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Original Article

Histopathological evaluation of melatonin as a protective agent in heart injury induced by radiation in a rat model

İclal Gürses[a,*], Murat Özeren[b,1], Meltem Serin[c,2], Neslihan Yücel[d,3], Haldun Şükrü Erkal[e,4]

[a] Mersin University Medical Faculty, Department of Pathology, Mersin, Turkey
[b] Mersin University Medical Faculty, Department of Cardiovascular Surgery, Mersin, Turkey
[c] Acıbadem University Adana Hospital, Department of Radiation Oncology, Adana, Turkey
[d] İnönü University Medical Faculty, Department of Emergency Medicine, Malatya, Turkey
[e] Sakarya University Medical Faculty, Department of Radiation Oncology, Sakarya, Turkey

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ABSTRACT

Introduction: Melatonin is a hormone which is known to be a powerful cardioprotective agent due to its free radical-scavenging properties. This study was carried out to evaluate whether melatonin administration prior to irradiation would have a protective effect on cardiac histopathological changes in an experimental rat model.
Methods: Rats were divided into four groups. Single dose of 18 Gy radiation and sham radiation exposure were used in related groups. 50 mg/kg dose of melatonin were injected intraperitonally 15 min prior to radiation exposure. Analyses and assessments were performed 6 months after radiation exposure.
Results: Severe myocardial fibrosis was observed prominently in three regions: the apex, tips of papillary muscles and adjacent to the atrioventricular valves. Inflammation was found to be more in irradiated groups. Increased inflammation and fibrosis were in concordance. The number of mast cells was found to be decreased in irradiated groups. Myocyte necrosis and fibrosis were diminished with melatonin while vasculitis was prevented.
Conclusions: Elementary pathological lesions of radiation-induced heart disease (RIHD) are fibrosis, vascular damage, vasculitis and myocyte necrosis. Development of vasculitis was prevented by the use of melatonin. Fibrosis and necrosis were prominently decreased. Prevention of RIHD with the use of melatonin at the long term is encouraging according to the histopathological results.

Introduction

Cancer and cardiovascular diseases are the two major causes of morbidity and mortality worldwide. Radiation therapy (RT) is an important treatment modality for many different types of malignancies, including breast, chest and mediastinum. In recent years, the indications and use of radiotherapy have gradually increased [1,2].

Long-term cancer survivors may suffer from late side effects of cancer therapy, one of them being radiation-induced heart disease (RIHD), which may occur after radiotherapy of thoracic and chest wall tumors whenever all or part of the heart is situated within the radiation field [3].

In the past, the heart was thought to be relatively resistant to the effects of radiation and damaged only by doses of radiation ≥30 Gy. Recent data from several independent sources have provided substantial evidence that mean heart doses of ≤20 Gy, and even ≤5 Gy, can increase the risk of cardiac damage [4].

Although modern RT techniques have reduced radiation exposure to the heart, they may not reduce the cardiotoxicity, and it appears that these methods may still give rise to cardiovascular disease [5]. Diagnosis of the RIHD is quite difficult, and this entity carries important side effects of RT. The disease can be acute

* Corresponding author at: Mersin University Hospital, Faculty of Medicine, Department of Pathology, 34.Cadde Çiftlikköy kampüsü, 33343 Mersin, Turkey. Tel.: +90 324 241000/0532 5792181; fax: +90 3243374305.
E-mail addresses: iclalgurses@gmail.com (İ. Gürses), mozeren@yahoo.com (M. Özeren), m_serin@hotmail.com (M. Serin), neslihan.yucel@inonu.edu.tr (N. Yücel), hserkal@sakarya.edu.tr (H.Ş. Erkal).
[1] Tel.: +90 0537 2116760.
[2] Tel.: +90 0532 6855056.
[3] Tel.: +90 0532 5037703.
[4] Tel.: +90 0532 2967902.

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[6], sub-acute [7] or chronic [6]. Manifestations of RIHD are pericarditis (both acute and chronic forms), coronary artery disease (accelerated atherosclerosis), conduction abnormalities, valvulitis, myocarditis and notably, pericardial and myocardial fibrosis. The disease is progressive; therefore, the manifestations may become clinically apparent several years after irradiation [8].

Melatonin, the chief secretory product of the pineal gland, has been shown to be a powerful cardioprotective agent due to its free radical-scavenging properties [9]. The present study was carried out to evaluate whether melatonin administration prior to irradiation would have a protective effect on RIHD in an experimental rat model.

Materials and methods

Animal model

All experimental procedures and protocols used in this investigation were reviewed and approved by the 'Animal Ethics Committee of İnönü University' following the approval of the study design. Study was designed as a late stage study comprising a time period of 6 months [10].

