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Biochemical Evaluation of Serum CK18 (M30/M65) Levels and Radioprotective Effect of Melatonin in Rats Irradiated at Different Dose Rate

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ABSTRACT

Purpose: Elucidating the mechanisms of radiation-induced cell death is of great importance for monitoring radiation-associated tissue damage through biomarkers and for developing novel radioprotective strategies. In this context, the present study aims to biochemically evaluate serum Cytokeratin-18 (CK18) levels (M30 and M65) in healthy rats irradiated with FF and FFF modes, and to investigate the radioprotective effect of melatonin against two different dose rates.

Methodology: A total of 48 healthy adult female Sprague Dawley rats were included in this experimental study. Rats in Groups 1 and 2 did not receive any radiotherapy. Groups 3 and 5 were exposed to radiotherapy alone at dose rates of 600 MU/min (LDR-FF) and 2400 MU/min (HDR-FFF), respectively. Group 4 received 50 mg/kg of melatonin (MLT) 15 minutes prior to LDR-FF irradiation, while Group 6 was administered the same dose of MLT 15 minutes before HDR-FFF exposure.

Findings and Conclusion: A significant increase in M30 and M65 levels was observed in Groups 3 and 5 as a result of radiation exposure. In both irradiated groups, melatonin treatment led to a reduction in these biochemical parameters. The findings demonstrate significant changes in serum M30 and M65 levels following melatonin administration, highlighting its potential as a radioprotective agent. CK18-based biomarkers are suggested for broader use in future preclinical and clinical research.

Keywords: *Radiotherapy, melatonin, radioprotection, biochemistry, Cytokeratin-18.*

INTRODUCTION

Radiotherapy has long been used in cancer treatment to destroy tumours, prevent cancer recurrence, reduce physical pain and improve patients' quality of life [1]. In parallel with the developments in modern technology, higher doses can be prescribed to the target tumour in radiotherapy applications, leading to a decrease in radiation-induced damage to surrounding critical organs. As a result of these developments, flattening filter-free (FFF) photon beams have been widely used in radiotherapy [2]. These FFF photon beams are obtained by removing the flattening filter. Flattening filters (FF) beams are beams with a homogeneous (flat) dose profile by using a flattening filter, while FFF beams are beams with a more dense-centred, inhomogeneous dose profile obtained directly without a flattening filter [3]. In stereotactic treatments (SRS/SBRT), FFF beams are preferred because of the need to deliver high doses in a short time [4].Despite these advances in radiotherapy, exposure of non-target tissues may lead to the development of undesirable side effects. This situation brings to the agenda the loss of organ function after radiotherapy and cellular damage mechanisms that trigger inflammatory response.

Cell death is one of the most fundamental biological effects of radiation. This death process usually occurs in the form of apoptosis and/or necrosis. In both

processes, destruction of cytoskeleton components occurs. One of the important markers of this destruction is cytokeratin 18 (CK18) [5]. CK18 is an intermediate filament protein found mostly in epithelial cells and is degraded in the process of cell death and passes into the circulation [6]. The cleaved form of CK18 can be detected by the M30 antibody and indicates apoptosis. On the other hand, the M65 antibody, which recognises both intact and cleaved forms of CK18, indicates total cell death (apoptosis + necrosis) occurring in the cell [7]. Therefore, measurement of M30 and M65 levels in serum is a valuable biochemical indicator in the characterisation of radiation-induced tissue damage.

Radiation causes serious cellular damage in living environments. Melatonin (MLT) can significantly reduce this damage with its antioxidant and cell protective effects [8]. MLT is a hormone secreted from the pineal gland and has strong antioxidant properties [9]. When given both endogenously (produced in the body) and exogenously (exogenous), it can reduce radiation-induced damage. MLT scavenges free radicals (ROS, RNS), activates DNA repair pathways and reduces cell death.In the literature, measurement of CK18 levels is becoming widespread in the evaluation of the effect of cytotoxic agents such as chemotherapy and radiotherapy, and is emerging as a unique approach to determine the toxic effects on non-tumour normal tissues. However, there are limited in vivo studies on how CK18 biomarkers change in fractionated radiotherapy, different dose rates and in combination with protective agents. Elucidating the mechanisms of radiation-induced cell death is of great importance for monitoring radiation-associated tissue damage through biomarkers and for developing novel radioprotective strategies.

