Antioxidant activity of melatonin in liver of male rabbits
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Abstract

Melatonin, [N-acetyl-5-methoxytryptamine] (ME), is an endocrine product of pineal gland. The present work was conducted to investigate the hepatoprotective effect of exogenous (ME). Thirty six healthy male rabbits weighting 1500-1700g were divided into six groups with 6 animals in each group. Animals in the first group served as control, animals in the second, third, fourth, fifth, and sixth groups were intraperitoneally (i.p) injected with D-Galactosamine (GalN) in a single daily dose of 50mg/kg for the period of 20 days for the induction of hepatocellular injury. Animals in the third and fourth groups in addition to GalN were orally treated with ME in a single daily dose of 300µg/kg, as follows: animals in the third group received ME at 9am; and those in the fourth group received ME at 9pm, for the period of 20 days. Animals in the fifth and sixth groups, in addition to GalN, were orally treated with ME in a single daily dose of 600µg/kg, as follows: animals in the fifth group received ME at 9am, and animals in sixth group received ME at 9pm for the period of 20 days. The level of Albumin, Total Protein, Alanintransferase (ALT), Asparatatetransferase (AST), and Alkalin-phosphatase (ALP) in serum was estimated. Results showed that ME significantly (P<0.01) reduced the toxicity of GalN, and that ME is more effective when given at evening times.

Key words: Melatonin, Antioxidant activity, Liver.

Introduction

Melatonin, [N-acetyl-5-methoxytryptamine] (ME), is a secretory product of the vertebrate pineal gland [30]. Although melatonin was discovered to be a free radical scavenger just over a decade ago [32], the data documenting its ability to overcome oxidative stress has accumulated at a rapid pace and it is now abundant[24,25,1,15]. The efficacy of melatonin functioning in this capacity is related to its direct free radical scavenging actions [1,27], its ability to enhance the activities of a variety of antioxidative enzymes [3,29,36], its stimulatory actions on the synthesis of another important intracellular antioxidant, glutathione [37], its efficacy in reducing electron leakage from the mitochondrial electron transport chain [20], and its synergistic interactions with other antioxidants [21]. Moreover, in recent years, it has become apparent that, when melatonin scavenges radicals and related reactants, the products are generated are also free radical scavengers thereby greatly exaggerating the antioxidant potential of melatonin [15]. Melatonin is a potent free radical scavenger, more than vitamin E, which is the reference in the field [30]. Melatonin directly scavenges the highly toxic hydroxyl radical and other oxygen centered radicals and displays antioxidative properties: it increases the levels of several antioxidative enzymes, including superoxide dismutase, glutathione peroxidase and glutathione reductase [36,37]. On the other hand; melatonin inhibits the pro-oxidative enzyme nitric synthase [13,11]. Since considerable experimental evidence supports the idea that oxidative stress is a significant component of specific heart, blood vessels and CNS diseases, the ability of melatonin to protect against cardio- and neurodegeneration was tested in a multitude of models [22,8]. At the present time, there is experimental evidence indicating that the quantity of melatonin endogenously produced is relevant as a physiological antioxidant in normal conditions; whereas, to experimentally evaluation the antioxidant activity of exogenous melatonin in mammals, the quantity of it should not exceed
Antioxidant activity of melatonin ………Shikoo Ebtisam Yassin and Alsakaf Galal Mohammed

1mg/kg to avoid possible side effects [22,14]. The administration of exogenous melatonin, should take into consideration that, antioxidant defense system displays a daily rhythm which is abolished by pinealectomy, or by light in mammals, including man [10]. Few controlled trials studies showed that, in chronic hemodialysis patients, the oxidative stress induced by iron and erythropoietin and given for treatment of anemia was prevented by oral administration of melatonin 0.3 mg/kg [16]. Preliminary results, in septic newborns showed that high melatonin doses (20 mg per subject) significantly reduces serum levels of lipid peroxidation products and inflammation markers, increased the survival rate and improves the clinical outcome of patients [13]. Similarly, increased blood levels of malondialdehyde and nitrite/nitrate observed in asphyxiated newborns were reduced by melatonin treatment (a total dose of 80 mg per infant). Asphyxiated newborns not given melatonin died within 72hrs. after birth, in percent three of the 10, whereas none of the 10 who received melatonin died [12].

