J Biochem Tech (2010) 2(3):171-174
ISSN: 0974-2328

Melatonin attenuates lipid peroxidation and enhances circulatory antioxidants during mammary carcinogenesis in rats

Sankaran Mirunalini*, Kandhan Karthishwaran, Ganesan Dhamodharan, Shalini Mohan

Received: 22 Nov 2009 / Received in revised form: 1 April 2010, Accepted: 30 August 2010, Published online: 30 August 2010

Abstract

The possible protective effect of Melatonin was investigated for its antioxidant and lipid peroxidation activity against 7,12-dimethylbenz(a)anthracene (DMBA) induced mammary carcinogenesis in female albino Wistar rats. Mammary tumor was developed to the animals by administering 5mg/kg body weight of DMBA orally at weekly intervals for one month. Intraperitoneal administration of melatonin 5mg/ml per animals for 15 days prior to the first oral administration of DMBA was continued for a month. After the experimental period, oxidative stress parameters were assessed in plasma of both control and experimental groups. A significant increase in lipid peroxidation levels were observed in cancer induced rats while the activities of enzymic and non-enzymic antioxidants were decreased in cancer-bearing animals when compared to control animals. Administration of melatonin remarkably reduced the lipid peroxidation activity and increased the antioxidants level in drug treated animals. This result suggests that melatonin shows antioxidant activity and play a protective role against 7, 12-dimethylbenz(a)anthracene induced breast cancer. The inhibitory effect of melatonin on tumor cells and its lack of side–effects, indicate that melatonin should be considered as an adjuvant drug in the treatment of neoplastic diseases.

Keywords: 7, 12-Dimethylbenz(a)anthracene, melatonin, lipid peroxidation, breast cancer, antioxidants

Abbreviations: CAT, Catalase; DMBA, 7,12-dimethylbenz (a)anthracene; GPx, glutathione peroxidase; GSH, glutathione; LPO, Lipid peroxidation; PAH, Polycyclic aromatic hydrocarbon; SOD, superoxide dismutase; ROS, Reactive oxygen species; TBARS, Thiobarbituric acid;

Sankaran Mirunalini*, Kandhan Karthishwaran, Ganesan Dhamodharan, Shalini Mohan

Department of Biochemistry and Biotechnology, Faculty of Science, Annamalai University, Annamalai Nagar-608 002, Tamil Nadu, India

*Tel: 0091 4144 238343 Ext-221, Email: mirunasankar@gmail.com

Introduction

DMBA, a polycyclic aromatic hydrocarbon (PAH) consistently produces carcinomas in animal models. Sources of PAHs include industrial and domestic oil furnaces, gasoline and diesel engines. PAHs are widely distributed in our environment and are implicated in various types of cancer. Enzymatic activation of PAHs leads to the generation of active oxygen species such as peroxide and superoxide anion radicals. Uncontrolled increase of these highly reactive molecules lead to free radical mediated chain reactions of which indiscriminately damage proteins, lipids and DNA resulting at last resort in cell death (Lenaz 2001).

Experimental investigations provide evidence supporting the role of reactive oxygen species (ROS) such as singlet oxygen ($^1O_2$), superoxide anions ($O_2^{\cdot-}$), hydrogen peroxide (H_2O_2), and hydroxyl radicals ($^{\cdot}OH$) in the etiology of cancer (Cheeseman and Slater, 1993). Human body is equipped with various antioxidants visualizing superoxide dismutase (SOD), glutathione peroxidase (GPx), catalase (CAT), glutathione (GSH), ascorbic acid (Vitamin C), α-tocopherol (Vitamin E), etc., which can counteract the deleterious action of ROS and protect from cellular and molecular damage. Antioxidants act as radical scavengers inhibiting LPO and other free radical–mediated processes thereby protecting the human body from various diseases (Mirunalini et al. 2004).

