ORIGINAL ARTICLES

Assessment of the damaging effect of single and fractionated doses of ultraviolet-B radiation on the rabbit's lens: protective role of melatonin

Amira A.M. 1, Saber S.G. 2, Abdelkawi S.A. 3, Nahed S.H. 4 and Mona S.T. 5

1 Elsafwa Higher Institute of Engineering, Cairo, Egypt.
2 Radiometry Department, National Institute for Standards (NIS), Cairo, Egypt.
3 Vision Sciences Department, Biophysics and Laser Sciences Unit, Research Institute of Ophthalmology, Giza, Egypt.
4 Biochemistry Department, Biophysics lab, National Research Centre, Cairo, Egypt.
5 Physics Department, Science faculty, Ain Shams University, Cairo, Egypt.

ABSTRACT

The aim of the present study was to investigate the damaging effect of single and fractionated dose of ultraviolet B (UVB) radiation on the eye lens and the effect of melatonin against oxidative stress in UVB radiation induced cataract development. 25 New Zealand male rabbits were used and divided into five groups (five rabbits each). One group was considered as control (n=10 eyes) the second group were received a single dose of 3.12 J/cm² UVB and the animals were left for one week. The 3rd group was daily injected interpritoneal with 4mg/Kg melatonin one week before and after exposure to a single dose of 3.12 J/cm² UVB .In the 4th group the rabbits were received three fractionated doses of 1.04 J/cm² UVB for one week (day 1, 3 and 5) and the rabbits were left for one week. Finally, in the 5th group the rabbits were injected with melatonin one week before and after each exposure to three fractionated doses of 1.04 J/cm² UVB. The melatonin uptake will continue for one week until the decapitation. After the demonstrated periods, rabbits were decapitated. The lenses were isolated for determination of total protein concentration, total antioxidant capacity (TAC), total oxidative capacity (TOC), malondialdhyde (MDA), oxidative stress index (OSI) and calcium concentration. The results indicate that rabbits exposed to UVB radiation without melatonin show very high significant decreased in protein content (P<0.001) and TAC. Moreover, there were very high significant increases in TOC, MDA and calcium concentration. Treatment of rabbits with melatonin reduces the degree of lipid peroxidation and elevates the antioxidant capacity. In addition, the data indicate that fractionated dose groups without melatonin had strongly affected than single dose groups without melatonin. The data conclude that melatonin may protect the eye lens from the damaging effects of UVB exposure, and its actions protect lens from oxidative stress, and prevent the elevation of Ca²⁺ level, which are considered as important causes of cataractogenesis.

Key words: ultraviolet B radiation; Melatonin; rabbit; lens; oxidative stress; cataract.

Introduction

Ultraviolet (UV) radiation is the most frequent cause of radiation injury to the eye, which is affected by oxidative stress due to its physical and metabolic characteristics. Particularly, the lens is most affected by oxidative damage caused by UVB radiation, because it is an avascular structure and has a constant and spare production of lenticular proteins (Cabrera and Chihualef, 2011). The ultraviolet radiation from sunlight and other ambient sources is considered a major causative factor for the onset of lens opacification and cataract processes (Kohli et al., 1996). Taylor et al. (1988) reported that, exposure to UVB is associated with cataract formation. Many investigators supported that UV-induced cataractogenesis through the elevation of free radicals and induction of photooxidation in the lens (Vrensen, 1994 and Bardak et al., 2000). The effect of UV radiation may lead to cell death that may be responsible for the observed decrease in the cell density in all three zones of the lens epithelium (Johar et al., 2003).