This study aims to biochemically evaluate serum CK18 levels (M30 and M65) in FF and FFF irradiated healthy rats. In addition, the radioprotective effect of MLT against two different dose rate exposures will be analysed. By analysing these parameters, the effect of radiation on cell death mechanisms will be tried to be understood, thus providing basic data for both biochemical monitoring of radiation toxicity and development of potential protective strategies.

MATERIAL AND METHODS

Animals and Groups

Forty-eight healthy adult female Sprague Dawley rats were used in this experimental study. The rats were obtained from the Selcuk university experimental medicine research and application centre and ethical approval was obtained from the same institution. Animals were maintained under standard conditions such as 20±1◦C room temperature, 60±10% humidity, and 12/12 h light/dark cycle, and allowed free access to food and water [10, 11]. The rats were divided into six groups with 8 rats in each group. Only distilled water was given intraperitoneally (i.p.) (Group 1). A single dose of 10 mg/kg melatonin (Sigma, St. Louis, MO, USA) diluted in 1% ethanol physiological saline solution was administered to rats in this group (Group 2). Rats in group 1 and group 2 did not receive any radiotherapy. Rats in group 3 and group 5 received radiotherapy only at dose rates of 600 MU/min low dose rate (LDR-FF) and 2400 MU/min high dose rate (HDR-FFF), respectively. Group 4 rats received 50 mg/kg MLT 15 minutes before LDR-FF radiotherapy. In group 6, 50 mg/kg MLT was administered 15 minutes before HDR-FFF radiotherapy exposure.

Irradiation procedure

The rats in Group 1 and Group 2 were provided to be in the same environment with the rats in the other groups, but no radiation was applied to these groups. Before radiotherapy, the dose efficiency of the device was calibrated as 1MU = 1cGy. Output measurements were checked for two different dose rates. Except for these two groups, the other four groups were placed under general anaesthesia by administering 80mg/kg ketamine and 5mg/kg xylazine ip for immobilisation before radiotherapy. Animals in the irradiation group were immobilised in supine position. In the radiation groups, a total of 16 Gy radiotherapy was applied to the whole body in a single fraction with 10 MV photon energy using Elekta Versa HD linear accelerator device (Elekta AB, Stockholm, Sweden). The dose rate was 600 MU/min for Groups 3 and 4 and 2400 MU/min for Groups 5 and 6.

Collection of Blood Samples and Biochemical Analysis

Rats in all groups were sacrificed 48 hours after irradiation by taking blood samples from the heart under high dose anaesthesia. Blood samples were centrifuged at 3000 rpm for 5 minutes and the plasma samples obtained were stored at -80 °C until analysis. M30 and M65 assays were analysed using commercial test kits according to the manufacturer's instructions. M30 and M65 assays were performed using

commercial ELISA kits obtained from MyBiosource (San Diego, CA, USA).

Statistical analysis

Data analysis was conducted using the SPSS 25 software package. The normality of data distribution was assessed using the Shapiro–Wilk test. Since the data showed a normal distribution, differences between the groups in the biochemistry results were analysed by one-way ANOVA. In case of significant differences between the groups, pairwise comparisons were performed using the Tukey post-hoc test. Differences were considered statistically significant at $P < 0.05$.

RESULTS

The mean ± standard deviation values for the biochemical parameters M30 and M65 for cytokeratin 18 (CK18) obtained from the serum samples of the rats in the experimental groups are presented in Table 1. No significant difference was observed between the control and melatonin alone groups. A significant increase in M30 and M65 parameters was observed in Group 3 and Group 5 due to radiation exposure. For these radiotherapy groups, a decrease was observed for two biochemical parameters with MLT effect. Statistically significant difference was observed in the control group and radiotherapy groups. No significant difference was observed between the LDR-FF and HDR-FFF groups, radiotherapy alone and MLT in addition to radiotherapy groups. The comparison of statistical significance between the experimental groups is shown in Table 2. The changes between the groups for M30 and M65 biochemical parameters are presented in Figure 1.

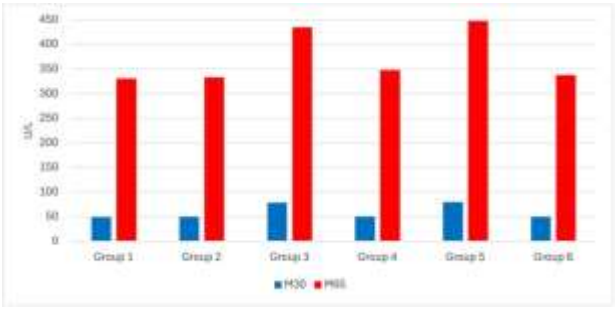

Figure 1: Variation between groups for biochemical parameters M30 and M65.