Various molecules can inhibit the formation of free radicals associated with carcinogenesis. Chemoprevention using drugs is a promising approach to control the mortality and occurrence of cancer that accounts for millions of death Worldwide. Many compounds have been identified as good protectors against the free radicals by triggering antioxidants gene expression (Sharma et al. 1994). Melatonin, a hormone of the pineal gland has been known to be a chemopreventive, anticancer agent in *in vitro* studies and experimental animal models (Cos et al. 2005). In pharmacological amounts, melatonin effectively reduces oxidative stress through several mechanisms [Martinez et al. 2005]. In this juncture reports suggest that melatonin possesses cytotoxic and anticancer activity in MCF-7 breast cancer cell lines (Blask et al. 2002).

The present study was undertaken to evaluate the antioxidant and anticancer activity of melatonin against DMBA induced mammary cancer in experimental rats.

Materials and Methods

Chemicals

DMBA was purchased from Sigma chemical company, St. Louis, MO, USA. Melatonin and other chemicals used were of analytical grade and purchased from SRL, Mumbai, India.

Animals

Female albino Wistar rats (100g) body weight was procured from Tamilnadu Veterinary Science Institute, Chennai, India. Animals were housed in well ventilated large polypropylene cages (45 × 24 × 15 cm) and were handled according to the University and Institutional Legislation, regulated by the Ethical Committee on Animal care of Annamalai University. All the procedures performed on the animal were approved and conducted in accordance with the National Institute of Health Guide (Reg. No. 486/160/1999/CPCSEA). The animals were fed with standard pellet diet and water *ad libitum* throughout the experimental period and replenished daily.

Tumor Induction

Mammary tumor was induced by dissolving 5mg of DMBA in 1ml corn oil, administered through oral gavage once in a week with a total dose of 20mg/kg body weight (Loscher et al. 1997).

Experimental group

A total of 24 Female albino Wistar rats were divided into 4 groups of 6 animals each. Group I animals served as control without any treatment. Group II animals received a dose of 5mg/kg of DMBA at weekly intervals for one month (Loscher et al. 1997). Group III animals received a daily intraperitoneal administration of melatonin 5mg/ml per animal for one month prior to the first oral administration of DMBA (Aoyama et al. 1987). Group IV animals received a daily intraperitoneal administration of melatonin 5mg/ml per animal beginning the next day after DMBA administration for one month (Aoyama et al. 1987).

Animals were monitored every day. After the termination of the experiment, all animals were anesthetized with 24 mg/kg ketamine chloride and sacrificed by cervical dislocation after an overnight fast. Plasma and serum were collected from blood after centrifugation.

Biochemical Parameters

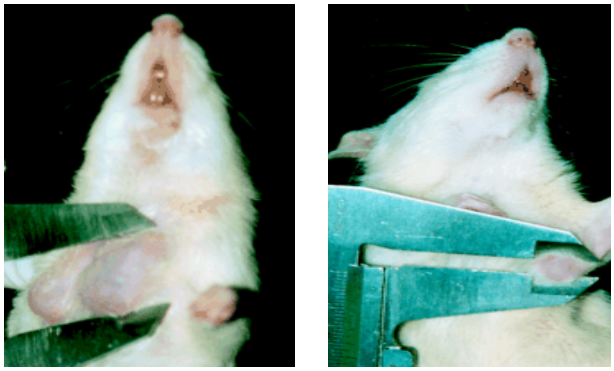
Lipid peroxidation was estimated by the method of Yagi (Niehaus and Samuelson 1968). 2-thiobarbituric acid from lipid hydroperoxides was read at 540 nm. The antioxidant enzyme SOD was analyzed by the method of Kakker et al. (Kakkar et al. 1984). SOD activity was measured at absorbance 560 nm. Catalase activity was assayed by the method of Sinha (Sinha, 1972). The colour developed was read at 620 nm. GPx was measured by the method of Rotruck et al. 1973. Glutathione oxidation by the enzyme was read at 420 nm. Total GSH was estimated by the method of Ellman 1959. Amount of ascorbic acid (vitamin C) was estimated by the method of Omaye et al. 1979 and α-tocopherol (vitamin E) levels were estimated by the method of Baker et al. 1980.