Melatonin (N-acetyl-5- methoxytryptamine) is an indoleamine originally found in the pineal gland (Axelrod, 1974) and other organs including the lens (Abe et al., 1999). Melatonin was identified as a powerful direct free radical scavenger (Tan et al., 2002 and Allegra et al., 2003) and indirect antioxidant (Reiter et al., 2000c and Rodriguez et al., 2004). Melatonin may protect against the UVB-induced cataract development by directly quenching lipid peroxides and indirectly by enhancing the production of the endogenous antioxidant glutathione (GSH) (Bardak et al., 2000). Anwar and Moustafa (2001) concluded that melatonin may protect the
eye lens from the damaging effects of UV exposure, and its actions protect lens from oxidative stress, elevating Ca^{2+} levels, which are considered as an important causes of cataractogenesis. The aim of the present work was to study the damaging effect of single and fractionated dose of UVB on the eye lens and investigate the effect of melatonin against oxidative stresses in UVB radiation induced cataract development.

**Materials and Methods**

**Chemicals:**

All reagents were of the highest purity available. Melatonin, chemicals for measurement of antioxidant, oxidant, lipid peroxides and calcium concentration were purchased from Biodiagnostic Chemical Co, Cairo, Egypt.

**Exposure to ultraviolet radiation:**

UVB lamp was calibrated at the Radiometry Department, National Institute of Standard, Giza, Egypt. The wavelength produced from the anterior surface of the lamp was ranging from 320 to 280 nm. The single dose of ultraviolet radiation that reached the rabbit's eye after 100 minutes at a distance 18 cm was 3.12 J/cm^2 and the fractionated dose of ultraviolet radiation was 1.04 J/cm^2 after 33.5 minutes.

**Animals and methods:**

Twenty-five New Zealand male rabbits weighing 1.5-2 kg were selected from the animal house of Research Institute of Ophthalmology, Giza, Egypt. The rabbits were maintained in a standard 12 hr light-dark cycle with free access to water and balanced diet. All procedures were conducted according to the principles enunciated in the Guide for Care and Use of Laboratory Animals, NIH publication No. 85-23. They were subjected to experimental protocols approved by the local experimental ethics committee of ophthalmic and vision research. Rabbits were divided into five groups of 5 rabbits each. The first group was kept as control and twenty rabbits (n=40 eyes) were generally anesthetized using intramuscular Xylaject (0.2 ml/kg) and Benoxinate eye drops (0.4%) was used for local anesthesia before UVB exposure. The second group was received a single dose of 3.12 J/cm^2 UVB for 100 min and the rabbits were left for one week. The third group was injected intraperitoneal with 4mg/Kg melatonin daily for one week before exposure then they received a single dose of 3.12 J/cm^2 UVB for 100 min. Following the exposure the rabbits were injected intraperitoneal with 4 mg/Kg melatonin daily for one week. The fourth group was received three fractionated doses of 1.04 J/cm^2 UVB radiation for 33.5 min (day 1, 3 and 5) and the rabbits were left for one week. The fifth group was injected intraperitoneal with 4mg/Kg melatonin daily for one week before exposure then they received three fractionated doses of 1.04J/cm^2 UVB radiation for 33.5 min (day 1, 3 and 5) followed by injection intraperitoneal with 4 mg/Kg melatonin after each exposure. The melatonin uptake will continue for one week until the decapitation. After the demonstrated periods, rabbits were decapitated then the eyes were enucleated.

Lenses were accurately isolated, weighed and homogenized using cell homogenizer (type Tübingen 7400, Germany), in a 10-fold volume of 20 mM ice-cold tris-HCl buffer, pH 7.4. The homogenate was centrifuged for 20 minutes at 10,000 rpm in a bench centrifuge (Awel centrifuge MS 20, France). The resultant supernatant was used for total protein concentration, total antioxidant capacity, total oxidant capacity, oxidative stress index, malondialdehyde and calcium concentration.

**Total protein concentration:**

Lens protein concentration was determined according to the method of (Lowry et al., 1951). The developing color was measured with a spectrophotometer (type UV-visible recording 240 Graphical, Shimatzu, Japan) at 750 nm.

