
Exogenous melatonin effects on Glomerular Filtration Barrier in male albino rats.

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Abstract:

Background: In kidney, basal lamina is the main component of the glomerular filtration barrier and podocytes have a major role in its synthesis.

Objective: To study the effects of different oral doses of exogenous melatonin on glomerular filtration barrier by using methylene blue injections.

Material & methods: In this study, sixteen male rats of five weeks of age were used, they were divided into three groups: Group I was control group, groups II and III were treated orally with melatonin in a dose of 250 & 500 µg/kg body weights subsequently.

Results: Focal segmental glomerulosclerosis was caused by 500 µg/kg body weight melatonin, but when methylene blue was given intraperitoneally; its particles appeared in renal interstitial tissue in both 250 & 500 µg/kg body weights treated rats.

Conclusion: Melatonin has an effect on the glomerular filtration barrier. It is recommended to use immunohistochemical techniques to evaluate the subcellular level changes.

Key Words: Kidney, glomerular filtration barrier, melatonin, methylene blue.

Introduction:

Previous studies showed that the renal corpuscle is an important unit in the process of filtration in which the glomerular filtration barrier is found within it, this barrier includes the followings:

1-Fenestrated endothelium of the glomerular capillaries and the mesangial cells: The fenestrae of endothelial cells are large (70-90 nm) in diameter and are more numerous than in the fenestrated capillaries of other organs. .

They lack the thin diaphragm commonly observed spanning the openings of other fenestrated capillaries ^[1]. Besides endothelial cells, there are the mesangial cells that are considered as a major constituent of renal glomerulus, they play a critical role in the regulation of glomerular filtration rate and renal blood flow within the glomerular capillaries ^[2&3]. These cells represent a contractile smooth muscle-derived like cells ^[4] that contain α -smooth muscle actin and myosin ^[5]. They synthesize the amorphous matrix to support the capillary walls and also act as macrophages to clean the endothelial side of the basal lamina from particulate material that accumulates during the filtration process ^[1].

2-Basal lamina: This basal lamina is derived from the fusion of capillary- and podocytes- produced basal lamina ^[1]. It is about (3) times the usual thickness of other basal laminae found else where in the body. It is the main barrier that is newly synthesized by adding new material to it on epithelial side (podocytes) and removal of old material from its endothelial side by phagocytic activity of mesangial cells ^[6&7].

3-The visceral layer of Bowman's capsule or the podocytes that have cell body from which arises

several primary processes which give rise to secondary processes called pedicels.

They are highly specialized cells whose functions include support of the glomerular capillaries, synthesis of the glomerular basal lamina, regulation of glomerular permeability and phagocytic function to remove large molecules which become trapped in the outer layer of the filter ^[2&8], they have a very limited potential for repair and are unable to replicate postnatally ^[1]. Junqueira (2003) found that the secondary processes (foot processes) of these cells interdigitate at a regular distance of about (25nm) wide which are called as the filtration slits that bridged by diaphragm of about (6nm) thick ^[1].

The aim of the present research is to study the effects of different oral doses of exogenous melatonin on glomerular filtration barrier by using methylene blue injections.

Materials & Methods:

Sixteen male Wistar albino rats of five weeks old (before puberty) were used, four rats in each group, their average body weight was (64) gram at the beginning of the experiment. The drug used in the experiments was; N-acetyl-5-methoxytryptamine (melatonin) tablets, from Nature's Bounty INC, Bohemia, NY11716, U.S.A. The animal house was of constant controllable temperature (ranging between 20-24°C). The light-dark cycle was (12:12) with free access to food, except for one and half-hour prior to meal containing melatonin supply. Water was provided *ad libitum*. The study included (3) groups of animals: control group which was divided into two subgroups (Ia) not received methylene blue injections, (Ib) received intraperitoneal injections of methylene blue, second

and third groups (II & III) treated with 250µg/kg and 500µg/kg body weight subsequently of melatonin hormone that was given as a single oral dose per day, two hours prior to sunset. Intraperitoneal injections of methylene blue were given in a dose of (1mg/kg) on alternate day (6 injections) for (14) days for control group (Ib) and treated animals. The animals were sacrificed by dissection under effect of diethyl ether, left kidney was removed when the heart was still beating, it was separated from the surrounding connective tissues, fat and suprarenal gland, and then bisected longitudinally through the pelvis of the kidney and used for paraffin sections. Transverse serial sections of kidneys of (3) µm in thickness were stained with H. & E. for control subgroups and treated groups.