Statistical Analysis

Statistical analysis was performed and data were presented as mean ±S.D. Differences between groups were assessed by ANOVA followed by DMRT using SPSS software package (SPSS South Asia (P) Limited, Bangalore, India). A value of $P<0.05$ was considered to indicate a significant difference between groups

Results

Animals in the breast cancer control group II attained a tumor promotional stage after 120 days. At the end of the experiment, DMBA induced breast tumors increased to the maximum in terms of tumor incidence (100%) compared to the normal control rats. While the animals administered melatonin (5mg/mL) individually achieved 64% of tumor reduction after 1 month treatment. Melatonin post treated animals achieved a significant decrease in the mammary tumor size. Melatonin pretreated animals show no tumor incidence without any change in the total body weight of the animals. Morphological changes are shown in figure 1.

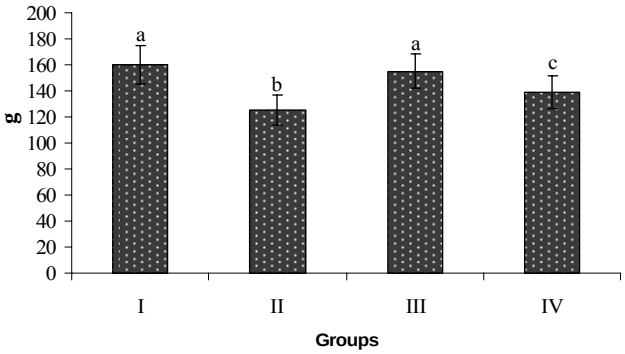


Group II, DMBA induced mammary tumors with 100%

Group IV, Melatonin post treated animals with a reduction in mammary tumor size

Figure 1: Morphological examination of mammary glands of DMBA induced rats

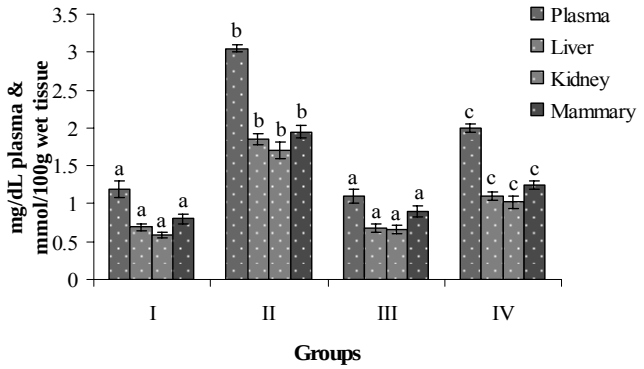
Figure 2 presents the body weight of control and experimental animals. The body weight was found to be significantly decreased in Group II tumor induced animals when compared with control animals ($P<0.05$). Conversely, the administration of melatonin increased the body weight in Groups III and IV when compared to Group II animals ($P<0.05$). No significant changes were observed in Groups III animals when compared to control animals.



Group I: control, Group II: DMBA, Group III: Mel+DMBA, Group IV: DMBA+Mel. Each column is mean ± S.D for 6 rats in each group. Column not sharing a common letter (a, b, c) differ significantly with each other ($P<0.05$, DMRT)

Figure 2: Effect of Melatonin on body weight of control and experimental animals

Lipid peroxidation as evidenced by the formation of TBARS in tissues (liver, kidney and mammary) and plasma are presented in Figure 3. The level of TBARS in rats treated with DMBA alone (group II) were the highest among all groups and significantly higher than those of untreated controls (group I). In groups III and IV, the levels were significantly lower than those in group II. Moreover, the lipid peroxidation activity was significantly ($P<0.05$) higher in group IV animals when compared to group III animals. No significant changes were found in group III animals when compared to control animals.



