Histological Changes in the Thoracic Aorta of Adult Rats Treated with Melatonin

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Abstract:

Background: The main pineal hormone, Melatonin, is an important cardioprotective hormone whose receptors have been located in the vasculature & have been suggested to inhibit vascular reactivity through different mechanisms. It can prevent the initiation & the progression of atherogenicity.

Objective: the study aims to explore the histological effects of pharmacological & high doses of dietary melatonin on the thoracic aorta of adult rats.

Materials & Methods: melatonin was given to adult albino rats for successive 4 weeks at daily single doses ranging from 250-1000 µg / kg body weight. The thoracic aorta was then studied under light microscopy for histological changes.

Results: Studied specimens showed that 250 µg / kg body weight doses didn't cause a significant change in the histology of the vasculature while 500 µg / kg body weight doses resulted in significant but regular vascular thickening. The high doses resulted in irregular smooth muscle hyperplasia, disruption of the connective tissue patterns & signs of intimal injuries.

Conclusion: Low doses of melatonin may be protective & supportive to the vasculature but higher doses carry the risk of vascular damage.

Key Words: Melatonin, vasculature, thoracic aorta, smooth muscle hyperplasia.

Introduction

Melatonin is a methoxyindole synthesized and secreted principally by the pineal gland at night under normal environmental conditions. The primary physiological function of melatonin, whose secretion adjusts to night length, is to convey information concerning the daily cycle of light and darkness to body physiology [1]. Melatonin can be transported everywhere by platelets and, thanks to its lipophilicity, can cross cellular membranes easily, thus regulating blood-tissue exchanges [2].

Melatonin receptors have been located in the vasculature & have been suggested to inhibit vascular reactivity through different mechanisms [3]. The expression of vascular melatonin receptors in the rat is differentially regulated by factors such as strain and age, and in the female by reproductive hormones. Functional studies using the caudal artery of the rat suggest that melatonin regulates vascular tone [4].

An important cardiovascular protective effect of melatonin is mediated via its direct free radical scavenger & its indirect antioxidant activity. Melatonin efficiently interacts with various reactive oxygen & reactive nitrogen species (receptor independent actions) and it also upregulates antioxidant enzymes & downregulates pro-oxidant enzymes (receptor-dependant actions) [5]. Melatonin effects surpass the protective use to therapeutic applications. Sener *et al* demonstrated that nicotine-induced dysfunction of the rat aorta was reversed by melatonin treatment [6]. Combined with other antioxidants, melatonin can reduce the alcohol-related oxidative stress on the vasculature [7]. The direct scavenger activity of melatonin on free radical is evident by its ability to remove hydroxyl & peroxyl radicals. These radicals oxidize low-density lipoproteins (LDL) which in turn become endocytosed by resident macrophages in the subendothelial vascular wall leading to atherosclerosis. Therefore, Melatonin can prevent the initiation & the progression of atherogenicity [8]. Besides its antioxidative effects, melatonin exhibits a hypolipidemic activity that further reduces the atherogenic risk in aging animals [9].

This study aims to explore the histological effects of both therapeutic & high doses of melatonin on the aorta.

Materials & Methods

40 adult Wistar albino rats (aged 10 weeks with an average weight of 355 grams at the beginning of the experiment) were used & kept in an environmentally controlled animal room (12-12 hour light cycle & temperature of 25^{o}C). They were fed a controlled diet with free access to water & diet except for 90 minutes prior to the melatonin meal. Dietary melatonin was given as a single daily dose, 2 hours before sunset. The animals were divided into 5 groups, each consisting of 8 rats. Group I served as a control group. The other four groups (Group II, III, IV & V) were given melatonin in a single oral daily dose of 250, 500, 750 & 1000 µg / kg body weight

respectively, for successive 4 weeks. After the last day of treatment, the animals were sacrificed under diethyl ether anesthesia. The thoracic aorta was removed & prepared for paraffin sections using Bouin' solution for fixation and Haematoxylin & Eosin for staining. Magnified digital images of the stained aortic slices were obtained using a digital colour attached to an optical microscope. These digital images were calibrated using an objective micrometer (DigiZeiss®). Measurements of media thickness (from the internal to the external elastic laminae) were obtained from 10 areas of 5 sections of aortic wall from each rat. Statistical analyses were performed using SPSS Software. The results were then tabulated & statistical significance was inferred at $P<0.05$