Table 1. Mean ± standard deviation values of M30 and M65 biochemical parameters obtained in the experimental.

Groups	M30 (U/L) Mean ± SD	M65 (U/L) Mean ± SD	p-values
G1	49.14±1.27	330.70±19.78	<0.001*
G2	49.24±1.49	332.60±13.56	
G3	78.16±6.61	434.95±18.64	
G4	50.30±1.14	347.38±27.44	
G5	79.49±2.19	447.76±22.86	
G6	59.85±5.60	337.74±29.91	

*Statistically significant values (p <0.05).

Table 2. p values for biochemical parameters between experimental groups.

Examined parameters	G1 vs. G2	G1 vs. G3	G3 vs. G4	G1 vs. G5	G5 vs. G6	G3 vs. G5	G4 vs. G6
M30	1.000	<0.001*	<0.001*	<0.001*	<0.001*	1.000	1.000
M65	1.000	<0.001*	<0.001*	<0.001*	<0.001*	0.982	0.998

*Statistically significant values (p <0.05).

DISCUSSION

In the present investigation, the biochemical assessment of cytokeratin 18 (CK18) concentrations in irradiated rat models was conducted utilizing M30 and M65 parameters, which serve as indicators of apoptosis and necrosis respectively. The findings elucidate that ionizing radiation precipitates both apoptotic and necrotic forms of cellular demise. Notably, the elevation in M30 concentrations signifies that radiotherapy activates intracellular caspase activities, thereby initiating programmed cell death mechanisms [12]. The pronounced increase in M65 levels suggests that radiation contributes to necrotic cell destruction [13]. This outcome implies that tissue damage following radiotherapy is not confined solely to a regulated process, but also encompasses abrupt forms of cellular death that jeopardize tissue integrity. Furthermore, this investigation demonstrates that melatonin exerts a statistically significant mitigating effect on the radiotherapy-induced elevation of CK18 levels [14]. This finding implies that melatonin serves a protective role against apoptosis and necrosis by preserving cell membrane integrity through its antioxidant and anti-inflammatory properties.

In the literature, CK18 (M30/M65) levels are generally included in the measurement of toxicity of chemotherapy drugs [15], and its use in radiotherapy is limited. The findings of this study suggest that

CK18 may be useful as a lead biomarker in radiation biology. Combined analysis of M30 and M65 may provide a more comprehensive assessment of the type of cell death.

Aras et al. observed an increase in M30 and M65 biochemical parameters due to radiotherapy in rats to which 6 MV photon energy was applied at different dose rates. M30 and M65 levels decreased significantly ($p < 0.05$) due to the use of MLT, one of the radioprotective agents, indicating that melatonin reduces radiation-induced cell death [16]. In our current study, 10 MV photon energy was preferred in order to reach higher dose rates. Although there was a difference in energy between the two studies, an increase in M30 and M65 parameters was observed, and the reduction of radiation-induced cell death by MLT was confirmed with parallel results in both studies.

Pagano et al. M30 immunohistochemistry showed a significant increase in the number of apoptotic cells directly proportional to the dose in rat liver after 20 Gy. In the comparison of 1×2 Gy vs 5×2 Gy, they showed that the apoptotic index measured by M30 was significantly increased. This study reveals that M30 may be an indicator of early apoptotic response even to low dose radiation [17]. In our current study, in parallel with Pagona et al. a significant increase was observed in M30 and M65 parameters due to cell damage as a result of radiotherapy exposure. Significant improvement in cell damage was observed with the use of melatonin.

CONCLUSION

It was observed that serum M30 and M65 parameters can be used safely in the determination of radiotherapy-induced cell damage. In rats exposed to LDR-FF (600 MU/min) and HDR-FFF (2400 MU/min) dose rates, a significant increase was observed in M30 and M65 parameters at both dose rates compared to the control group. Significant changes were observed in serum M30 and M65 biochemical parameters due to the use of melatonin, one of the radioprotective agents. CK18-based markers are recommended to be used more widely in further preclinical and clinical studies.

Conflict of Interest

There are no conflicts of interest and no acknowledgements.

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