**Measurement of the Total Antioxidative Capacity (TAC):**

The supernatants were subjected to biochemical analysis of the total antioxidative capacity using a colorimetric method performed by the reaction of antioxidants in the sample with a defined amount of exogenously provided hydrogen peroxide (H_2O_2). The TAC was determined at 505nm by an enzymatic reaction and the results are expressed in terms of mM/g tissue (Koracevic, 2001).
Measurement of the Total Oxidant Capacity (TOC):

The TOC levels of the lens were determined using a colorimetric method (Aebi, 1984). Therefore, the color intensity, measured spectrophotometrically at 510 nm, is related to the total number of oxidant molecules present in the sample. The results are expressed in terms of mM/g tissue.

Measurement of Malondialdehyde (MDA):

Quantitative determination of lipid peroxide was carried out colorimetrically, according to method described by (Ohkawa et al., 1979 and Martinez et al., 2002). The determination of lipid peroxidation product (MDA) was measured by thiobarbituric acid assay, which is based on MDA reaction with thiobarbituric acid in acidic medium at temperature of 95°C for 30min to form thiobarbituric acid reactive product. The absorbance of the resultant pink product can be measured at 534 nm.

Measurement of the calcium concentration:

The calcium levels of the lens were determined using a colorimetric method. Calcium ion produces with methylthymol blue, in an alkaline medium, a blue color the intensity of which is proportion to the calcium concentration. The absorbance of the resultant product can be measured at 585 nm.

Determination of oxidative stress index (OSI):

The ratio of TOC to TAC was accepted as the OSI. The OSI values were calculated according to the following formula: OSI = TOC (mM / g tissue) / TAC (mM / g tissue) (Demirbag et al., 2007).

Statistical analysis:

Statistical analysis was performed using Students’ t-test. The results were expressed as the mean ± standard deviation (SD). Statistical significance was assumed at a level of $P < 0.05$ (Snedecore and Cochran, 1976).

Results:

Protein content:

The total protein concentration of the control lens was 270.4±2.8 mg/g tissue. This value was very high significant decreased in single and fractionated dose without melatonin groups to (227.8±1.6 and 218.1±1.1 mg/g tissue respectively). The protein content for single and fractionated dose with melatonin groups showed progressive recovery with values of 249.3±3.9 and 259.4±2.3 mg/g tissue, respectively (Fig.1).

![Fig. 1: Protein content of rabbits’ lens for control, single dose without melatonin, single dose with melatonin, fractionated dose without melatonin, fractionated dose with melatonin](image)

**Oxidative stress markers:**

Exposure of lenses to UVB radiation, reduced lenticular levels of antioxidant capacity while lipid peroxidation and oxidant capacity was markedly elevated. Mean and SD values of TAC, TOC and MDA levels for control and all groups were presented in Table 1.

There were significant differences than the control in TAC and TOC levels in untreated groups. The data showed very high significant reduction in TAC ($P <0.001$) from 3.8±0.062 x10⁻³ mm/g tissue for the control to
2.9±0.069 x10^{-3} and 2.09±0.07 x10^{-3} mm/g tissue for single and fractionated dose without melatonin groups respectively. Also very high significant increase in TOC (P<0.001) from 232±4.9 x10^{-3} mm/g tissue for the control to 319±8.3 x10^{-3} and 331±10.5x10^{-3} mm/g tissue for single and fractionated dose without melatonin groups respectively.

Treatment of rabbits with melatonin reduces the degree of lipid peroxidation and elevates the antioxidant capacity. There were progressive increases in TAC for single and fractionated dose with melatonin groups with values of 3.2±0.62 x10^{-3} and 3.6±0.049 x10^{-3} mM/g tissue, respectively. In addition, there were a significant decrease in TOC for single and fractionated dose with melatonin groups with values of 247±5.1± x10^{-3} and 236±3.7x10^{-3} mm/g tissue, respectively. Table 1 shows the levels of MDA are very high significantly correlated with TOC for single and fractionated dose without melatonin groups and significantly correlated with TOC for single and fractionated dose with melatonin groups. In contrast, and there is an inverse relationship between MDA and TAC levels in all groups.

