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Effectiveness of Melatonin, as a Radiation Damage-Mitigating Drug in Modulating Liver Biochemical disorders in γ-Irradiated Rats

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MELATONIN has an antiperoxidative effect on several tissues as well as a scavenger effect on reactive oxygen species (ROS). Whilst radiation-hazards due to free radical generation, present enormous challenges for biological and medical safety. Therefore, rats were classified into four groups; control (n= 8), (received 0.5 ml of alchoholic saline as a vehicle for 5 days). Melatonin-treated rats received 10 mg/ kg body wt, for 5 days (given to the animals in the morning via stomach tube). γ-irradiated rats received 0.5 ml of the melatonin vehicle followed by one shot dose of 3 Gy γ-rays. Each of these groups was compared with a further group, which-received melatonin for 5 days after 3 Gy γ-irradiation exposure. The results revealed that all considered biochemical parameters were not changed significantly in melatonin-treated group as compared with control one. In the liver tissue of the γ-irradiated animals (3 Gy), the oxidative stress markers malondialdehyde (MDA) and protein carbonyl (PC) were significantly increased, while a marked decrease occurred in the contents of deoxy- & ribo-nucleic acids (DNA & RNA) and glutathione (GSH) as well as activity of glutathione-S-transferase (GST). In addition, catalase (CAT) and myeloperoxidase (MPO) activities were increased. Activities of aspartate transaminase (AST), alkaline phosphatase (ALP) and gamma-glutamyltransferase (GGT) were significantly increased in sera of the irradiated rats. Treatment with melatonin for 5 days after γ-rays exposure significantly modulated the radiation-induced elevations in MDA and PC levels in the liver tissue and significantly restored hepatic GSH content, GST, CAT and MPO activities. Post-irradiation treatment with melatonin showed significant higher hepatic DNA and RNA contents than irradiated rats. The activities of AST, ALP, and GGT in serum were significantly ameliorated when melatonin was administrated after irradiation.

Conclusion: Melatonin has effective mitigating effects against γ- radiation induced oxidative stress and liver injury.

Key words: Melatonin, mitigation, liver, γ-rays, rats.

Based on animal models, radiobiologists have long been interested in identifying novel non-toxic and convenient compounds to protect and/ or mitigate humans from radiation induced normal tissue and organ injuries and to allow the use of higher doses of radiation during radiotherapy for a better curative ratio.

The properties of melatonin suggest that this molecule is an important effector of stress responses. In this way, its actions may counteract or buffer both environmental and endogenous stressors to maintain organ integrity (Slominski1 *et al.,* 2008). Melatonin is distributed ubiquitously throughout an organism because its small size and amphiphilic nature facilitate permeability to all cellular compartments. Moreover, it has pleiotropic bioactivity as a biological-response modifier and in energy expenditure (Pandi-Perumal *et al.,* 2006). Furthermore, melatonin is reported to influence a variety of inflammatory and immune responses (Esposito *et al.,* 2008).

Radiation is a source of exogenous free radicals and these radicals with other radicals damage biological macromolecules (Hannig *et al.,* 2000). Irradiation caused marked decrease in serum melatonin and its pineal biosynthesis (Ahlersova *et al.,* 1998). Total antioxidant capacity of plasma was reduced in rats exposed to whole body γ-radiation (Zahran *et al.,* 2006). Consequently the cellular antioxidant capacity is decreased and the organs become more susceptible to the deleterious effects of ROS (Karbownik and Reiter, 2000). Similarly, total body irradiation in rats has been reported to cause oxidative tissue damage (Sener *et al.,* 2004). The aim of the present study is to investigate the radiation damage mitigating-role of melatonin (delivered after irradiation) against oxidative stress induced liver injury in rats.

Materials and Methods

All animal treatment procedures met the guidelines of the National Research Council (1996). The experiments were performed in male rats weighing 120-140 g, which were kept at a constant temperature (22± 1°C). They were fed with a maintenance rat diet, had access to water *ad libitum* and were synchronized by maintenance of controlled environmental conditions (light, temperature, feeding time, etc) for at least 1 week prior to and throughout the experiment. The lighting regimen was 12 h of light attenuated with 12 h of darkness. Melatonin and chemical reagents used in this study were purchased from Sigma-Alderich Chemical Co. (St. Louis, MO, USA).

