ORIGINAL RESEARCH PAPER
Volume-7 | Issue-3 | March-2018 | PRINT ISSN No 2277 - 8179
INTERNATIONAL JOURNAL OF SCIENTIFIC RESEARCH

THE COMPARISON OF L-CARNITINE AND AMIFOSTINE IN PREVENTION OF RADIATION INDUCED CARDIAC INJURY

Oncology

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ABSTRACT

We aimed to compare the effects of amifostine (AMI) and L-carnitine (LC) in prevention of radiation induced cardiac injury. Sixty-four rats were divided into 6 groups: Control (CONT), radiotherapy (RT), AMI+RT, LC+RT, and LC. The animals in RT groups received 15 Gy/fraction by 6 MV photon, to the cardiac area. After 6 months follow-up, electrocardiograpy and histopatologic evaluation were carried out. Significant difference was found between AMI+RT and RT, LC+RT and RT, and LC+RT and CONT in terms of myocardial degeneration. Atrial fibrosis was found to be significantly different between AMI+RT and RT. Significant difference was found between groups in terms of ventricular fibrosis. LC and AMI obviously prevented the prolongation of JTa, JTac and QTc intervals after irradiation. The cardioprotective effect of AMI was superior to LC in terms of myocardial degeneration, atrial fibrosis and ventricular fibrosis.

KEYWORDS

Amifostine, carnitine, heart, radiotherapy

Introduction

Cardiac toxicity is a well-known and the most severe adverse effect of irradiation during the treatment of malignancies related to chest. It has been shown in lots of clinical studies such as Hodgkin's disease, breast cancer, esophagus, or lung cancer. This toxicity depends on whether a part of the heart remains in the field of treatment. A rare, yet serious, and late complication of irradiation is radiation induced heart disease (RIHD) which is a clinical and pathological condition.[1-4)] RIHD is most frequently encountered in the form of pericardial disease. It can occur either during or immediately after the administration of radiotherapy (RT) as acute pericarditis, and can also be encountered as effusive- constrictive pericarditis months or years after receiving radiation treatment. [5-7] Other late complications are myocardial fibrosis, cardiomyopathy, accelerated coronary artery disease, conduction anomalies, and valve dysfunctions. [7,8] Due to the prolonged survival through the new treatment methods in cancer patients, the risk of cardiovascular complications in patients who receive RT to the chest, increases by years. [9]

There has been clear evidence that thorax irradiation causes acute inflammation, and progressive fibrosis in pericardial, myocardial, and endocardial tissues (valvular and arterial). [6,10] Ionized irradiation gives rise to oxygen free radicals (OFR) in irradiated tissue. The free radicals react with molecules such as deoxyribonucleic acid (DNA), ribonucleic acid (RNA), proteins, and membranes causing either deterioration in the cell function or death of the cell. [11] To be able to control OFR, aerobic cells use a defense mechanism, which is called the "antioxidant system", including components that are both enzymatic and non-enzymatic.

Amifostine (AMI) (Ethyol; WR-2721) is an organic thiophosphate ester prodrug and must be activated by alkaline phosphatase to convert into an active sulfhydryl compound (WR-1065).[8,10] WR-1065 selectively protects normal cells against antineoplastic drug toxicity by scavenging free radicals, donating hydrogen ions to free radicals, depleting oxygen and binding to active derivatives of antineoplastic agents.[12] The protective effect of AMI against doxorubicin induced heart toxicity has been shown in several preclinical and clinical studies.[13-18] Its radioprotective effectiveness against to local heart irradiation has been also shown in previous two studies.[19,20]

L-Carnitine (LC) (3-hydroxy-4-trimethylammoniumbutyric acid) is a small water-soluble molecule that facilitates the transfer of long-chain fatty acids into the mitochondria where they undergo beta-oxidation.[11] LC decreases damage to the cell membrane by preventing the formation of OFR produced by the xanthine/xanthine oxidase system. [12] LC and its short chain esters Propionyl-L-Carnitine (PLC) and acyl L-Carnitine (ALC), due to its antioxidant and OFR cleaning qualities,

can play the role of a modulator against OFR, which may occur in connection with the ionization radiation in cells. [21] It has been demonstrated that LC played a protective role in oxidative stress induced by radiation as well as in cataract cases induced by radiation in several studies.[22,23]

We hypothesized that LC might have a potential radioprotective activity in cardiac tissue. Therefore the aim of this study is to determine and compare the radioprotective effects of AMI and LC against radiation induced cardiac toxicity with ECG analysis and histopathological method, to the best of our knowledge for the first time in the literature.

