PROTECTIVE ROLE OF MELATONIN IN L-NAME INDUCED HYPERTENSION IN MALE ALBINO RATS

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(Accepted for publication: April 17, 2016)

Summary:

The objective for the present study is to investigate the effects of melatonin (MEL) on systolic blood pressure (SBP), some biochemical parameters; serum (malondialdehyde (MDA), superoxide dismutase (SOD), reduced glutathione (GSH), nitric oxide (NO)) in NG-nitro-L-arginine methyl ester hydrochloride (L-NAME) treated rats. The male albino rats divided into five groups treated for 4 weeks: Group 1: Control rats. Group 2: L-NAME (35 mg/100 ml drinking water). Group 3: L-NAME (35 mg/100 ml drinking water) + melatonin (30 mg/Kg diet). Group 4: L-NAME (35 mg/100 ml drinking water) + melatonin (60 mg/Kg diet). Group 5: L-NAME (35 mg/100 ml drinking water) + melatonin (120 mg/Kg diet). A significant elevation in SBP and serum MDA were detected in L-NAME treated rats. Co-administration of melatonin with L-NAME prevented increasing in SBP and serum level of MDA in a dose dependent manner. On the other hand serum levels of SOD and GSH were decreased in response to L-NAME treatment, while, co-treatment with melatonin increased SOD and GSH in a dose dependent manner. The decrease serum NO level in response to L-NAME was significantly increased by melatonin but its level was decreased by increasing melatonin doses.

In conclusion: L-NAME induced hypertension model was associated with decreased NO level, interestingly; melatonin increased serum NO in L-NAME treatments, but with increasing dose of MEL, NO level was decreased. Furthermore; MEL through its antioxidant properties reduced oxidative stress and prevented lipid peroxidation.

Keywords: *melatonin, L-NAME, hypertension, NO, oxidative stress.*

INTRODUCTION

Hypertension is a most common cardiovascular disease and a major public health issue in developing countries; it can often lead to lethal complications if left untreated (Badyal *et al*., 2003). Nitric oxide is known to be synthesized in many cells and tissues from L-arginine by the action of NO synthase, which is non-specifically inhibited by L-arginine analogues such as L-NAME. Inhibition of NO synthesis in experimental animals results in sustained elevation of blood pressure (BP) (Kunes *et al*., 2004). Nitric oxide production by vascular endothelium is particularly important in the regulation of blood flow (Huk *et al*., 1997). Nitric oxide has multiple roles including regulation of vasomotor tone, inhibition of platelet and leukocyte adhesion to vascular endothelium and anti-proliferative effect (Andrew and Mayer, 1999). Therefore NO deficiency leads to increase accumulation of superoxide anion ($O^{\cdot}_2^{-}$) in biological tissue which causes oxidative stress in the body which in turn involved in pathophysiology of many forms of hypertension (Kopkan and Majid, 2005).

Melatonin plays a crucial role in several physiological functions such as sleep induction, vasoregulation, immunomodulation, control of sexual maturation, temperature regulation, aging (Pierpaoli and Regelson, 1994) and mood enhancement (Guyton and Hall, 2006). The suprachiasmatic nucleus (SCN) and possibly, the melatoninergic system can modulate cardiovascular rhythmicity; during the night, when melatonin is at its highest level, the heart rate decreases, the cardiac output is higher, the BP drops, the level of cholesterol declines (Chuang *et al*., 1993).

The potent antioxidant ability of melatonin can be explained by the potential to scavenge hydroxyl, superoxide, peroxynitite anion, singlet oxygen but also NO free radical (Paulis and Simko, 2007). Rodriguez *et al*., (2004) showed that melatonin effectively protects against lipid peroxidation and decrease the synthesis of MDA which is an end product of lipid peroxidation.

The objective for the present study was to investigate the effects of MEL on systolic blood pressure (SBP) and some biochemical parameters (MDA, SOD, GSH and NO) in L-NAME treated rats.

