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Melatonin as a protective agent in spinal cord damage after gamma irradiation

Sara Aghazadeh[1], Mahnaz Azarnia[2], Alireza Shirazi[3], Seied Rabie Mahdavi[4], Bagher Minaee Zangii [5]

[1] Department of Biology, Faculty of Sciences, Azad University of North Tehran, Tehran, Iran
[2] Department of Biology, Faculty of Sciences, Tarbiat Moalem University, Tehran, Iran
[3] Department of Medical Physics, Tehran University of Medical Sciences, Tehran, Iran
[4] Department of Radiotherapy, Babol University of Medical Sciences, Babol, Iran
[5] Department of Histology, Tehran University of Medical Sciences, Tehran, Iran

Summary

Background Radiation causes serious damage to the spinal cord and several agents are used for protection.

Aim The aim of this study was to assess the neuro-radioprotective effect of melatonin on the cervical spinal cord.

Materials/Methods A sample of 32 male adult Wistar albino rats weighing 200–250g was used. They were divided into four groups of eight animals: control, melatonin (30mg/kg per day) and radiation (single Gamma dose of 10Gy) groups and the group that received radiation plus melatonin. After 72 hours, all rats were sacrificed for histopathological analysis of malondialdehyde, glutathione and protein biochemicals.

Results Malondialdehyde and protein levels were decreased after melatonin treatment while glutathione level was increased ($p<0.005$). Overall histopathological changes were markedly decreased after melatonin treatment in comparison to radiation group ($p<0.05$).

Conclusions In conclusion melatonin may be useful in preventing the spinal cord against radiation toxicity due to its potential for free oxygen radical scavenging.

Key words melatonin • free radical • radiation protection • MDA • GSH

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Author's address: Bagher Minaee Zangii, Faculty of Medicine, Tehran University of Medical Sciences, Tehran, Iran, e-mail: minaezb@sina.tums.ac.ir

Background

The central nervous system is irradiated in many malignant pathologic conditions and deleterious effects of gamma irradiation are common in nervous and normal supporting tissues. The extent of these side effects depends upon the radiation dose frequency and the size of the exposed area [1,2]. The most threatening types of damage in irradiated spinal cord tissue are necrosis, gliosis and white matter changes. Gamma irradiation dominantly conducts tissue damage through free radical production. Investigations have shown that oxygen free radicals may cause lipid peroxidation and oxidative stress. Melatonin, a secretory product of the pineal gland in the brain, was reported to be an effective agent in the regulation of a number of physiological and pathological processes [3]. Melatonin has been shown to scavenge hydroxyl and peroxyl radicals and peroxy nitrite anions; nitric oxide, singlet oxygen and lipid peroxyl radicals, and it acts as on antioxidant [2–4]. Melatonin has also been shown to stimulate enzymes involved in metabolizing reactive oxygen intermediates by further increasing its ability to protect against free radicals [2–7].

Aim

In this study spinal cord damage after total body irradiation in rats was investigated as well as the response of MDA, GSH and protein to such injury.

Materials and methods

The experiment was performed on 24 male adult Wistar albino rats weighing 200g to 250g. The rats were randomly divided each into four groups of eight animals. Group I was the control group, group II only received whole body radiation with a single dose of 10Gy gamma rays, group III was only injected with melatonin in a daily dose of 30mg/kg, and group IV received both melatonin and radiation simultaneously. Rats were irradiated with a Cobalt–60 teletherapy unit and after 72h they were autopsied under ketamine and chlorpromazine decapitation.

Histopathological investigation

The tissue samples after normal and routine processing were embedded in paraffin wax. Slices 4µm thick were prepared and stained with hematoxylin and eosin (H&E) for evaluation with light microscopy. Morphological evaluation of the spinal cord tissue was done blindly. Histopathological damage was scored on a scale of 0–3 (0 – none, 1 – mild, 2 – moderate, 3 – severe).

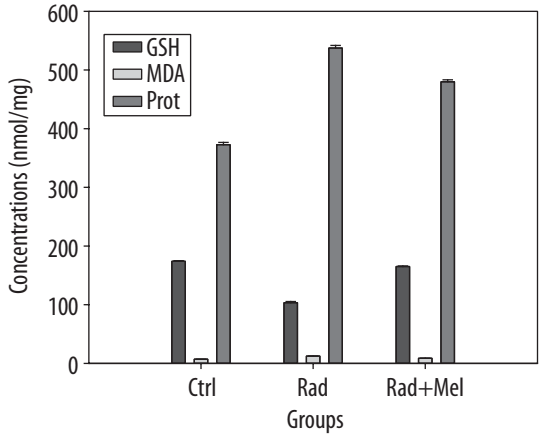

Figure 1. Comparison of mean GSH, MDA and protein concentrations of the groups vs controls.

Biochemical survey

Malondialdehyde (MDA) levels of tissue and glutathione (GSH) activity were estimated. Lipid peroxidation was determined according to the thiobarbitionic acid (TBA) method. Glutathione content was determined as explained by Kuo and Hook (1982). Total protein concentration was estimated by the Bradford method (Bradford 1979).