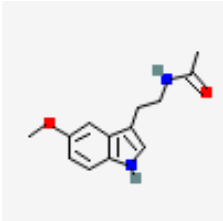

Fig. 1. Melatonin ($C_{13}H_{16}N_2O_2$) structure.

Melatonin treatment

In the present study, a freshly prepared melatonin dose (10 mg/ kg body wt) was dissolved in 0.5 ml of alchoholic saline as a vehicle (20 part of 0.9 % NaCl and 1 part of 100% ethanol v/v) was given to each animal at 10.30 a.m. using stomach tubes according to El-Missiry (2007).

Irradiation technique

Animals were placed in a specially designed well-ventilated acrylic container and the whole body of the animals was exposed to a dose level of 3 Gy, given at a dose rate of 0.52 Gy/ min from a biological-irradiator gamma cell-40, ^{137}cesium source (Atomic Energy Agency, Canada), belonging to NCRRT, Egypt.

Experimental design

Control group: Animals received daily 0.5 ml of the vehicle solution for 5 days. Melatonin treated group: Animals received melatonin dosages for 5 days. γ-irradiated groups: Animals were exposed to a dose level of 3 Gy whole-body γ-rays, thereafter administrated 0.5 ml of the vehicle. γ-irradiated & melatonin-treated group: Animals received melatonin dosages for 5 days after radiation exposure.

Animals were sacrificed 6 days after the various treatments and 12 h after overnight fasting. Blood samples were collected in non-heparinised dry tubes, which were centrifuged and the sera were frozen at -20 °C for the following biochemical evaluations. The liver homogenates were prepared in a 10-fold volume of ice-cold (20mM) tris-HCl buffer, pH 7.4.

Biochemical evaluations

The activities of serum AST, ALP and GGT were estimated according to the methods of Reitman and Frankel (1957), Roy *et al.* (1970) and Rosalk

(1975). Lipid peroxidation product, malondialdehyde (MDA), was measured by TBARS method (Ohkawa *et al.*, 1979). The protein carbonylation (PC), a major product of oxidized protein was measured as quantified by Levine *et al.* (1990). The GSH content was determined according to Beutler (1982). GST activity was performed according Habig *et al.* (1974). CAT activity was determined as described by Bock *et al.* (1980). DNA and RNA contents were detected as described by Burton (1956) and Thoresen *et al.* (1983) methods.

Determination of myeloperoxidase (MPO) activity

100 mg of tissue was homogenized in 1 ml ice-cold 0.02 M EDTA, pH 4.7, for 60 seconds. 1 ml homogenate was centrifuged at 20,000 *g* for 15 min at +4 °C to obtain the pellet of the insoluble cellular debris. The pellet was then re-homogenized in an equivalent volume of 0.05 M potassium phosphate buffer, pH 6.0, containing 0.5% hexadecyl trimethyl ammonium bromide. This homogenate was centrifuged at 20,000 *g* for 15 min at +4 °C and the supernatants were used in the MPO assay by the method of Grisham *et al.* (1986). Protein assays in the samples were determined by the method of Lowry *et al.* (1951).

Statistical analysis

Statistical significance of the data was analysed using Student's *t*-test (Sokal and Rahif, 1981). Data are shown as means± SD of 8 rats and the level for statistical significance was $P< 0.05$.

Results

All considered biochemical parameters were not changed significantly after administration of melatonin alone for 5 days (Table 1, 2, 3 & 4).

TABLE 1. Hepatic levels of TBARS, PC and GSH of male irradiated rats (3 Gy) and the response to post γ-irradiation treatment with melatonin.

Groups	TBARS nmol/ g protein	PC µmol/ g protein	GSH mg/ g protein
Control	351± 26.2	151± 10.1	0.18± 0.013
Melatonin-treated	321± 22.5	141± 10.3	0.18± 0.012
γ-irradiated	602± 46.9[a,b]	366± 29.6[a,b]	0.14± 0.011[a,b]
γ-irradiated & melatonin-treated	422± 40.6[a,b,c]	191± 14.5[a,b,c]	0.16± 0.013[c]

All values are expressed as mean± SD of 8 animals.