Results:

Cellular filtration barrier components, which include podocytes, endothelial cells of the

glomerular capillaries and the mesangial cells, were seen under light microscope in transverse sections of kidneys of the control group of rats, stained with H. & E., podocytes appeared as spindle-shaped cells, had fine processes, their cytoplasm was thin with dark nucleus. However, endothelial cells appeared as squamous cells, and mesangial cells appeared as polygonal cells with large euchromatic nucleus between capillary loops. Histological examination of treated groups (with 250 and 500µg/kg body weight) showed that they were affected. The hormone induced significant structural changes in the kidney, in a dose dependent manner i.e. more histological changes appeared in those treated with 500µg/kg body weight represented by decreasing in the number of mesangial cells per field (under magnification power of x800), table (I), and dilated their capillaries, but the most obvious change was the detachment of podocytes from its glomerular basement membrane into urinary space, figure (1).

Table I: The effects of dietary exogenous melatonin on number of mesangial cells per glomeruli in five weeks old male rats.

Daily dose of melatonin (µg/kg) body weight	Number of mesangial cells per glomerulus
Group Ia & Ib	39.4 ± 2.12
Group II (250µg/kg)	28.75 ± 2.73 ***
Group III (500µg/kg)	15.067 ± 1.79 ***

Values were presented as mean ± SD.

Multiple groups comparisons were performed by analysis of variance (ANOVA).

Inter group's difference was assessed by the Student's t-test. Significance of difference in comparison with the control groups: *** (P<0.0001).

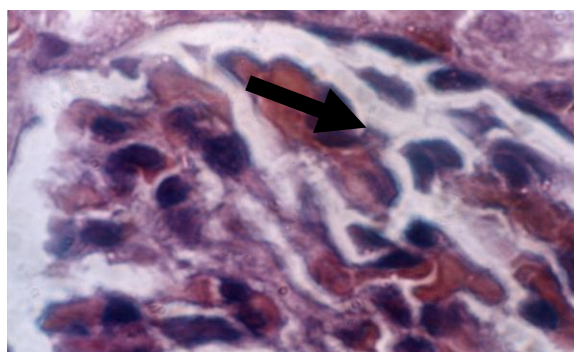


Figure 1: Transverse section of kidney's rat (five weeks old male rat treated with a dose of 500µg/kg) shows shed podocyte in urinary space (arrow). H. & E. stain, x1000.

In control group (Ib) there were no particles of methylene blue seen inside renal tissue and no histological difference from

control group (Ia), figure (2), while small dark particles of methylene blue were seen scattered through renal tissues of rats treated with

250 μ g/kg body weight, figure (3), which become larger and more in number in those

treated with 500 μ g/kg body weight, figure (4).

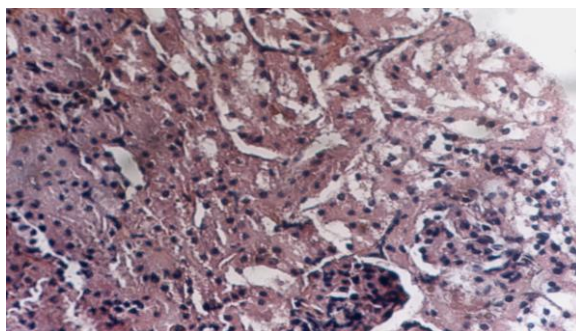


Figure 2: Transverse section of kidney's rat (five weeks old male rat, control) demonstrates no particles of methylene blue. H. & E. stain, x200.