MATERIALS AND METHODS

Animals

Twenty five adult male albino rats (250-300 g) were used in the current study. Animals were housed in plastic cages bedded with wooden chips. This work was conducted in the Laboratory of Advanced Physiology at the Department of Biology/ College of Science/ University of Salahaddin-Erbil, Kurdistan Region-Iraq. Rats were bred in the animal house, and maintained in plastic cages. They were kept under standard laboratory conditions at 22 ± 2 ºC and exposed to a photoperiod of 12 hrs. light followed by 12 hrs. of darkness, using an automated light-switching devise. The rats were fed on standard rat pellets with free access to dechlorinated tap water *ad libitum*.

Experimental Design

This experiment was designed to study the effect of three doses (30, 60 and 120 mg/ Kg diet) of melatonin on SBP and some biochemical parameters (serum MDA, SOD, GSH and NO) in L-NAME (35 mg/100 ml drinking water) treated rats. Melatonin and L-NAME were given at the same time, and animals were assigned randomly to five different treatment groups and were continued for 4 weeks as the following:

Group I: Control

The rats were given a standard rat chow and tap water *ad libitum.*

Group II: L-NAME

The rats were given standard rat chow and L-NAME at dose (35 mg/100 ml drinking water).

Group III: L-NAME + melatonin (30 mg/Kg diet)

The rats were supplied with standard rat chow with melatonin (30 mg/kg diet) and L-NAME at dose (35 mg/100 ml drinking water).

Group IV: L-NAME + melatonin (60 mg/Kg diet)

The rats were supplied with standard rat chow with melatonin (60 mg/kg diet) and L-NAME at dose (35 mg/100 ml drinking water).

Group V: L-NAME + melatonin (120 mg/Kg diet)

The rats were supplied with standard rat chow with melatonin (120 mg/kg diet) and L-NAME at dose (35 mg/100 ml drinking water).

Collection of blood samples

At the end of experiment, the rats were anesthetized with ketamine hydrochloride (100 mg/kg). Blood samples were taken by cardiac puncture into test tubes and centrifuged at 3000 rpm for 15 minute; then serum samples were stored at -80 ºC (Sony, Ultra low, Japan) until use.

Measuring SBP, serum MDA, SOD, GSH and NO

Systolic blood pressure was measured weekly by the tail-cuff method in all groups using a PowerLab Data Acquisition System (ADInstruments, PowerLab 2/25) with computer running chart software. Serum MDA, SOD and GSH were determined spectrophotometrically using thiobarbituric acid (TBA) solution, modified biochemical Nitroblue tetrazolum (NBT) method and modified Ellman's reagent respectively. Serum total NO was determined by NO non–enzymatic assay kit (US Biological, USA).

Statistical analysis

All data were expressed as means $\pm$ standard error (SE) and statistical analysis was carried out using available statistical software (SPSS version 11.5). Data analysis was made using one-way analysis of variance (ANOVA). The comparisons between groups were done using Duncan post hoc analysis. P values <0.05 were considered significant.

Results

Long term blockade of NOS by administration of L-NAME (35 mg/100ml drinking water) for four weeks greatly increased SBP that reached a maximum level within the last two weeks as compared with control. Co-administration of L-NAME along with a low dose of melatonin (30 mg/kg diet) decreased SBP significantly versus L-NAME treated rats within the same period of treatment. Also a greater reduction in SBP was observed on each week throughout the four weeks of the study when the rats supplemented with intermediate (60 mg/kg diet) and high doses (120 mg/kg diet) of melatonin (Table 1).