D-Galactosamine (GalN), an amino sugar, was found to cause liver damage, increase oxidative stress of LPO products [2].

The goal of this study is to investigate the hepatoprotective and antioxidant role of exogenous ME; against GalN induced liver injury in male rabbits.

Materials and methods

Chemicals
D-Galactoseamine, white crystal powder, and Melatonin, white crystal powder, were manufactured by Sigma, St.Louis, MO, USA, obtained from Faculty of Sciences, Cape town University, South Africa.

Experimental animals
A total number of 36 healthy male rabbits local breed weighed 1500-1700g.were used in the present study. Animals were randomly assigned to 6 groups as follows:

Group1: (n = 6) control animals, they received 10 ml. Normal Saline once a day period of 20 days.

Group2: (n = 6) animals in this group were intraperitoneally (i.p.) injected with D-Galactosamine (GalN), in dose 50mg/kg./day, dissolved in Nacl for a period of 20 days, for the induction of hepatocellular injury.

Group3: (n = 6) animals in this group were intraperitoneally (i.p.) injected with D- Galactosamine (GalN), in dose 50mg/kg./day, orally treated with ME in dose 300µg/kg./day, dissolved in distilled water at 9am, for 20 days.

Group4: (n = 6) animals in this group were intraperitoneally (i.p.) injected with D- Galactosamine (GalN), in dose 50mg/kg./day, orally treated with ME in dose 300µg/kg./day, dissolved in distilled water at 9pm, for 20 days.

Group5: (n = 6) animals in this group were intraperitoneally (i.p.) injected with D- Galactosamine (GalN), in dose 50mg/kg./day dissolved in Nacl and orally treated with ME in dose 600µg/kg./day dissolved in distilled water at 9am, for 20 days.

Group6: (n = 6) animals in this group were intraperitoneally (i.p.) injected with D- Galactosamine (GalN), in dose 50mg/kg./day dissolved in Nacl and orally treated with ME in dose 600µg/kg./day dissolved in distilled water at 9pm, for 20 days.

All animals were maintained in standard environmental conditions; they were housed in a glass house under normal light and dark cycle of day, and kept a standard commercial diet with water ad libitum.

The experiment was administrated in the Animal Physiology Laboratory, Department of Biology, Faculty of Science and Education, Aden University.

After 20 days, the animals were fast over night for12hrs. Then they were sacrificed, the blood was immediately collected and centrifuged, and serum was discarded and kept at - 21 ° C for the biochemical testes.
Antioxidant activity of melatonin .......... Shikoo Ebtisam Yassin and Alsakaf Galal Mohammed

Analysis

Alanine-aminotransferase (ALT) and Aspartate-aminotransferase (AST) Assay:
The estimation was carried out according to the method originally developed by Reitman and Frankel [28].

Alkaline phosphatase Assay:
ALP was determined using a colorimetric method as described by Kind and King [19].

Total Protein Assay:
The total protein was determined by Biuret method explained by Tietz [35].

Albumin Assay:
Serum albumin was determined according to the method of Doumas et al., [9].

Statistical analysis:
The statistical analysis was performed by SPSS; continuous data were expressed as mean ± S.E. Data were compared using one – way ANOVA. P value <0.01 was considered to be statistically significant.

Results

Table 1. Level of studied parameters, after 20 days of GalN and ME administration at morning and evening times in dose 50mg/kg and 300µg/kg respectively

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Treatments</th>
<th>GalN+ Control</th>
<th>GalN+ ME at 9 am</th>
<th>GalN+ ME at 9 pm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Albumin g/dl</td>
<td>3.7±0.23</td>
<td>1.2±0.04</td>
<td>1.9±0.09</td>
<td>2.4±0.11</td>
</tr>
<tr>
<td>T. Protein g/dl</td>
<td>7.1±1.01</td>
<td>2.1±0.88</td>
<td>3.1±0.96</td>
<td>4.3±0.98</td>
</tr>
<tr>
<td>ALT U/L</td>
<td>41±4.93</td>
<td>111±8.76</td>
<td>97±6.77</td>
<td>81±8.44</td>
</tr>
<tr>
<td>AST U/L</td>
<td>36±3.44</td>
<td>101±9.73</td>
<td>92±8.65</td>
<td>82±6.44</td>
</tr>
<tr>
<td>ALP U/L</td>
<td>61±5.32</td>
<td>173±11.01</td>
<td>149±9.79</td>
<td>122±9.91</td>
</tr>
</tbody>
</table>

Values are mean of 6 animals ± S.E. P<0.01 vs. control.