Results

The thoracic aortae of animals from group II (doses of 250 µg / kg body weight, **Figure 1**) showed normal histological features of the tunical layers as the control group (group I, **Figure 2**). The tunica intima was composed of a continuous layer of endothelial cells. The tunica media appeared to have normal & healthy numerous distinct elastic laminae, which were wavy & arranged concentrically, with smooth muscle cells seen in the interspaces between the concentric lamellae. There was no significant increase in the media thickness. The tunica adventitia was recognized by the normal-looking fibrous tissue elements.

In group III (dose of 500 µg / kg body weight, **Figure 3**) there was vascular wall thickening resulting from an increase in the thickness of tunica media observed as smooth muscle hyperplasia among regular concentric elastic laminae, on the expense of a relatively thin tunica adventitia.

In group IV & V (high doses of 750 & 1000 µg / kg body weight, respectively) the increase in the tunica media was more pronounced but the hyperplasia was irregular & was associated with disruption of the concentric pattern of the elastic laminae (Figure 4). Irregular luminal layers of endothelial cells were observed & the internal elastic laminae showed areas of discontinuity (**Figure 5**). In group V, few fibrocytes were spotted in the subendothelial regions (**Figure 6).** Fibrous elements of the tunica adventitia were also thickened in both groups as compared to the controls.

There was a statistically significant increase in media thickness of groups III, IV & V as shown in **table 1**.

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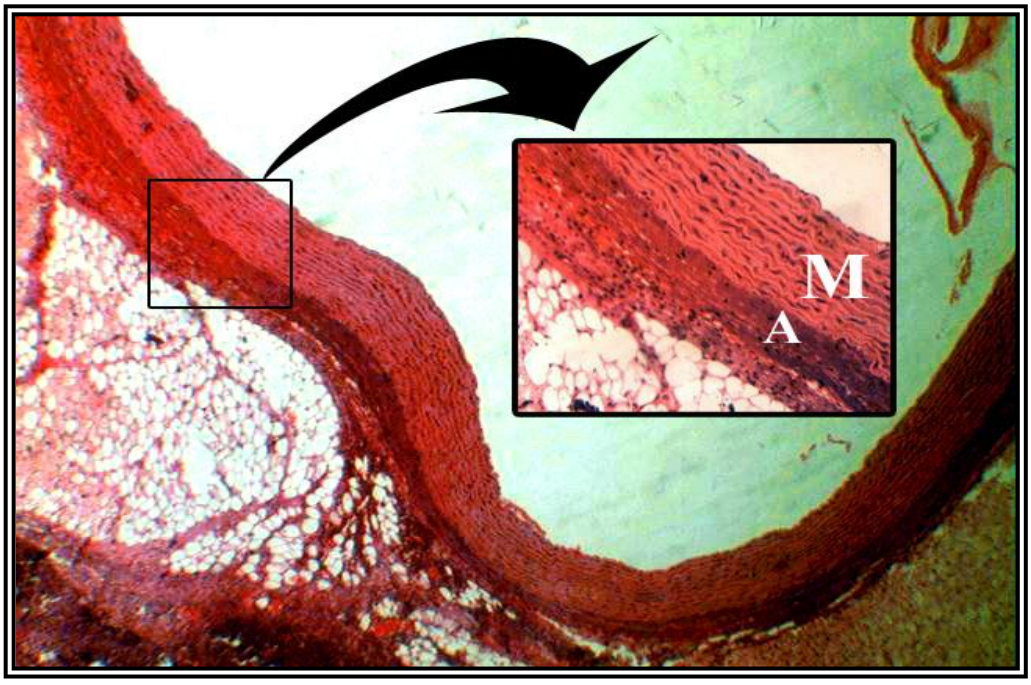

Figure 1: Transverse section the thoracic aorta of rats treated with 250 µg / kg body weight melatonin showing normal histological features of the vascular layers X150. The inset picture (black magnifying arrow) shows the tunica media (M) with the concentric elastic laminae & smooth muscle cells and the tunica adventitia (A) with the nuclei of fibrous tissue cells X300.