Table 1: Malondialdehyde (MDA), total oxidative capacity (TOC), total antioxidative capacity (TAC) of rabbit's lens for control, single dose without melatonin, single dose with melatonin, fractionated dose without melatonin, fractionated dose with melatonin.

<table>
<thead>
<tr>
<th>GROUPS</th>
<th>MDA nM/g tissue</th>
<th>TOC x10^{-3} mM/g tissue</th>
<th>TAC x10^{-3} mM/g tissue</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>279.3±9.7</td>
<td>252±4.9</td>
<td>3.8±0.062</td>
</tr>
<tr>
<td>Single dose without melatonin</td>
<td>370.3±11.6</td>
<td>319±8.3</td>
<td>2.9±0.069</td>
</tr>
<tr>
<td>Single dose with melatonin</td>
<td>301.1±6.9</td>
<td>247±5.1</td>
<td>3.2±0.062</td>
</tr>
<tr>
<td>Fractionated dose without melatonin</td>
<td>396.1±12.2</td>
<td>331±10.5</td>
<td>2.09±0.07</td>
</tr>
<tr>
<td>Fractionated dose with melatonin</td>
<td>286±49.1</td>
<td>236±3.7</td>
<td>3.6±0.049</td>
</tr>
</tbody>
</table>

Oxidative stress index (OSI):

Oxidative stress index (OSI) illustrates the ratio of TOC to TAC and determined the oxidative/antioxidative balance. In case of single and fractionated dose without melatonin groups, the data indicate shifting towards oxidative status (106.6±0.3 and 161.6±10.1), and oxidative stress was very high compared with the control group (60.8±1.2). While the data of single and fractionated dose with melatonin groups indicated shifting towards antioxidative status with values of 75.7±2.9 and 65.1±1.5 respectively (fig.2).

Fig. 2: Oxidative stress index (OSI) of rabbits' lens for control, single dose without melatonin, single dose with melatonin, fractionated dose without melatonin, fractionated dose with melatonin.

The calcium concentration:

The calcium concentration in the control lens was 2.19±0.12 mmol/L. This value was increased in the groups with single and fractionated dose without melatonin to 4.34±0.18 and 4.41±0.3mmol/L respectively. Moreover, the calcium concentration showed recovery in single dose with melatonin group (2.27±0.12 mmol/L) and in fractionated dose with melatonin group (2.26±0.12 mmol/L) fig.3.
Fig. 3: calcium concentration of rabbits’ lens for control, single dose without melatonin, single dose with melatonin, fractionated dose without melatonin, fractionated dose with melatonin.

Discussion:

The exposure of the lens to ultraviolet radiation is considered an important cause of cataract induction in vertebrates (Vrensen, 1994 and Kohli et al., 1996). The method of cataractogenesis induction has been used to test the role of melatonin in preventing lenticular damage. The efficacy of UVB radiation in lenticular damage derives from the imbalance between oxidant and antioxidants level represented by OSI. As observed in this study the exposure of the eye lens to UVB radiation produced a marked reduction in protein content and TAC with an elevation in the levels of lipid peroxidation as an indicator of the degree of lens damage. Johar et al. (2003) revealed that, the effect of UV radiation might lead to cell death that may be responsible for the observed decrease in the cell density in all three zones of the lens epithelium.

Michael et al. (1999) found that two, near-threshold UVR exposures at 0 or a 6 h interval produce the same degree of lens opacification. When the second exposure follows 24 or 48 h after the first, lenticular damage increases. Repair processes between 24 and 48 h after exposure appear to be sensitive to UVR, and an additional exposure during this time may aggravate cataract development. Repeated UVB exposure of the rabbit eye had a stronger effect on the lenticular metabolic profile than a single irradiation of the same overall dose. Fris et al. (2008) revealed the cumulative effect of repeated UVB irradiations, and shows that even a 48 hour interval between subsequent UVB exposures is not sufficient for the healing processes to restore lenticular integrity. In the present study, we observed in the groups without melatonin that fractionated dose without melatonin groups was strongly affected than single dose without melatonin groups. Treatment of rabbits with melatonin before, in-between and after UVB exposure-reduced lipid peroxidation, elevated antioxidant enzymes activities and reduced oxidative damage.