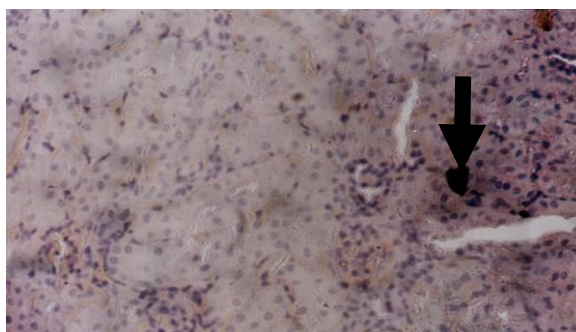


Figure 3: Transverse section of kidney's rat (five weeks old male rat treated with a dose of 250 μ g/kg) demonstrates small particle of methylene blue (arrow). H. & E. stain, x200.

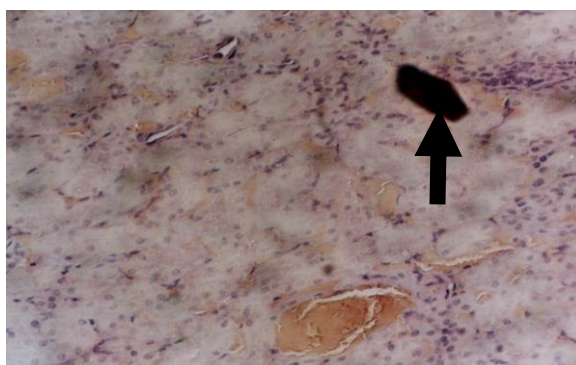


Figure 4: Transverse section of kidney's rat (five weeks old male rat treated with a dose of 500 μ g/kg) demonstrates large particle of methylene blue (arrow). H. & E. stain, x200.

Discussion:

In this experiment, methylene blue was used to assess the membrane integrity of the kidney^[9, 10&11]. Song et al, (1993)^[12] has identified iodomelatonin binding sites in the kidneys of mammals which supports the possibility of the direct action of melatonin on the renal system, especially on glomerular filtration barrier^[13]. The kidneys of rats treated with the dose of 500 μ g/kg

body weight were more affected than those treated with the dose of 250 μ g/kg body weight, in which melatonin effects are dose-dependent^[4&14], the effects is in the form of focal segmental glomerulosclerosis that one of its feature is detachment of podocytes^[15]. Glomerular filtration depends on well cell-cell and cell-matrix contacts of glomerular podocytes; the structural link between the cell-matrix and cell-cell contacts is

maintained by the actin cytoskeleton^[16]. Wong et al, (2000)^[17] has shown that podocytes are highly specialized cells that make up a major portion of the glomerular filtration barrier in the normal physiological and pathological kidney functions of human being. Recent evidence suggests that the important role of podocyte in glomerular filtration barrier comes through its main cell adhesion protein *nephrin*^[18, 19&20]. This protein is a transmembrane protein and it is an essential component of the glomerular filtration barrier in maintaining the normal function of the kidney^[21,22&23], the exact physiological role of the filtration barrier which consists of endothelial cells of glomerular capillaries, a basement membrane and a visceral epithelium (podocytes), has not yet been determined^[24,25&26]. However, the location of nephrin has been identified as the filtration slit-diaphragm of the glomerular podocyte which is considered as the site of renal ultrafiltration^[18, 19&23].

Damage to nephrin occurs in focal segmental glomerulosclerosis^[19] leading to increase permeability (damage) of the filtration barrier and appearance of methylene blue particles within renal tissue. Thus, it is preferable to give antioxidant agent in diseased conditions that cause increase in the production of free radicals, to counteract their damage effects on human body cells^[27]. It is not advisable to give antioxidant to healthy individuals, because they have normal oxidant-antioxidant balance and this supplement or additional antioxidant can produce oxidant-antioxidant imbalance.

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