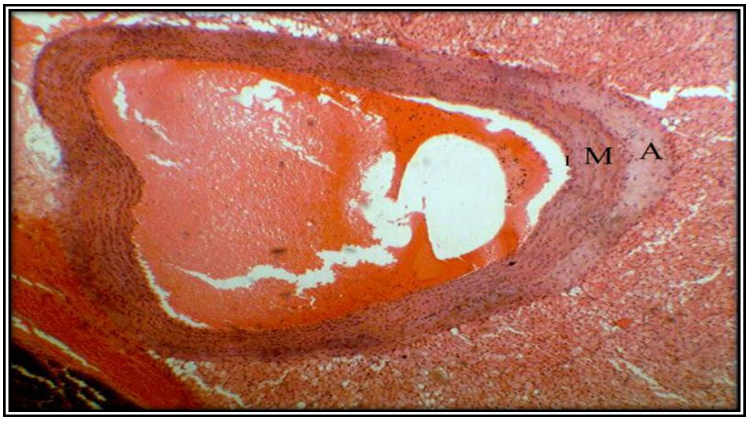

Figure 2: Transverse section of the thoracic aorta of control rats showing normal histological features of the tunica intima (I), tunica media (M) & tunica adventitia (A). X150

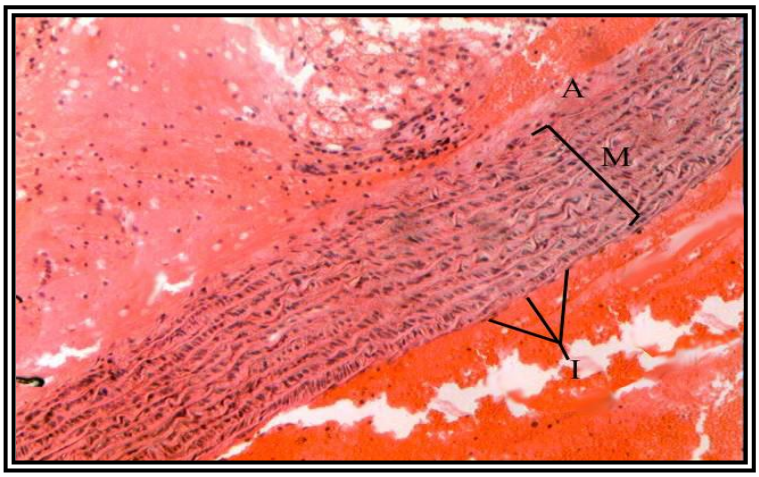

Figure3: Transverse section of the thoracic aorta wall of rats treated with 500 µg / kg body weight showing vascular wall thickening with increase in the thickening of the tunica media (M) as smooth muscle cell hyperplasia but with maintenance of the concentric pattern of the elastic laminae. The tunica advetitia (A) is relatively thin while the tunica intima (I) is normally regular as indictaed by the continuous shadow of the internal elastic lamina. X300

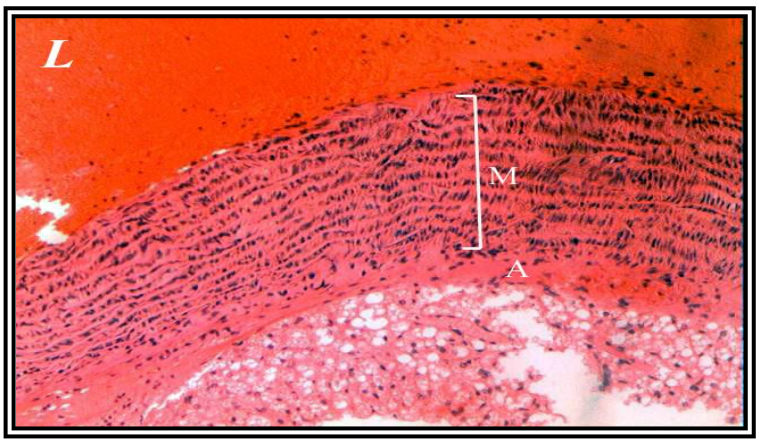

Figure4: Transverse section through the thoracic aorta of rats treated with 750 µg / kg body weight showing thinning of the tunica advintitia (A), pronounced smooth muscle hyperplasia of the tunica media (M) but with irregularities in the patterns of the elastic laminae. X300 [L=vessel lumen].