Melatonin can reach the lens through the anterior chamber fluid of the eye exhibiting a circadian rhythm like that seen in the blood (Yu et al., 1990). Melatonin’s antioxidant properties may well benefit from the fact that it is highly soluble in lipids and partially in water (Menendez-Pelaez and Reiter, 1993; Menendez-Pelaez et al., 1993; Ceraulo et al., 1999 and Leon et al., 2004). Thus, this indole is readily accessible to all parts of the cell unlike some other antioxidants. Melatonin’s antioxidant mechanism of action implies a free radical scavengers cascade in case of UV irradiation (tan et al., 2002). There are two antioxidant actions of melatonin (Fischer et al., 2001 and 2004) a powerful direct free radical scavenger (Hardeland et al., 1993 and 1995; Tan et al., 2002 and Allegre et al., 2003) and indirect antioxidant (Reiter et al., 2000c and Rodriguez et al., 2004). Melatonin as a powerful free radical scavenger, it is able to remove H$_2$O$_2$, ·OH, peroxinitrite anion (ONOO$^-$), singlet oxygen and peroxyl radical.

From the present study, melatonin may prevent lenticular damage by reducing lipid peroxidation, elevating antioxidant enzymes activities and reducing oxidative damage. The present results agree with previous reports that, melatonin may protect against the UVB-induced cataract development (Bardak et al., 2000; Anwar and Mostafa, 2001 and Karsioglu et al., 2005). Bron et al. (2000) reported that, exposure to UV increased glutathione hydroxykynurenine glycoside, which is the major factor responsible for the increased yellowing of the lens. Moreover, oxidative stress is involved in the age-related cataract (Ottonello et al., 2000). UV radiation can induce single and double strand breaks in the DNA and can induce pyrimidine dimers. This affects lens cell differentiation and protein synthesis (Michael et al., 1998). Lipid peroxidation and the inhibition of oxidative enzymes alter membrane functions and cell metabolism and, finally, lead to cellular edema (Bardak et al., 2000).

All these changes disturb the order and placement of the lens cells, and lead to an increase of light scattering in the lens. High levels of Ca$^{2+}$ cause protein aggregation, which results in water insoluble, high
molecular weight proteins (Hightower and Reddy, 1982 and Garner and Spector, 1986). Increased lenticular Ca\(^{2+}\) levels may lead to activation and induction of the calpains proteolytic system, which may induce lens opacification (Truscott et al., 1990). Transparent protein solutions have been shown to lose their transparency in a medium with high Ca\(^{2+}\) concentration (Jedziniak et al., 1972). Bardak (2000) found that, there were no significant differences between the control and the UVB + melatonin groups for the mean lens calcium and magnesium levels. In the present study, there is a significant difference between the control and the UVB + melatonin groups for the lens calcium content while there were very high significant difference between the control and the UVB without melatonin groups for the lens calcium content. Melatonin treatment lowered lenticular Ca\(^{2+}\) and this may be due to the inhibitory effect of Ca\(^{2+}\) influx and/or increased stability of the membrane by reduction of lipid peroxidation and subsequent reduced lenticular Ca\(^{2+}\) levels. Melatonin could block Ca\(^{2+}\) mobilization from the inositol trisphosphate-sensitive pool, or block Ca\(^{2+}\) influx through the voltage-sensitive channels (Vanecek and Klein, 1992 and Slanar et al., 1997). Therefore, the best approach to protect cells against damaging oxidative challenge is a combination of antioxidant and Ca\(^{2+}\) blocker (Halliwell, 1987). These properties seem to be fulfilled by melatonin and our results suggest that melatonin may protect against UVB radiation-induced cataract development by directly quenching lipid peroxides, and lowering cytosolic calcium, and indirectly by enhancing the production of endogenous antioxidants (elevating antioxidant level).

References