Table (1): Effect of melatonin on SBP in L-NAME treated rats

Parameters / Treatments	Week 1 **	Week 2 **	Week 3 *	Week 4 **
Control	107.4 ^{a} ± 0.871 ^{ab}	106.6 ^{a} ± 1.4 ^{a}	109.8 ^{a} ± 1.019 $_{ab}$	110 ^{a} ± 0.632 ^{b}
L-NAME **	128.2 ^{d} ± 0.734 ^{a}	131.4 ^{d} ± 0.6 ^{a}	168 ^{d} ± 4.658 ^{b}	165.8 ^{d} ± 1.356 ^{b}
L-NAME + MEL (30mg/kg diet) **	118.2 ^{b} ± 1.019 ^{a}	133.4 ^{d} ± 1.208 ^{c}	127.4 ^{c} ± 1.166 ^{b}	147.2 ^{c} ± 1.593 ^{d}
L-NAME + MEL (60mg/kg diet) *	121.8 ^{c} ± 0.86 ^{a}	121.8 ^{c} ± 0.734 ^{a}	125.4 ^{bc} ± 0.748 ^{b}	126.8 ^{b} ± 1.624 ^{b}
L-NAME + MEL (120mg/kg diet) **	116.4 ^{b} ± 0.509 ^{a}	115.4 ^{b} ± 2.249 ^{a}	119.8 ^{b} ± 1.319 ^{ab}	123.4 ^{b} ± 1.363 ^{b}

Data presented as mean ± S.E
The same letters mean no statistical differences
The different letters mean statistical differences
*=P<0.05 **=P<0.01

Serum total NO decreased greatly in L-NAME treated rats compared with control. Serum total NO increased significantly (P<0.01) in animals provided with diet supplemented with melatonin when compared with L-NAME group. Total NO level was significantly (P<0.01) higher in rats treated with low melatonin dose than intermediate and high melatonin doses, also in intermediate melatonin dose the serum total NO level was higher than high melatonin dose group (Table 2).

Superoxide dismutase activity was significantly (P<0.01) decreased by L-NAME treatment versus control. While melatonin significantly (P<0.01) enhanced SOD activity in a dose dependent manner as compared with L-NAME treated animals (Table 2). In L-NAME group serum GSH level significantly (P<0.05) decreased versus control animal. Melatonin significantly (P<0.05) increased GSH level in a dose dependent manner when compared with L-NAME treated animals (Table 2).

The L-NAME hypertensive rats showed a significant (P<0.01) increase in serum MDA level as compared with control. This variable was significantly reduced in three dietary melatonin treated groups when compared with L-NAME group. Rats treated with high dose of melatonin significantly lower MDA level than intermediate dose and low dose (Table 2).

Table (2): Effects of melatonin on serum NO, SOD, GSH and MDA in L-NAME treated rats

Parameters / Treatments	Serum NO (□mol/L) **	Serum SOD (U./mg protein) **	SerumGSH (□mol/ml) *	Serum MDA (□mol/L) **
Control	17.6 ± 0.039 ^{e}	0.013 ± 0.0007 ^{b}	119.88 ± 0.225 ^{c}	2.896 ± 0.22 ^{a}
L-NAME	9.483 ± 0.035 ^{a}	0.0048 ± 0.0003 ^{a}	112.38 ± 0.335 ^{a}	5.862 ± 0.366 ^{c}
L-NAME + MEL (30mg/kg diet)	14.67 ± 0.044 ^{d}	0.0068 ± 0.0015 ^{a}	117.83 ± 0.386 ^{b}	4.642 ± 0.22 ^{b}
L-NAME + MEL (60mg/kg diet)	14.05 ± 0.036 ^{c}	0.0156 ± 0.0004 ^{b}	118.88 ± 0.232 ^{bc}	4.442 ± 0.121 ^{b}
L-NAME + MEL (120mg/kg diet)	13.13 ± 0.04 ^{b}	0.0226 ± 0.001 ^{c}	122.71 ± 1.32 ^{d}	3.348 ± 0.188 ^{a}

Data presented as mean ± S.E
The same letters mean no statistical differences
The different letters mean statistical differences
*=P<0.05 **=P<0.01