Group I: control, Group II: DMBA, Group III: Mel+DMBA, Group IV: DMBA+Mel. Each column is mean ± S.D for 6 rats in each group. Column not sharing a common letter (a, b, c) differ significantly with each other ($P<0.05$, DMRT)

Figure 3: Effect of Melatonin on TBARS in circulation and tissues of control and experimental animals

Table 1 indicates the activities of enzymic antioxidants (SOD, CAT and GPx) and non-enzymic antioxidants (GSH, vitamin C and vitamin E) in serum of control and experimental animals. The activities of antioxidants were markedly decreased in group II when compared to all other groups. Administration of melatonin in group IV animals ($P<0.05$) significantly increased the antioxidant levels when compared to group II animals. However, the antioxidant levels were decreased significantly ($P<0.05$) in group IV versus group III animals. No significant changes were observed in group III animals when compared to group I animals.

Table 1. Effect of melatonin on enzymic and non-enzymic antioxidants in circulation of control and experimental animals

Parameters	Group I Control	Group II DMBA	Group III Mel + DMBA	Group IV DMBA +Mel
SOD	10.16 ± 0.64^{a}	5.00 ± 0.42^{b}	9.23 ± 0.76^{a}	7.12 ± 0.56^{c}
CAT	53.29 ± 4.36^{a}	27.69 ± 2.24^{b}	49.40 ± 3.98^{a}	37.18 ± 3.16^{c}
GPx	6.60 ± 0.54^{a}	3.22 ± 0.26^{b}	6.50 ± 0.49^{a}	4.51 ± 0.31^{c}
GSH	7.76 ± 0.64^{a}	4.20 ± 0.22^{b}	7.00 ± 0.58^{a}	5.86 ± 0.42^{c}
Vitamin C	3.10 ± 0.23^{a}	0.80 ± 0.04^{b}	2.60 ± 0.23^{a}	0.86 ± 0.05^{c}
Vitamin E	4.40 ± 0.38^{a}	2.20 ± 0.18^{b}	4.00 ± 0.37^{a}	2.90 ± 0.13^{c}

Each value is mean ± S.D. for six rats in each group. Values not sharing a common superscript (a, b, c) differ significantly with each other ($P<0.05$, DMRT).

Units: SOD (*units/mg protein), CAT (µmol of H_2O_2 consumed/min/mg protein), GPx (µg of GSH oxidized/min/100 mg protein), GSH (mg/dL), Vitamin C (mg/dL), Vitamin E (mg/dL), *units-the amount of enzyme required to inhibit 50% reduction/mg protein.

Group II cancer bearing animals showed a significant reduction in both enzyme and non-enzyme antioxidant levels ($P<0.05$) when compared to control animals. Administration of melatonin in Group IV animals ($P<0.05$) significantly elevated the antioxidant levels, we compared to Group II animals. No significant changes were observed in Group III animals when compared to Group I animals.

Discussion

The generation of reactive oxygen species (ROS) and the peroxidation of membrane lipids are well associated with the initiation of carcinogenesis affecting the normal biochemical process, which further leads to the reduction of body weight (Davis and Kuttan 2001). These results agree with the hypothesis that the administration of DMBA to rats brought changes in body weight and enzyme activities which may serve as markers to evaluate the chemopreventive role of compounds of a potential clinical use. Oxidative stress induced due to the generation of free radicals and/or decreased antioxidant level in the target cells and tissues has been suggested to play an important role in carcinogenesis (Huang et al. 1999). Increased incidence of oxidative stress and lipid peroxidation are implicated in carcinogenic processes (Datta et al. 2000).

Naturally, there is a dynamic balance between the amount of free radicals generated in the body and antioxidant defense system that quench or scavenge them and thereby protect the body against pathogenesis. Free radicals are involved both in the initiation as well as promotion stage of tumourigenesis and their biochemical reactions in each stage of the metabolic process are associated with cancer development (Mates and Gomez 1999). It is evident from the results that increased level of LPO was found in cancer-bearing animals when compared to control group. On the contrary, reduced levels of LPO were observed in melatonin treated animals indicating that it is a good free radical scavenger. Both physiological and pharmacological concentration of melatonin *in vivo* are and commonly effective in reducing total oxidative burden with in organisms (Chen et al. 2006). During the process of neutralizing toxic reactants, melatonin protects proteins, lipids, mitochondrial DNA and nuclear DNA from oxidative damage (Okatani et al. 2002).