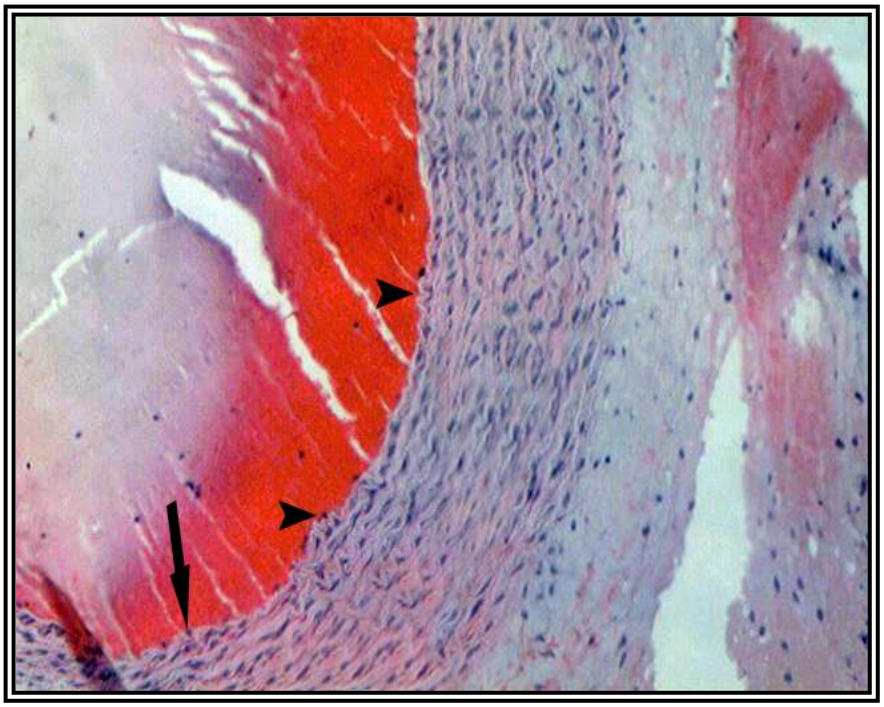

Figure 1: Transverse section through the thoracic aorta of rats treated with 1000 µg / kg body weight showing intimal irregularities (short arrow heads) and disruption of the internal elastic lamina with endothelial sloughing (long arrow). X300