[a]Significant when compared with the control group at $P< 0.05$.

[b]Significant when compared with the melatonin-treated group at $P< 0.05$.

[c]Significant when compared with the γ-irradiated group at $P< 0.05$.

Exposure of rats to γ-radiation (3 Gy) resulted in significant increases in TBARS & PC and a decrease in GSH in the liver tissue 6 days after irradiation comparing with control groups. Daily treatment with melatonin (10 mg/ kg body wt) for 5 days post irradiation (3 Gy) significantly modulated these radiation-induced changes in TBARS, PC and GSH levels in the liver comparing with irradiated groups (Table 1).

TABLE 2. Hepatic enzymes activities of GST, CAT and MPO of male irradiated rats (3 Gy) and the response to post γ-irradiation treatment with melatonin.

Groups	GST μmol/ g protein	CAT mmol/ g protein	MPO U/ g tissue
Control	18.7± 1.12	4.5± 0.31	1.1± 0.07
Melatonin treated	19.2± 1.11	4.1± 0.29	1.1± 0.05
γ-irradiated	12.3±1.06[a,b]	6.7± 0.44[a,b]	2.4± 0.14[a,b]
γ-irradiated & melatonin-treated	16.1± 1.09[a,b,c]	5.1± 0.53[a,b,c]	1.2± 0.06[a,b,c]

Legends as in Table 1.

In livers of irradiated groups (3 Gy), GST activity was significantly inhibited comparing with control group. On the other hand, CAT and MPO activities were significantly increased comparing with control groups. Melatonin-treatment modulated hepatic GST and maintained CAT and MPO activities within the control levels (Table 2). In 3 Gy-irradiated groups, there were significant decreases in hepatic DNA and RNA contents comparing with control groups. Daily treatment with melatonin for 5 days following irradiation showed significantly higher DNA and RNA content in the liver of γ-irradiated & melatonin-treated groups than irradiated rats (Table 3).

TABLE 3. Hepatic contents of DNA and RNA of male irradiated rats (3 Gy) and the response to post γ-irradiation treatment with melatonin.

Groups	DNA mg/ g protein	RNA mg/ g protein
Control	21.6± 1.41	6.9± 0.17
Melatonin treated	21.1± 1.32	6.8± 0.19
γ-irradiated	15.2± 1.01[a,b]	4.1± 0.12[a,b]
γ-irradiated & melatonin-treated	18.3± 1.14[a,b,c]	5.5± 0.18[a,b,c]

Legends as in Table 1.

The activities of AST, ALP, and GGT in serum showed a significant rise at the 6th day following γ-irradiation comparing with control groups. The treatment with melatonin after irradiation displayed significant amelioration in the elevation of these enzyme activities in serum comparing with irradiated groups (Table 4).

TABLE 4. Serum activities of AST, ALP and GGT of male irradiated rats (3 Gy) and the response to post γ-irradiation treatment with melatonin.

Groups	AST U/L	ALP U/L	GGT U/L
Control	8.6± 0.19	52± 1.3	30± 1.4
Melatonin treated	8.6± 0.22	50± 1.4	31± 1.3
γ-irradiated	19.8± 1.11^{a,b}	113± 2.2^{a,b}	49± 1.7^{a,b}
γ-irradiated & melatonin-treated	13.1± 0.88^{a,b,c}	84± 1.7^{a,b,c}	39± 1.5^{a,b,c}

Legends as in Table 1.

Discussion

The biological consequences of ionising-radiation are attributable to chemical changes in biological molecules as a result of energy absorption (Reiter *et al.,* 2004). The generation of the reactive oxygen metabolites plays an important role in the pathogenesis of irradiation-induced tissue injury (Agrawal *et al.,* 2001). γ-rays induces both an exponential burst of ROS from cells and an exponential increase in intracellular Ca^{2+} levels (Claro *et al.,* 2008), the exponential increase of both oxidative stress and Ca^{2+} influx causes a massive, sudden cell death (Liu *et al.,* 2011). Blockade of any of these steps is sufficient to prevent cell death (Tan *et al.,* 2001[a]).