Results

The differences in mean GSH values of the control, radiation and radiation plus melatonin groups were found to be statistically significant ($p<0.05$). The same analysis for MDA showed significant differences ($p<0.05$) and for changes in protein concentration it was again significant ($p<0.05$) (Figure 1).

Histopathologically, mean and quantitative summation values (or total effect) of different variables in terms of inflammation, mono-nuclear infiltration, congestion, necrosis, vesiculation, demyelination and thrombosis are shown in Table 1. There were statistically significant differences in total effects of radiation and control groups ($p<0.05$). The differences between control and radiation plus melatonin groups were significant for inflammation, mono-nuclear infiltration, congestion, necrosis, vesiculation, and demyelination ($p<0.05$). Statistical differences were not significant for inflammation and mono-nuclear infiltration between radiation and radiation plus

Table1. Histopathological values in all groups.

Groups	Histopathological values: mean ±standard error (95% confidence interval)							Total effect
	Inflammation	Mononuclear infiltration	Congestion	Necrosis	Vesiculation	Demyelination	Thrombosis	
Control	0.33±0.21 (0.54)	0.33±0.21 (0.54)	0.00±0.00 (0.00)	0.00±0.00 (0.00)	0.00±0.00 (0.00)	0.00±0.00 (0.00)	0.00±0.00 (0.00)	0.66±.21
Radiation	2.50±0.22 (0.57)	2.67±0.21 (0.54)	1.67±0.21 (0.54)	2.17±0.17 (0.43)	1.34±0.21 (0.54)	3.00±0.00 (0.00)	0.83±0.17 (0.43)	13.35±.22
Melatonin	0.00±0.00 (0.00)	0.00±0.00 (0.00)	0.00±0.00 (0.00)	0.00±0.00 (0.00)	0.00±0.00 (0.00)	0.00±0.00 (0.00)	0.00±0.00 (0.00)	0.00±.00
Radiation + Melatonin	1.83±0.17 (0.43)	1.67±0.34 (0.85)	1.00±0.00 (0.00)	0.17±0.17 (0.43)	2.00±0.00 (0.00)	1.33±0.21 (0.54)	0.00±0.00 (0.00)	8.00±.34

Table 2. Correlation coefficients between histopathologic and enzymatic changes after irradiation.

Enzyme	Total pathologic effect	
	Correlation coefficients (R's)	P-values
GSH	–0.94	0.83
MDA	0.94	0.057
Protein	0.94	0.057

melatonin groups ($p>0.05$). However, t-test results showed a significant difference for demyelination and necrosis ($p<0.05$). Data of correlation coefficients (R's) from Spearman analysis are shown in Table 2 in addition to their p-values. An inverse correlation was found between histopathologic data and change in GSH concentration. However, correlation coefficients were positive for MDA and protein concentrations (Table 2). In other words, an increase in pathologic changes secondary to irradiation was associated with a decrease in GSH enzyme but an increase in MDA and proteins.

Qualitatively, irradiation significantly caused an inflammatory condition including vasodilatation, increase in vascular density, necrosis associated with neuronal degeneration and gitter cell formation (Figure 2). Melatonin may be an efficient protective agent in all pathologic conditions except for vesiculation.

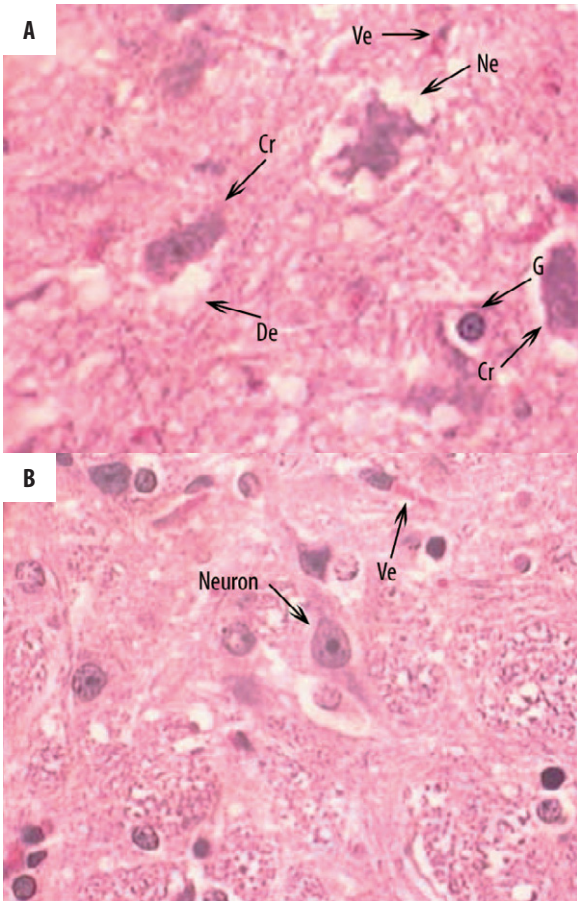

Figure 2. Some of the white matter changes are shown: (**A**) Necrotic neural cells, chromatolysis, demyelination and vascular changes are demonstrated in the irradiated group (H&E, ×100). (**B**) Changes in neural cells and in the number of vessels are shown in the melatonin treated group (H&E, ×100). Ne – Necrosis; Cr – chromatolysis; De – Demylination; ve – vessel; G – Gitter cell.