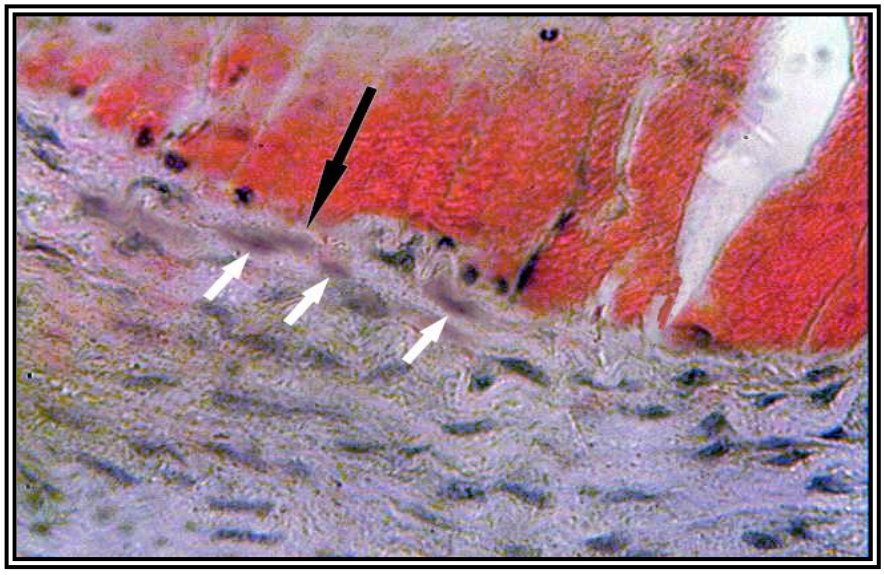

Figure 2: Transverse section through the thoracic aorta of rats treated with 1000 µg / kg body weight showing subendothelial fibrocytes (short white arrows) & intimal discontinuity (long black arrow). X600

Table 1: The effect of different doses of dietary melatonin on the media thickness (µm) of the thoracic aorta of adult rats (Data are Mean± S.D., *P<0.05 compared with control)

Group / Dose	Group I (Control)	Group II (250 µg / kg)	Group III (500 µg / kg)	Group IV (750 µg / kg)	Group V (1000 µg / kg)
Media thickness (µm)	125.6±24.1	127.4±21.2	142.6±29.3*	146.6±26.3*	144±23.3*

Discussion

Melatonin inhibits central sympatho-adreno-medullary outflow in rats [10]. It's also hypothesized that it activates MT2 receptors that induce relaxation in vascular smooth muscles [11]. The resulting effect of lowered blood pressure may initiate a response of the vasculature to regain its tone by smooth muscle hypertrophy or hyperplasia. Our finding of smooth muscle hyperplasia & the tunica media thickening in animal receiving 500 µg / kg body weight of melatonin but not at lower doses (250 µg / kg body weight) suggest that high therapeutic doses of melatonin affect the vascular tone & blood pressure to the level that entails a body response antagonize the relaxant effect of melatonin. Melatonin also induces the production of sulphated glycosaminoglycans (GAG), fibrillin, elastic & collagenous fibers and other components of connective extracellular matrix [12] and may have directly contributed to the perspicuity & increase of the elastic lamina in the treated animals.

Melatonin effect on vascular tone also involves other indirect mechanisms. An endothelium-dependent mechanism involves an inhibition of the metabolism & thus subsequent increase in cGMP leading to vascular relaxation in a concentration-dependent manner [13]. At high concentrations, melatonin also inhibits nitric oxide (NO) production from endothelial cells by affecting Ca^{++} ion mobilization [14] but without influencing nitric oxide synthetase activity [15]. Nitric oxide inhibits the proliferation of vascular smooth muscle cells & protein synthesis in these cells, in part by a cGMP-mediated mechanism [16]. Therefore, the prominent smooth muscle hyperplasia seen in animals treated with high doses of melatonin may be related to the loss of the inhibitory effect of (NO) on smooth muscle proliferation through a cGMP-mediated pathway. The disarrangement of the elastic lamina is probably related to the accelerated increase & re-arrangement of smooth muscle cells.

Through an endothelium-independent mechanism, melatonin causes a dose-dependent relaxation of pre-contracted vascular smooth muscles by impairing α-1 & α-2 adrenergic responses without changes in the β-adrenergic responses of the vascular smooth muscle fibers [17]. It is hypothesized that endothelial cells modulate the GAG composition of adjacent connective tissue and thereby influence its morphological and physiological properties. It is also suggested that the normal amounts, types and distribution of GAG in the arterial wall, and especially in the intima, may be partly dependent on interaction between the endothelium and smooth muscle cells [18]. It is possible that the relaxant effect of high doses of melatonin resulted in an abnormally high blood flow that caused endothelial injury & damage to the internal elastic lamina [18] which in turn added to the disruptions seen at the tunica media levels. The presence of fibrocytes in the subendothelial region of some animals treated with the maximum dose supports the suggestion of intimal injury [20].

We therefore conclude that low therapeutic doses of melatonin are probably beneficial & protective to the vasculature but caution should be taken as regards high melatonin dosage or over longer periods of time. Therefore, dietary doses of melatonin of 250-500 µg / kg body weight may be considered therapeutic while higher doses are toxic & harmful.

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