Results in Table 1 showed that the i.p. administration of GalN in dose 50mg/kg for period of 20 days (group 2) resulted in high significant P<0.01 decrease in the level of albumin and total protein, the mean percent decrease in albumin and total protein was 67%±0.87 and 70%±2.12 respectively, as compared to control. GalN i.p. administration resulted also in high significant P<0.01 increase in the level of ALT, AST and ALP, in the mean percent 170%±7.75, 180%±6.09 and 183%±8.34 respectively, as compared to control. The mean percent decrease in the level of albumin and total protein in the serum of animals, treated with ME in dose 300µg/kg at 9am (group 3) beside GalN, was 48%±0.65 and 56%±1.88 respectively, as compared to control, while the mean percent increase in the level of ALT, AST and ALP was 136%±6.06, 155%±5.87 and 144%±6.73 respectively, as compared to control. The mean percent decrease in the level of albumin and total protein in the serum of animals, treated with ME in dose 300µg/kg at 9pm (group 4) beside GalN, was 35%±0.85 and 39%±1.08 respectively, as compared to control, while the mean percent increase in the level of ALT, AST and ALP was 97%±4.22, 127%±6.05 and 100%±6.87 respectively, as compared to control.

Table 2. Level of studied parameters, after 20 days of GalN, and ME administration at morning and evening times in dose 50mg/kg and 600µg/kg respectively

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Treatments</th>
<th>GalN+ Control</th>
<th>GalN+ ME at 9 am</th>
<th>GalN+ ME at 9 pm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Albumin g/dl</td>
<td>3.7±0.23</td>
<td>1.2±0.04</td>
<td>2.9±0.88</td>
<td>3.3±0.91</td>
</tr>
<tr>
<td>T. Protein g/dl</td>
<td>7.1±1.01</td>
<td>2.1±0.88</td>
<td>5.1±1.16</td>
<td>6.1±0.13</td>
</tr>
</tbody>
</table>

Antioxidant activity of melatonin

Shikoo Ebtisam Yassin and Alsakaf Galal Mohammed

ALT U/L                        41±4.93           111±8.76         78±6.56                 53±6.45
AST U/L                        36±3.44           101±9.73         70±6.56     49±7.12
ALP U/L                        61±5.32           173±11.01     112±5.39                 70±6.98

Values are mean of 6 animals ±S.E. P<0.01 vs. control.

The obtained results in Table2 showed that the mean percent decrease in the level of albumin and total protein in the serum of animals, treated with ME in dose 600µg/kg at 9am (group5) beside GalN, was 21±0.17 and 28±1.54 respectively, as compared to control, while the mean percent increase in the level of ALT, AST and ALP was 92±6.34, 97±6.43 and 83±5.39 respectively, as compared to control. The mean percent decrease in the level of albumin and total protein in the serum of animals, treated with ME in dose 600µg/kg at 9pm (group6) beside GalN, was 10±0.66 and 14±1.98 respectively, as compared to control; while the mean percent increase in the level of ALT, AST and ALP was 29±6.18, 36±6.55 and 14±9.95 respectively as compared to control.

Discussion

Our results clearly showed the hepatotoxicity of GalN. In agreement with previous study [2], the i.p. injection of GalN to rabbits in group 2 in dose 50mg/kg for 20 days resulted in high significant p<0.01 increase in the level of ALT, AST, and ALP, and high significant p<0.01 decrease in the level of albumin and total protein. The noticed increase in the levels of aminotransferases (ALT and AST) and the level of ALP, as well as the decrease in the in the levels of total protein and albumin in the serum, are the major diagnostic symptoms of liver diseases [6]. Exposure to GalN leads to increase the oxidative stress and excessive of free radicals production, which attack many organic molecules in cells membrane, including polysaturated fatty, acid leading to increase in LPO and damage of cells and their function [2]. Several studies reported that reactive oxygen species (ROS) initiate LPO through the action of hydroxyl radicals [17,18,15].