Material and methods

Animals

The experimental protocol was approved by the Ethical Committee of Trakya University Medical Faculty. Sixty-four, male, Wistar Albino rats, aged three to four months old and with an average weight of 200±28 g were used. The rats were obtained from Trakya University Animal Care and Research Unit. Throughout the experiment all rats were kept in cages that can hold up to five rats under constant laboratory conditions of 22±1 ºC room temperature, 50-60% humidity and in a 12/12 hour light/dark cycle. The rats were cleaned daily and they were all provided with feed and water containing 20% protein. A total of 64 rats were randomly assigned to six groups: control (CONT, n=10), RT (n=10), AMI+RT (n=11), LC+RT (n=12), AMI (n=11), and LC (n=10). One rat in the AMI+RT and another one rat in RT died during the study, so the analysis was done on 62 rats.

Administration of anesthesia

Rats were administered intramuscular anesthesia by means of 50-60 mg/kg Ketamine (Ketalar, Pfizer, Istanbul, Turkey) and 10 mg/kg Xylazine (Rompun, Bayer, Istanbul, Turkey).

Amifostine and L-carnitine administration

After achieving anesthesia, the animals in the LC+RT were administered 300 mg/kg LC (Cartinine ampule, Sigma-tau, Roma) 30 minutes intraperitoneal (i.p.) prior to RT, while the animals in the AMI+RT were given 200 mg/kg AMI (Ethyol, Erkim, Istanbul, Turkey) 30 minutes i.p. prior to RT.[20,21] On the day of the application, 0.9% of saline solution was administered to the CONT and RT groups.

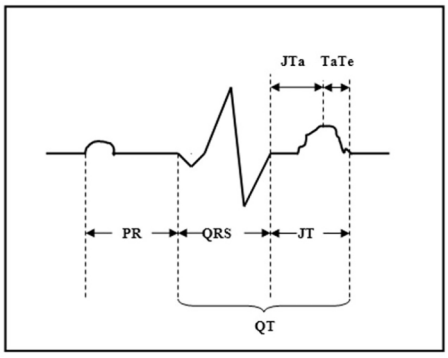
Irradiation

RT was applied externally to the heart area of the animals in the RT, LC+RT and AMI+RT. Following the administration of anesthesia each rat was stabilized in prone position on blue foam (Styrofoam, Med-Tec, Orange City, IA). Irradiation was delivered by a 6 MV-X radiation via a linear accelerator device (2100 C/D, Varian Medical Systems, USA) at a source–skin distance of 100cm. Rats were irradiated individually using an anterior 3×3cm sized single field with a depth of 2.5cm with a 1.5cm bolus. Through the field, single doses of 15Gy were given at a dose rate of at 600cGy/min to the whole heart. Radiographs of rats in the prone position were obtained during anesthesia with a simulator, and multileaf collimators were used to protect lungs. Special dosimetry was done for the irregular field. The dose homogeneity across the heart was ±3%. The control rats were sham irradiated.

Electrocardiography

At the end of the six-month period rats in all groups were administered anesthesia after and fixed in supine position on a wooden surface. The electrode constructed from 26 gauge hypodermic needle were placed subcutaneously in the gently extended limbs of the supine animal. Standard leads (I,II,III) and augmented limb leads (aVR,aVL,aVF), and precordial leads (V1,V3, and V5) were used to record ECG at paper speed of 50mm/sec by using a dedicated ECG device (MP35 USB Data Acquisition Unit, Biopac System, USA). For precordial leads electrodes were placed subcutaneously on chest sites corresponding to the position in a human subjects. Sensitivity was adjusted to provide a deflection of 20mm for 1mv standard square wave. Eleven parameters were measured and evaluated from the ECG records (PR interval, QRS wave, QT interval, JT interval, JTa interval, TaTe interval, RR interval, and QTc, JTc, JTac, TaTec parameters that are corrected according to the heart rate; Figure 1). All ECG parameters were evaluated by an expert cardiologist was unaware of the treatment groups.