Antioxidants act as the primary line of defense against ROS and suggest their usefulness in eliminating the risk of oxidative damage induced during carcinogenesis. SOD and CAT acts mutually supportive antioxidative enzymes, which provide protective defense against reactive oxygen species (Reiter et al. 2005). An increased level of superoxide radicals in tumor cells decreases the antioxidant activity when compared to normal cells. The present study reveals that SOD levels are increased in cancer-bearing animal, which may be due to altered antioxidant status caused by carcinogenesis. Similar result was observed for CAT in group II, which may be due to utilization of antioxidant enzymes in the removal of H_2O_2 by DMBA. GPx is an important defense enzyme which catalyses the oxidation of GSH to GSSG at the expense of H_2O_2 (Weydert et al. 2006). Decreased GPx activity was observed in cancerous conditions (Cerutti et al., 1994). Our findings agree well with this observation.

The non-enzymic antioxidant systems are the second line of defense against free radical damage. GSH in conjunction with GPx plays a regulatory role in cell proliferation (Bewick et al. 1987). GSH serves as a marker for the evaluation of oxidative stress and it act as an antioxidant at both intracellular and extra cellular levels (Comporti 1989), we also observed decrease activities of GSH in cancer-bearing animals. Melatonin enhanced the GSH levels, which clearly suggest their antioxidant property. Decreased levels of water-soluble antioxidants in cancer-bearing animals may be due to the utilization of antioxidants to scavenge free radicals. Vitamin C undergoes a synergistic interaction with tocopheroxyl radical in the regeneration of α-tocopherol. Vitamin E protects cell membranes from oxidative damage initiated by carcinogens (Schindler and Mentlein 2006). The

free radical clearing capacity of vitamin E is due to the localization of an unpaired electron on its conjugated double bond.

Administration of melatonin significantly reversed the alteration to near normal level in cancer-bearing animals and substantially inhibited the breast tumor incidence or decrease in initiation of tumorigenesis in pretreated group. From the results it can be inferred that melatonin positively modulated antioxidant activity by quenching and detoxifying the free radicals induced by DMBA. It is worth emphasizing the protective role of melatonin against the side-effects of chemo and/or radiotherapy. Although majority of the studies involved experimental animals, the results could suggest the applicability of melatonin in humans. All the above-mentioned data, i.e. the inhibitory effect of melatonin on tumor cells and its lack of side–effects, indicate that melatonin should be considered as an adjuvant drug in the treatment of neoplastic diseases. Further investigation on the anticancer activity mechanisms of melatonin remains to be studied in our laboratory.

References

Aoyama H, Mori N, Mori W (1987) Anti-glucocorticoid effects of melatonin on adult rats. Acta Pathol Jpn 37:1143-48

Baker H, Frank O, De Angelis B, Feingold S (1980) Plasma tocopherol in man at various times after ingesting free or acetylated tocopherol. Nutr Rep Int 21:531-536

Bewick M, Coutie W, Tudhope GR (1987) Superoxide dismut*ase, glutathione peroxidase and* catalase in the red cells of patients with malignant lymphoma. Br J Haematol 65:347- 350

Blask DE, Sauer LA and Dauchy RT (2002**)** Melatonin as a chronobiotic/anticancer agent: cellular, biochemical and molecular mechanisms of action and their implications for circadian-based therapy. Curr Top Med Chem 2(2):113-132

Cerutti P, Ghosh R, Oya Y, Amstad P (1994) The role of cellular antioxidants defence in oxidant carcinogenesis. Environ Health Perspect 102(10):123-129