Discussion

Radioprotectors have a number of potential applications in radiotherapy. Some substances although they do not directly affect the radiosensitivity of cells nevertheless may protect whole animals because they cause vasoconstriction or

in some way upset the normal process of metabolism to such an extent that the oxygen concentration in critical organs is reduced. There is another group of radioprotectors, such as amifostine, that scavenge free radicals generated by ionizing radiation [8].

Radiation myelopathy is known as one of the most important complications of radiation in radiotherapy of patients and recent studies have demonstrated dose and time dependent radiation effects. Destructive changes of the white matter infrastructure constitute the main histopathological aspect [9,10]. Dose response curves for treatment of malignancies profoundly show a strict margin between virtual 100% tumoricidal dose and neurotoxicity occurrence in spinal cord as a normal tissue [11]. Free radical damage, particularly activated oxygen derivatives, was reported to be the significant mechanism in cell injury. Cell oedema (reversible injuries) and cell necrosis (irreversible ones) are caused by oxygen free radicals [12].

Melatonin synthesized by the pineal gland is known to play a significant role in the regulation of many physiological events [13]. It also has an antioxidant effect by scavenging hydroxyl and peroxyl radicals and peroxy-nitrite anions [14]. The *in vitro* investigation of Tan et al., 1993, was the first report of melatonin protective effects to scavenge the ·OH radicals generated when hydrogen peroxide was exposed to ultraviolet light [15]. Investigations on blood lymphocytes also showed a reduction in the incidence of abnormal cells with genetic damage. Irradiated lymphocytes exhibited significant reduction in incidence of chromosomal aberrations and micronuclei formation after the administration of melatonin (60–65%) [16]. Satisfactory results of *in vitro* investigations led to extensive *in vivo* studies [17]. The data from Mehmet et al., 2003, suggest that pretreatment with melatonin significantly lowers the extent of oxidative damage as determined by malondialdehyde levels, as well as the activities of superoxide dismutase and gluthatione peroxidase in the liver tissue of rats subjected to whole body 6Gy gamma radiation [18].

Our results revealed that the GSH level was decreased following irradiation but in the presence of melatonin it was increased in comparison to the control group. GSH was shown to be a major protective agent in oxidative injury by participating in the cellular system of defence against oxidative damage [13]. It was reported that GSH scavenges O_2. and protects protein thiol groups from oxidation. GSH's role in restoring other free radical scavengers and artioxidants, such as vitamin E and ascorbic acid, to their reduced state was also investigated [14]. It was reported that tissue GSH levels and the activities of glutathione reductase and glutathione peroxidase, which are critical constituents of the GSH redox cycle, were significantly reduced due to oxidative stress, and we propose that impairment of antioxidant defence mechanisms could permit enhanced free radical induced tissue damage [15]. In the present experiment, the decrease in spinal cord GSH concentration after irradiation can be explained by the interaction of this enzyme with free radicals induced by radiation.

Tissue level of malodialdehyde (MDA) is an index of lipid peroxidation [15]. It was recently shown that administration of melatonin before irradiation reduced MDA levels in rat liver [16]. Melatonin treatment may also prevent the tissues from morphometric damage such as that reported for seminiferous tubules and Leydig cells in rats [17]. The present study showed an increase in MDA after irradiation; however, the elevation of MDA level in the spinal cord may be significantly controlled by melatonin treatment. The MDA change shows a direct relationship with histopathological effects of radiation.

Total protein concentration was found to be reduced *in vitro* after whole body irradiation plus melatonin in comparison to the radiation group. The contribution of the immune system to defence against oxidative stress may not be a direct one [4]. On the other hand, the efficiency of the immune system improves the function of the other systems and functions in healthy tissues and the relation between antioxidant agents and tissue immune system is well known [4]. A positive correlation was demonstrated in our results between the antioxidant capacity and melatonin contents of the irradiated samples. So, supplementing the irradiated tissues with melatonin may have some benefits to overcome the serious damage with further emphasis on radiotherapy patients. Compared with radiotherapy alone, administration of melatonin in combination with radiation therapy for untreatable glioblastoma was shown to increase the survival rate with a concomitant reduction in radiation-induced toxicity [19].

Conclusions

Due to the antioxidative and free radical scavenging properties of melatonin, we suggest that

it is able to reduce irradiation-induced toxicities in cervical spinal cord. Moreover, it was shown that melatonin has the potential to reduce MDA levels and also regulate GSH concentration after irradiation. Therefore, the results of this study suggest that melatonin therapy could potentially be an ameliorating agent to reduce radiation effects due to free radicals in the spinal cord when it exists as a normal tissue in the irradiated volume.

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