Figure 1. Parameters of Electrocardiography



Histopathological analysis

A total of 62 rats were sacrificed after six months. After 24 hours of formaldehyde fixation, heart tissues of the rats were sliced by means of three transverse cuts passing through the apex, basis and center of the ventricle after which tissue follow-up was initiated. Following the tissue follow-up, the tissues were immersed in paraffin and sections with a thickness of 4 microns were taken. The sections were subjected to haematoxylin-eosin staining to be examined under an optical microscope for myocardial and vascular changes. The tissues were also subjected to staining by means of Masson Trichrome and Gieson plastic dyes for purposes of fibrosis evaluation.

The myocardial degeneration was graded and evaluated according to the semi-quantitative method of Billingham.[24] This grading system is based on the percentage of myocytes showing myofibrillar loss, and cytoplasmic vacuolization: 0 = no damage, 1 = < 5%, 1.5 = 5-15%, 2 = 16-25%, 2.5 = 26-35% and 3 = >35%. Vascular damage, ventricular fibrosis, and atrial fibrosis were evaluated according to the study of Kruse et al.who researched the effects of AMF in heart damage due to radiation (Table 1).[19]

Table 1. Vascular damage, ventricular fibrosis and atrial fibrosis were evaluated according to Kruse et al.[19]

Grade	Vascular damage	Ventricular fibrosis	Atrial fibrosis
0	no fibrosis; thickness of the adventitia ≈50% media	no areas affected	no fibrosis
1	mild fibrosis; adventitia = media,	one small area affected	fibrosis in the epicardial layer
2	moderate fibrosis; adventitia ≈2 x media	less than 10% affected	fibrosis in the epi- and myocardial layer
3	severe fibrosis, adventitia > 3 x media.	up to 20% affected	fibrosis in the epi-, myo- and endocardial layers

Statistical analysis

The statistical evaluation was performed by using STATISTICA AXA 7.1 Statistics Program. On the data which could be measured the test to determine compliance with normal distribution was performed. As the data was not compliant with normal distribution, Kruskal-Wallis variance analysis was used in comparisons between the groups and on the ones that were revealed to be significant, the Mann Whitney U test for comparison of two groups was applied while Kolmogorov Smirnov Two Sample test was used when the expected value was less than 5 in tables of 2xn arrangement. As defining statistics, mathematical average ±SS values were provided. The level of significance for all statistics was taken as $p < 0.05$.

Results

Histopathological analysis

The comparison of the CONT and RT groups showed significant difference according to myocardial degeneration, vascular differentiation, ventricular fibrosis, and atrial fibrosis ($p<0.001$; Table 2-5).

Table 2. Myocardial degeneration grades and rates in different groups

Myocardial Degeneration Grades	Groups					
	RT* (n=9)	CONT** (n=10)	LC+RT (n=12)	LC (n=10)	AMI+RT (n=10)	AMI (n=11)
0	-	5 (%50)	-	6 (%60)	3 (%30)	6 (%55)
1	-	5 (%50)	4 (%33)	4 (%40)	3 (%30)	4 (%36)
1,5	-	-	2 (%17)	-	3 (%30)	1 (%9)
2	1 (%11)	-	5 (%42)	-	1 (%10)	-
2,5	3 (%33)	-	1 (%8)	-	-	-
3	5 (%56)	-	-	-	-	-

RT: Radiotherapy, CONT: Control, LC: L-Carnitine, AMI: Amifostine
*RT group; Comparison of CONT, LC+RT with AMI+RT (p<0,01)
**Comparison of CONT with LC+RT (p<0.02)

Table 3. Vascular degeneration grades and rates in different groups

Vascular Degeneration Grades	Groups					
	RT* (n=9)	CONT (n=10)	LC+RT (n=12)	LC (n=10)	AMI+RT(n=10)	AMI (n=11)
0	-	8 (%80)	4 (%33)	7 (%70)	2 (%20)	5 (%45)
1	1 (%11)	2 (%20)	4 (%33)	3 (%30)	3 (%30)	5 (%45)
2	6 (%67)	-	3 (%25)	-	4 (%40)	1 (%9)
3	2 (%22)	-	1 (%8)	-	1 (%10)	-

RT: Radiotherapy, CONT: Control, LC: L-Carnitine, AMI: Amifostine
*Comparison of RT group with CONT (p<0.01)