Discussion

The obtained results of the present study show that L-NAME caused a significant elevation in SBP during the first three weeks of treatment and during the fourth week SBP remained more or less (Table 1). It has been found that long-term inhibition of NO synthase may due to increase in plasma epinephrine and norepinephrine, which are in turn increase BP (Zanchi *et al*., 1995). Kurtz and Wagner, (1998) suggested that renin-angiotensin system is activated during long-term NO blockade. Consequently, plasma renin activity (PRA) are frequently found to be elevated if the treatment with NOS inhibitors is extended over several weeks and this elevation of PRA is associated with severe hypertension.

The data of the present study revealed that different doses of melatonin administration caused a significant decrease in SBP in a dose dependent manner in L-NAME treated rats. Ding *et al*., (2001) documented that melatonin acts as a hypotensive factor and its effects are mainly due to activation of MEL1 receptor in rat brain and also they observed that the anterior hypothalamic area may be one of the important central areas where melatonin can exert modulatory effects on BP. It had been concluded that the hypotensive effect of melatonin in rats may be mediated by its anti-oxidative effect rather than its receptor (Wu and De Champlain, 1998). As reported previously, melatonin decreases plasma renin and serum nor-epinephrine concentrations (K-Laflamme *et al*., 1998). The improved NO production and decrease oxidative load after melatonin administration may lead to BP reduction (Girouard *et al*., 2001).

In this study, it has been demonstrated that serum levels of SOD, GSH and NO were significantly decreased, whereas MDA level was increased significantly in L-NAME treated rats. Landmesser *et al*., (2003) linked the impairment endothelium mediated vasodilatation in hypertension to decrease NO bio-availability; this may be secondary to decrease NO synthesis or to increase NO degradation because of its interaction with O^{-}_{2} to form peroxynitrite ($ONOO^{-}$). Furthermore, Uzun *et al*., (2005) found that NO levels were negatively correlated with SOD, this result is consistent with those of the present study.

The data of the present study showed that L-NAME increased MDA level which is in agreement with (Deniz *et al*., 2006) observing that NOS inhibition induced hypertension increases MDA level. The mechanisms through which melatonin reduces oxidative stress involve scavenger of hydroxyl radical and peroxynitrite, the latter has direct toxic effects leading to lipid peroxidation, protein oxidation, DNA damage

and can inhibit SOD (Pacher *et al*., 2007). Experimental evidences have shown that not only does melatonin not consume cellular GSH, but also preserves or even increases the content of GSH in tissues (Tan *et al*., 2002), by promoting the activity of glutathione reductase (GSH-Rd) (Hara *et al*., 2001).

Melatonin was increased NO synthase activity and decreased reactive oxygen species (ROS) production (Pechanova *et al*., 2004). It has been reported that melatonin increases NO synthase activity without up-regulation of endothelial NOS (eNOS) or inducible NOS (iNOS) protein expression (Pechanova *et al*., 2006). It seems that both increased NO synthase activity and ROS reduction is responsible for preventive effects of melatonin on the development of hypertension (Kojsova *et al.*, 2006).

In conclusion: L-NAME induced hypertension was associated with decreased NO level, interestingly; melatonin increased serum NO in L-NAME treatments, but with increasing dose of MEL, NO level was decreased. Furthermore; MEL through its antioxidant properties reduced oxidative stress and prevented lipid peroxidation.

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پوختە:

لەم توێژینەوەیەدا بیست و پێنج (25) جورجی نێرەی سپی بەکارهێنران، بۆ دۆزینەوەی کاریگەری میلاتۆنین بە سێ بڕی جیاواز (30، 60 و 120 ملگم / کلگم خۆراك) لەسەر پەستانی خوێن و هەندێك پارامیتەری کیمیایی ژیانی لە جورجی نێرەی سپی کە مامەلەکرابوون بە ماددەی L-NAME. بەرزی پەستانی خوێن دروستکرا بەپێدانی L-NAME بە جورجەکان بەبڕی (35 ملگم / 100 مل ئاوی خواردنەوە) بۆ ماوەی چوار هەفتە. پەستانی خوێن هەفتانە دەپێورا بەبەکارهێنانی پێوەریی پەستانی خوێن (Tail-cuff plethysmography).