Our results showed that the toxicity of GalN was reduced in the animals treated with ME animals (groups 3, 4, 5 and 6), the protective effect of ME in our experiment was the time of administration dependent effect. The high significant protective effect of ME was clear in the animals that received it at evening time (9pm), as compared to the animals that received it at morning time (9am). The protective effect of ME is related to its antioxidant activity. Melatonin was shown to be effective in neutralizing a number of oxygen-based and nitrogen-based toxic agents, some of which are free radicals and some of which are related metabolites [26,1]. ME was originally shown to detoxify the highly toxic hydroxyl radical (· OH) [33]. Since this discovery, its scavenging repertoire has been expanded to include hydrogen peroxide (H2O2) [31], hypochlorous acid (HOCl) [39], single oxygen (¹O2) [23], superoxide anion radical (O2·−), nitric oxide (NO·) [38,4], peroxynitrite anion (ONOO−) (Reiter et al., 2001a) and other free radicals [15].

The high significant efficacy of ME at the evening time is due to the well known fact that light/dark cycle is the main regulating system of ME secretion, function and receptors in the body [7]. Light suppresses ME secretion and ME receptors activity, and vice versa, dark stimulates ME secretion and it receptors [5,34,10].

References

Antioxidant activity of melatonin

Antioxidant activity of melatonin

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المختصر

الميلاتونين، هرمون تنتجه الغدة الصنوبرية، وقد أجري هذا العمل البحثي لمعرفة الأثر الوقائي للميلاتونين على الكبد. لهذا الغرض استخدم ستة وثلاثون من ذكور الأرانب تراوح وزن أوزانهم بين 500-1700 جرام. قسمت الحيوانات إلى ستة مجموعات ضمت كل مجموعة سته حيوانات. عولمت حيوانات المجموعة الأولى مجموعة ضرورية وأعطت وجبة 10 مل من محلل ملحي يومياً لمدة عشرة أيام. الحيوانات في المجموعات الثانية والثالثة والرابعة والخامسة والسادسة حققت بداخل الغشاء الريتوني (i.p) بمركب D-Galactoseamine (GalN) جرعة واحدة في اليوم مقدارها 50 مجم/كجم ووحدة عشرة يوماً، وذلك لتفعيل اضرارها في هضماً (GalN) الكبد. الحيوانات في المجموعات الثالثة والرابعة بالإضافة إلى حقنها بمركب (GalN) أعطت الميلاتونين عن طريق الفم بجرعة يومية مقدارها 300 ميكروجرام/كجم ووحدة عشرة يوماً على النحو التالي: حيوانات المجموعة الثالثة أُعطيت الميلاتونين الساعة التاسعة صباحاً، وحيوانات المجموعة الرابعة أُعطيت الميلاتونين (GalN) الساعات التاسعة مساءً. الحيوانات في المجموعات الخمسة والسادسة بالإضافة إلى حقنها بمركب (GalN) أُعطيت الميلاتونين عن طريق الفم بجرعة يومية مقدارها 600 ميكروجرام/كجم ووحدة عشرة يوماً على النحو التالي: حيوانات المجموعة الخامسة أُعطيت الميلاتونين الساعة التاسعة صباحاً، وحيوانات المجموعة السادسة أُعطيت الميلاتونين الساعات التاسعة مساءً. بعد عشرة يوماً تم فحص المؤشرات الأentionsفي مصل جميع حيوانات التجربة: الألبرونين، البروتين الكلوي، الأنزيمات الناقلة للأمين ALT وAST. أشارت النتائج إلى أن الميلاتونين يُضعف من التأثير السمي لمركب (GalN). أشارت النتائج إلى أن الميلاتونين يكون أكثر فعالية عندما يعطى في المساء.

الكلمات المفتاحية: ميلاتونين، الكبد، مضاد للأكسدة.