In the present study, TBARS, PC and GSH levels in the liver altered in γ-irradiated group comparing with control and melatonin treated groups in agreement with results report by El-Missiry *et al.* (2007). However, melatonin treatment after irradiation markedly restored TBARS and PC levels. In addition, it maintained hepatic GSH level was maintained close to the control level and showed insignificant changes compared to control and melatonin groups. In contrast, intra-peritoneal melatonin (10 mg/ kg) administrated to rats after whole body gamma-irradiation (6 Gy), resulted in no changes in GSH content in liver tissue (Bhatia and Manda, 2003).

Reduction in the amount of lipid peroxidation products and elevation in GSH level due to melatonin treatment suggested that melatonin could scavenge the free radicals formed during oxidative stress. GSH with its sulfhydryl-group functions in the maintenance of sulfhydryl-groups of other molecules (especially proteins), as a catalyst for disulfide exchange reactions, and in the detoxification of foreign compounds, hydrogen peroxide and free radicals. When GSH acts as a reducing agent, its SH becomes oxidized and forms a disulfide link with other molecules of GSH (Bhatia and Manda, 2004).

The present study demonstrates that whole body γ-irradiation (3 Gy) significantly decreased hepatic GST activity in comparison to controls but melatonin treatment post-irradiation significantly countered radiation-induced decrease in the activity of this enzyme in the liver. A significant decrease in hepatic GST activity was recorded after exposure to 3.5 Gy (Sridharan and Shyamaladevi, 2002). Melatonin enhanced the activity of GST in the livers of the γ-irradiated & melatonin-treated group. This is supported by the report of Reiter *et al.* (2001) that melatonin bound to DNA. Thus, it is proposed that GST might be influenced by melatonin. Taken together, these findings support the conclusion that melatonin offers radioprotection by modulating hepatic GST activity.

On the other hand, CAT and MPO activities were significantly increased comparing with control groups. Increased CAT activity is probably related to the inability of the cell to cope with overproduction of H_2O_2 or *OH (Weiss *et al.*, 2003). Oxidative injury of the colon is accompanied with neutrophil infiltration in both early and later phases after irradiation, whereas contribution of neutrophils to the irradiation injury of the liver occurs at the later phase (Sener *et al.*, 2004). Melatonin-treatment modulated hepatic GST and maintained CAT and MPO activities within the control levels. CAT is maintained within control level by post-treatment with melatonin, indicating controlled H_2O_2 generation in the liver or it's scavenging directly by melatonin (Tan *et al.*, 2000). In addition, melatonin treatment, suppresses radiation-induced elevation in the hepatic MPO activity.

The major forms of cellular damage induced by radiation are DNA damage, lipid peroxidation, and protein oxidation. The present study demonstrates significant decreases in hepatic DNA and RNA contents comparing with control groups. Ionising radiation generates ROS as a result of water radiolysis. These ROS can induce oxidative damage to vital cellular molecules and structures including DNA, lipids, proteins, and membranes **(**Cadet *et al.*, 2004). Melatonin mitigated the loss of DNA and RNA from rat liver in accordance with the El-Missiry *et al.* (2007) report.

The present study demonstrates as well a significant increase in serum AST, ALP and GGT activities after radiation exposure. GGT is a key enzyme in the catabolism of GSH (Lee *et al.*, 2002). It has been reported that the extra cellular cleavage of GSH by GGT induces the production of ROS, suggesting that GGT plays a pro-oxidant role (Lee *et al.*, 2004). Melatonin treatment

stimulates several antioxidants that raise the total antioxidant capacity of the **body** (Reiter *et al.,* 2004). Accordingly, melatonin might reduce serum GGT level to exert its antioxidant effect. Gamma-radiation elevates serum AST and ALP level in accordance with other studies (Bhatia and Manda, 2004). The present result demonstrates that melatonin treatment after irradiation ameliorates oxidative stress evidenced by decreased AST and ALP activities the liver.