پێدانی L-NAME بووە هۆی بەرزبوونەوەیەکی بەرچاوی پەستانی خوێن بەشێوەیەك کە بە تێپەربوونی کات زیاتر بەرز دەبووەوە.هەروەها ئاستی MDA لە زەردەوی خوێن بەشێوەیەکی بەرچاو بەرزبووەوە لەو جورجانەی کە مامەلەکرابوون بە L-NAME . پێدانی میلاتۆنین بە جورجەکان لەرێگەی خۆراکەوە لەگەل L-NAME بووە هۆی بەرگری کردن لە بەرزبوونەوەی پەستانی خوێن و MDA لە زەردەوی خوێن بەپشت بەستن بەو برەی کە بەکارهێنرابوو. لەلایەکی ترەوە ئاستی SOD و GSH لە زەردەوی خوێن بەشێوەیەکی بەرچاو نزمبوونەوە لە جورجانەی کە مامەلەکرابوون بە L-NAME، لەکاتێکدا مامەلەکردنی جورجەکان بە L-NAME و میلاتۆنین بووە هۆی بەرزبوونەوەیەکی بەرچاوی SOD و GSH لە زەردەوی خوێن بەپشت بەستن بەو بڕەی کە بەکارهێنرابوو. ئەو نزمبونەوە یەی کە لەئاستی نایتریك ئۆکساید (NO) رووبدا لە زەردەوی خوێن بەهۆی L-NAMEبە شێوەیەکی بەرچاو بەرزبۆوە بەهۆی میلاتۆنین، بەلام ئاستی NO نزم بووەوە بەزیادکردنی بری میلاتۆنین لە خۆراك.

الخلاصة

اشتملت الدراسة الحالية على 25 من ذكور الجرذ البيض، لدراسة تاثيرات جرعات مختلفة (30، 60و 120ملغم/كلغم الغذاء) من مادة الميلاتونين على ضغط الدم الانقباضي وبعض المتغيرات البيوكيميائية في الجرذان المعاملة بمادة L-NAME. استحدثت فرط ضغط الدم بواسطة معاملة الجرذان بمادة L-NAME بجرعة (35 ملغم/100 مل) في ماء الشرب لمدة اربعة اسابيع. تم قياس ضغط الدم الانقباضي اسبوعيا عن طريق جهاز قياس الضغط (Tail-cuff plethysmography).

اضهرت النتائج ان مادة L-NAME ادت الى ارتفاع معنوي في الضغط الدم الانقباضي اعتمادا على الوقت وكذالك ارتفعت مستوى مالون داي الديهايد (MDA) بصورة معنوية في الجرذان المعاملة ب L-NAME. ادت اعطاء ميلاتونين مع L-NAME الى منع رفع الضغط الدم ومستوى MDA في مصل الدم وحسب الجرع. ادت المعاملة بمادة L-NAME الى خفض مستويات كل من سوبراوكساید دسميوتيس (SOD) وكلوتاثايون المختزلة (GSH) في مصل الدم بينما ادت المعاملة مع الميلاتونين الى رفع مستويات SOD وGSH حسب الجرع. في حين ادت المعاملة الجرذان ب L-NAME الى انخفاض مستوى النايتريك اوكساید الكلي (NO) معنويا في مصل الدم، بينما ادت المعاملة بالميلاتونين الى رفع مستوى NO الكلي في مصل الدم في الجرذان المعاملة ب L-NAME ولكن هذا المستوى انخفضت مع ازدياد جرعات الميلاتونين المستخدمة.