stainless steel cages and provided with standard rodent pellet diet (GAFCO, Baghdad) and the food was withdrawn 12 hr before the experiment, water was allowed *ad libitum*. All experiments were performed according to the guidelines of laboratory animals' care and the ethical guidelines for the investigations on experimental animals. In the present study, 54 rats were allocated into 9 groups (6 rats each) and treated as follow: First group treated with normal saline 2ml/kg served as control; second and third groups were treated with dexamethasone (Rotex Medica, Germany) 1mg/kg and piroxicam (ZMC, Germany) 5mg/kg respectively, served as standard comparators; the other 6 groups were treated with 0.25, 0.5, 1, 2, 5 and 10mg/kg melatonin (Rupal Chemicals Ltd, Tarapur, India) respectively, given by IP injection.

Formalin-induced Chronic Inflammation: The effect of melatonin in chronic inflammation was evaluated utilizing formalin-induced paw edema [12]. In this model, chronic inflammation was induced by injecting 0.1 ml of 2% formalin into the subplanter area of the right hind paw of ether anaesthetized rats. All drugs including melatonin (0.25, 0.5, 1, 2, 5 and 10mg/kg), dexamethasone 1mg/kg, piroxicam 5 mg/kg and the vehicle (normal saline) were administered IP to the rats, 30 min prior to formalin injection and continued for 7 consecutive days. Dexamethasone, normal saline, melatonin and piroxicam doses were administered once daily. In this model the increase in paw thickness was measured by using the vernier caliper method [13]. The ability of anti-inflammatory drugs to suppress paw inflammation was expressed as a percent inhibition of paw edema [14] and calculated according to the following equation:

$$\text{Percentage of inhibition (\%)} = 100 \times (1 - X/Y)$$

Whereas
X= mean increase in paw thickness of treated rats
Y= mean increase in paw thickness of control rats

All the results were expressed as mean ± SEM. The significance of difference between the control and treated groups were determined using paired Student's *t*-test and one-way analysis of variance (ANOVA). *P*-values <0.05 were considered significant.

RESULTS

In the animal model of chronic inflammation, the suppressive effects of different doses of melatonin and the standard anti-inflammatory drugs, piroxicam and dexamethasone were shown in Table 1 and Figure 1. After 6 days of inducing chronic inflammation with 2% formalin, all treatment groups (different doses of melatonin, piroxicam and dexamethasone) produced significant decrease in paw thickness ($P<0.05$) compared to saline treated group (Table 1). In this model, melatonin 5 mg/kg group produced highest level of anti-inflammatory activity, which is comparable to that produced by the active comparator, piroxicam 5mg/kg group. In Figure 2, the relationship between the doses of melatonin and the percentage of inhibition of paw thickness showed a linear

Table 1: Effect of different doses of melatonin, compared with dexamethasone or piroxicam in formalin-induced chronic inflammation in rats

Treatment groups	Mean increase in paw thickness after 6 days (mm)	% of inhibition
Normal saline 2 ml/kg	3.0 ± 0.1	-
Piroxicam 5 mg/kg	1.6 ± 0.1*[a]	45
Dexamethasone 1 mg/kg	1.3 ± 0.2*[b]	58
Melatonin 0.25 mg/kg	2.8 ± 0.1[c]	7
Melatonin 0.5 mg/kg	2.6 ± 0.04*[c]	14
Melatonin 1 mg/kg	2.4 ± 0.07*[d]	20
Melatonin 2 mg/kg	2.3 ± 0.14*[d]	23
Melatonin 5 mg/kg	1.8 ± 0.09*[a]	41
Melatonin 10 mg/kg	2.2 ± 0.18*[d]	25

Values expressed as mean ± SEM; number of rats in each group=6; * significantly different compared to control group ($P<0.05$); values with non-identical superscripts (a,b,c,d) are considered significantly different ($P<0.05$).