Melatonin is a potent antioxidant, which can exert its action directly or indirectly (Majsterek *et al.,* 2005). In addition to its direct free radical scavenging action, melatonin antioxidant capacity includes the indirect effect of up-regulating several antioxidative enzymes and down-regulating pro-oxidant enzymes (Storr *et al.,* 2002). There is evidence that melatonin acts not only as a hormone but also as a tissue factor (Pandi-Perumal *et al.,* 2006). Additionally, melatonin is an antioxidant nutrient. Although its redox properties are difficult to preserve in food, it has been suggested that certain of its metabolites, especially a substituted kynuramine formed by oxidative pyrrole-ring cleavage, may be stable enough to serve as a dietary supplement without a significant loss of its antioxidant effects (Hardeland and Pandi-Perumal, 2005). Receptor-independent actions of melatonin biology are the array of receptor-independent activities, which operates as a potent cytoprotector and/ or metabolic modulator in many different biological systems (Tan *et al.,* 2007).

Conclusion

In conclusion, melatonin has clear antioxidant properties and is likely to be a valuable drug for mitigating radiation induced oxidative stress. These findings indicate that melatonin supplementation may be beneficial postradiotherapy. Such application awaits further investigations to be undertaken.

Recommendations

It may be reasonable to use melatonin with adjusting the dose to achieve the best radio-mitigating effect with as few side effects as possible. Before employment, it should be extensively tested in *vitro* and in *vivo* models, using the same and other biomarkers for different radiation exposure levels.

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فاعلية الميلاتونين كعقار لعلاج أثار الإشعاع في تحسين اضطرابات الكبد الكيموحيوية في الجرذان المعرضة لأشعة جاما

نعمة محمد الفاتح و الهام الشامي

قسمي بحوث البيولوجيا الاشعاعية و *البحوث الصحية الاشعاعية ، المركز القومي لبحوث وتكنولوجيا الاشعاع، ص ب: ٢٩ مدينة نصر ، مصر.

يتميز الميلاتونين بتأثيره المانع لضرر التأكسد علي العديد من الأنسجة و بقدرته علي التقاط الشوارد الحرة منها. و من تم يمكن التصدي لمخاطر الإشعاع البيولوجية والطبية. تم تقسيم الجرذان إلى مجموعة ضابطة (تجرعت ٥ر٠ سم^{٣} من محلول كحل ملحي كمذيب للميلاتونين) لمدة ٥ أيام ، و مجموعة تجرعت الميلاتونين صباحا لمدة ٥ أيام (١٠ ملجم/ كيلوجرام) ، و مجموعة عرضت لأشعة جاما (٣ جراي) بعد التشعيع ثم ينم تجريعها محلول المذيب ، و المجموعة الأخيرة تجرعت الميلاتونين صباحا لمدة ٥ أيام بعد أن عرضت لـ ٣ جراي من أشعة جاما.

أوضحت النتائج عدم وجود تغييرات إحصائية في المعايير المخنبرة في مجموعة الجرذان التي تناولت الميلاتونين عند مقارنتها بالمجموعة الضابطة. ازداد مقدار كل من المالون داي آلدهيد (MDA) و كاربونيل البرونين (PC) زيادة إحصائية. بينما نقص محتوي كل من DNA ، و RNA و الجلوتاثيون (GSH) إحصائيا وكذلك نشاط إنزيم الجلوتاثيون-س- ترانسفيريز (GST) ، بالإضافة إلى زيادة نشاط انزيمي الكتاليز (CAT) و الميلوبيروأكسيديز (MPO) إحصائيا. أرتفع نشاط انزيمات الأسبرتات ترانس أمينيس (AST) و الفوسفاتيز القلوي (ALP) و الجاما جلوتاميل ترانس فيريز (GGT) إحصائيا في سيروم الجرذان.

أدي العلاج بالميلاتونين لمدة ٥ أيام بعد التعرض لأشعة جاما الي الحد من الزيادة التي حدثت في محنوي الكبد بكل من:- MDA و PC. و ايضا علي استعادة مستوي كل من GSH و GST و CAT وMPO. كما بينت المعالجة بالميلاتونين بعد التعرض لأشعة جاما وجود زيادة إحصائية في محتوي نسيج الكبد من DNA و RNA مقارنة بالمجموعة المعرضة للإشعاع. و كذلك تحسن قياسات نشاط إنزيمات AST و ALP و GGT في سيروم الدم.

الخلاصة: أوضحت فاعلية الميلاتونين في معالجة أضرار التأكسد و أضرابات الكبد الكيموحيوية تحت الأختبار و التي يسببها تعرض الجرذان لأشعة جاما.