Chen YH, Xu DX, Wang H, Wei LZ et al. (2006) Melatonin protects against lipopolysaccharide induced intra-uterine fetal death and growth retardation in mice. J Pineal Res 40:40-47

Cheeseman KL and Slater TF (1993) An introduction to free radical biochemistry. Br Med Bull 49:481-483

Comporti M (1989) Three models of free-radicals induced cell injury. Chem Biol Interact 72:51-56

Cos S, Martinez-campa C, Mediavilla MD, Sanchez-Barcelo EJ (2005) Melatonin modulates aromatase activity in MCF-7 human breast cancer cells. J Pineal Res 38(4):217-222

Datta K, Sinha S, Chattopadhyay P (2000) Reactive oxygen species in health and disease. Natl Med J Ind 13:304-310

Davis L, Kuttan G (2001) Effect of Withania Somnifera on DMBA induced carcinogenesis. J Ethnopharmacol 75:165-168

Ellman GL (1959) Tissue sulphydryl groups. Arch Biochem Biophys 82: 70-77

Huang YL, Sheu JY, Lin TH (1999) Association between oxidative stress and changes of trace elements in patients with breast Cancer Clin Biochem 32:31-36

Kakkar P, Das B, Viswanathan PN (1984) A modified spectrophotometric assay of superoxide dismutase. Ind J Biochem Biophys 21:130-132

Lenaz G (2001) The mitochondrial production of reactive oxygen species mechanisms and implications in human pathology. Life 52:159-164

Loscher W, Mevissen M, Hausler B (1997) Seasonal influence on 7, 12-dimethylbenz(a) anthracene-induced mammary carcinogenesis in Sprague-Dawley rats under controlled laboratory conditions. Pharmacol Toxicol 81:265-270

Martinez GR, Almeida EA, Klitzke CF, Onuki J, Prado FM, Medeiros MH, Mascio DP (2005) Measurement of melatonin and its metabolites: importance for the evaluation of their biological roles. Endocrinology 27:111-118

Mates JM, Gomez CP (1999) Antioxidant enzymes and human diseases. Clin Biochem 32:595-603

Mirunalini S, Kumaraguruparan R, Subapriya R, Nagini S (2004) Garlic oil enhances hepatic and blood antioxidants during Hamster buccal pouch carcinogenesis. Pharm Biol 42(3):240-245

Niehaus WG, Samuelson B (1968) Formation of malondialdehyde from phospholipids arachidonate during microsomal lipid peroxidation. Eur J Biochem 6:126-130

Okatani Y, Wakatsuki A, Reiter RJ and Miyahara Y (2002) Melatonin reduce oxidative damage of neural lipids and proteins in senescence accelerated mouse. Neurobiol Aging 23(4):639-644

Omaye S, Turnvull CD, Sauberhich HE (1979) Selected Method for the determination of Vitamin C in animal cells, tissue and fluid. In Mcormic DB, Wright VL, E ds. Methods Enzmol 62:32-11

Reiter RJ, Tan DX, Maldonado M (2005) Melatonin as an antioxidant: physiology versus pharmacology. J Pineal Res 38:145-152

Rotruck JJ, Pope AL, Ganther HE, Swanson AB (1973) Selenium: Biochemical rates as a component of glutathione peroxidase. Science 179:588-590

Schindler R, Mentlein R (2006) Flavanoids and vitamin E reduces the release of the angiogenic peptide vascular endothelial growth factor from human tumor cells. J Nutr 136:1477-1482

Sharma S, Stutzman JD, Kelloff GJ, Steele VE (1994) Screening of potential chemopreventive agents using biochemical markers of carcinogenesis. Cancer Res 54:5848-5855

Sinha KA (1972) Colorimetric assay of catalase. Anal Biochem 47:389-394

Weydert CJ, Waugh TA, Ritchie JM, Iyer KS, Smith JL, Li L (2006) Over expression of manganese or copper-zinc superoxide dismutase inhibits breast cancer growth. Free Radic Biol Med 41:226-237