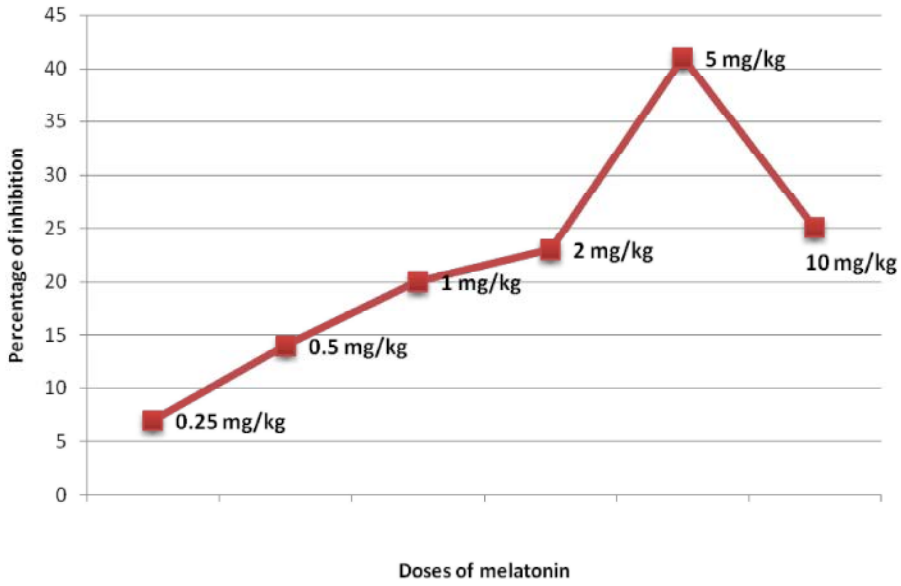

Fig. 1: Dose-response relationship of the anti-inflammatory activity of melatonin in formalin-induced chronic inflammation in ratsg2

relationship up to the dose of 5mg/kg (Maximum effect), after that a decline in the anti-inflammatory activity occurs with increasing the dose above this limit.

DISCUSSION

In chronic inflammatory model, inhibition of formalin-induced pedal edema in rats is considered as one of the most suitable test procedures to screen chronic anti-inflammatory agents as it closely resembles human arthritis [15]. Although the inflammatory process is a localized protective response vital for the survival of all complex organisms, the sustained production of inflammatory mediators can lead to sever pathological conditions [16]. The effect of melatonin in 5mg/kg dose on formalin-induced pedal edema, a chronic inflammatory model, was maximal compared to that produced by 5mg/kg piroxicam; this effect of melatonin seems to be mediated mainly by the inhibition of COX-2 enzyme [17]. Recent studies found that melatonin, when used in chronic inflammatory disorders as a potent antioxidant, allows a 10-fold reduction in the doses of steroids which are currently used in these situations [18]. Anatomical, physiological and pharmacological evidences support the existence of bilateral interactions between the endocrine and immune system [19]. In this context, melatonin plays an important role as modulator of a great number of cytokines [20]. These mediators may counteract stress-induced immunosuppression and other secondary immune-deficiencies as well as protect against lethal viral encephalitis, bacterial disease and septic shock. Hence, melatonin prevents endotoxin-induced circulatory failure in rats through an inhibition of TNF-α level [21] and reduces post shock level of IL-6 in mice [22]. Regarding inflammatory processes, melatonin is able to counteract LPS-induced expression of inducible nitric oxide synthase (NOS) [21] and mitochondrial NOS [23] in rats. Meanwhile, production of ROS causes cellular injury and necrosis through several mechanisms including peroxidation of membrane lipids during inflammation [24]. Accordingly, the possibility that melatonin effectively scavenges these ROS, or interfering with their harmful consequences might be one of the possible mechanisms for the reproducible anti-inflammatory activity reported in the present study or at least an additive pathway to the already involved ones. Melatonin is described to have a key regulatory role on cytokine production [20], as a strong antioxidant agent [25] and anti-apoptotic mediator [26]. Regarding modulation of pro- and anti-inflammatory cytokines level, melatonin has significant inhibitory effect on TNF-α, IL-12 and interferon-ã levels in peritoneal fluids [16]. Systemic administration of melatonin, in doses comparable with those of other known anti-inflammatory agents, exerted anti-inflammatory effects comparable with that of indomethacin 5mg/kg or the selective COX-2 inhibitor rofecoxib 2.25mg/kg [17]. Melatonin also displayed anti-nociceptive properties in a model of electrically induced pain in the rat and enhanced the anti-inflammatory and

anti-nociceptive effects of indomethacin [17]. The protective effect of melatonin is related, among other actions, to its function as an electron donor [27], as with the classical antioxidant vitamins C and E. The indole reacts directly with the activated form peroxynitrite ONOOH and also directly scavenges H2O2; its protective action include electron donation, electron capture, free radical trapping, as well as addition and substitution reactions [28]. All the previously mentioned data showed that melatonin could act mainly on the local site of inflammation, probably preventing the extravasations of inflammatory mediators, which promote systemic failure prior to death; melatonin through a multi-factorial pathway, in which its pleotropic effects as immunomodulator, antioxidant and anti-apoptotic agent are involved [16]. In conclusion, melatonin (when administered in pharmacological doses) exerts a well defined dose-dependent anti-inflammatory activity in animal model of chronic inflammation, which is comparable to that produced by the standard anti-inflammatory agent, piroxicam.

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