Forty male Wistar rats (ages between 10 and 12 weeks old and body weights between 200 and 250 g) were randomly divided into four groups. The number of rats in each group was ten. Each group was kept in separate cages in rooms with controlled light and temperature adjustments and was fed with standard chow and water ad libitum. Group 1 received no melatonin and underwent radiation exposure. The rats in group 2 received melatonin and underwent radiation exposure. The rats in group 3 received melatonin and underwent sham radiation. The rats in group 4 received no melatonin and underwent sham radiation.

Melatonin

For the rats in group 2 and 3, melatonin (Melatonin Crystalline, Sigma–Aldrich Corporation, Saint Louis, USA) was prepared at a concentration of 1% with dissolution in ethanol and dilution in 0.9% sodium chloride and administered at a dose of 50 mg/kg intraperitoneally 15 min prior to being exposed to radiation. 0.9% sodium chloride was prepared at an equal volume with melatonin, and the rest of the procedure was applied identically for group 1 and 4 rats.

Radiation exposure

Prior to radiation exposure or sham radiation, the rats received anesthesia using ketamine (Ketalar, Pfizer İlaçları Limited Şirketi, İstanbul, Turkey) at a dose of 80 mg/kg and xylazine (Rompun, Bayer Türk Kimya Sanayi Limited Şirketi, İstanbul, Turkey) at a dose of 5 mg/kg via an intraperitoneal injection. The rats were immobilized in the supine position on a rough surface by taping the extremities. For the rats in group 1 and group 2, radiation was delivered in the supine position on a Cobalt-60 unit using an anterior field size of 2.5 cm × 2.5 cm and a single fraction of 18 Gy defined for a depth of 2.5 cm through an anterior portal. The rest of the body was shielded with lead plates. For the rats in group 3, sham radiation was delivered on a Cobalt-60 unit over the same fraction duration. Following radiation exposure or sham radiation, the animals were closely observed until recovery from anesthesia.

Euthanasia

Euthanasia was performed to the rats at 6 months following radiation exposure or sham radiation. Prior to euthanasia, the rats received anesthesia with propofol (Propofol, Abbott Laboratuvarı Anonim Şirketi, Istanbul, Turkey) at a dose of 50 mg/kg administered via an intraperitoneal injection. Euthanasia was performed by transcardiac perfusion of 0.9% sodium chloride.

The histopathologic evaluation

Hearts were dissected rapidly from the mediastinum and kept in 10% buffered formaldehyde for 24 h. Average 3–4 mm thickness longitudinal tissue slices showing the four chambers of the heart were taken after following the routine tissue processing. Three different levels of section (5 μm thick) were obtained. Sections were stained using hematoxylin and eosin for general tissue characterization. Total collagen accumulation was determined by preparing tissue sections with Masson's trichrome stain. Mast cells were evaluated by using Toluidine blue stain. The blinded histopathological evaluation was performed under the light microscope (BX50, Olympus Corporation, Tokyo, Japan) using a semiquantitative scoring system for the severity and extent of histological parameters, in which left and right ventricles were examined separately according to the three layers of the heart (endocardium, myocardium, epicardium).

Inflammation, thrombus, fibrosis, myocyte necrosis and vascular damage were also the items used for the description of radiation injury for the ventricles. The degree of inflammation for each of layers of the ventricles was scaled from 0 to 3: (0) no, (1) mild, (2) moderate and (3) severe. Thrombus was defined as the existence (1) and absence (0). Myocyte necrosis was graded as (0) no necrosis, (1) single cell necrosis, (2) more than one cell. Description of fibrosis in the myocardium of ventricles was quantified by a graded scale from 0 to 4: (0) no fibrosis, (1) one small area affected, (2) less than 5% affected, (3) affected area between 5 and 10%, and (4) up to 10% affected (11). Mesh configuration was defined as the presence of the web form fibrosis covering each myofibril and was described as present or absent. Fibrosis of endocardial and epicardial layers were defined as the existence (1) and absence (0). In addition, vascular damage in the myocardium of ventricles was scored according to a graded scale from 0 to 3: (0) no fibrosis; thickness of the adventitia is up to 50% of the media, (1) mild fibrosis; adventitia = media, (2) moderate fibrosis; thickness of adventitia being greater than two fold of the media, (3) severe fibrosis; thickness of adventitia being greater than three fold of the media [11].

Total mast cell numbers were counted for each layer (endocardium, myocardium and epicardium) of ventricles in one longitudinal cross section in all rats.

Statistical analysis

Median item scores for fibrosis, presence of mesh configuration, thrombus, inflammation, vascular damage and necrosis were compared using the Mann Whitney *U* test. Mean values for mast cell counts were compared using the one-way analysis of variance (ANOVA). Correlation between the parameters was determined using the Bi Variate Correlation (Pierson) test. Statistical analysis was performed using SPSS for Windows software package. Statistical significance was defined as the p value being less than or equal to 0.05.

Results

Histopathologic evaluation

Inflammation

Inflammation in all groups is presented in Tables 1a and 1b according to the above mentioned criteria. The statistical results of the left ventricles were found to be as follows: inflammation at the myocardial layer reached to a significance between groups 1 and