Table 4. Ventricular fibrosis grades and rates in different groups

Ventricular Fibrosis Grades	Groups					
	RT* (n=9)	CONT** (n=10)	LC+RT*** (n=12)	LC (n=10)	AMI+RT (n=10)	AMI (n=11)
0	-	10 (%100)	1 (%8)	9 (%90)	7 (%70)	9 (%82)
1	1 (%11)	-	9 (%75)	1 (%40)	2 (%20)	2 (%18)
2	7 (%78)	-	1 (%8)	-	1 (%10)	-
3	1 (%11)	-	1 (%8)	-	-	-

RT: Radiotherapy, CONT: Control, LC: L-Carnitine, AMI: Amifostine

* Comparison of RT group with CONT, LC+RT, and AMI+RT (p<0.01)

** Comparison of CONT with LC+RT group (p<0.01)
*** Comparison of LC+RT with AMI+RT (p<0.05)

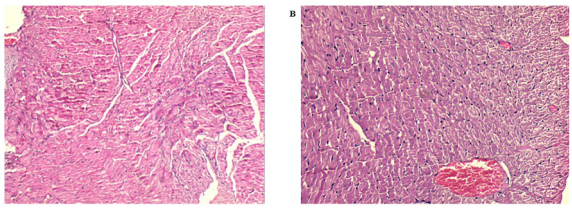
Table 5. Atrial fibrosis grades and rates in different groups

Atrial Fibrosis Grades	Groups					
	RT* (n=9)	CONT (n=10)	LC+RT (n=12)	LC (n=10)	AMI+RT (n=10)	AMI (n=11)
0	-	6 (%60)	4 (%33)	7 (%70)	3 (%30)	9 (%82)
1	2 (%22)	4 (%40)	4 (%33)	3 (%30)	6 (%60)	2 (%18)
2	6 (%67)	-	4 (%33)	-	1 (%10)	-
3	1 (%11)	-	-	-	-	-

RT: Radiotherapy, CONT: Control, LC: L-Carnitine, AMI: Amifostine
*Comparison of RT group with CONT and AMI+RT (p<0.03)

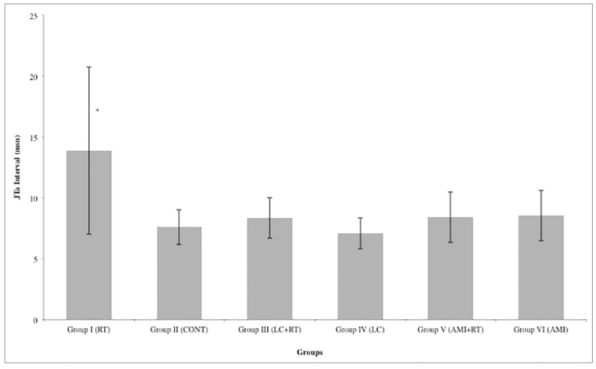
For myocardial degeneration definite myofibril loss in the muscles as well as cytoplasmic vacuolization was observed particularly in the RT group. In the LC+RT group there was a nominal amount of myocardial degeneration (Figure 2A). In the AMI+RT group a clear improvement in myocardial degeneration was seen (Figure 2B). There were significant differences between AMI+RT and RT (p=0.001), LC+RT and RT (p=0.003), and LC+RT and CONT (p=0.016). Additionally, results were similar between CONT and AMI+RT (p=0.4), and AMI+RT and LC+RT (p=0.347) (Table 2). The most important difference between the AMI+RT and LC+RT groups is in the rate of the grade 0 degeneration: when AMI is added to RT, is seen at the rate of 30% while the addition of LC did not generate any protection at grade 0 level.

Figure 2. A- Partial improvement of myocardial degeneration in L-Carnitine + Radiotherapy Group (H&E x 50), B- Improvement of the alterations of myocardial muscle bundles in Amifostine + Radiotherapy group similar to Saline group (H&E x 50)



For vascular differentiation, in the RT group definite vasculitis findings and fibrosis were determined with grade 2 and 3 vascular damage in majority. There is no statistical difference between AMI+RT and LC+RT with CONT and RT in vascular differentiation (AMI+RT-CONT, p=0.759; LC+RT-CONT, p=0.186; AMI+RT-RT, p=0.471; LC+RT-RT, p=0.084). The effects of LC and AMI were not observed in preventing vascular damage (Table 3). Furthermore, in a comparison of the LC+RT and AMI+RT groups a statistically significant difference could not be identified (p=0.998).

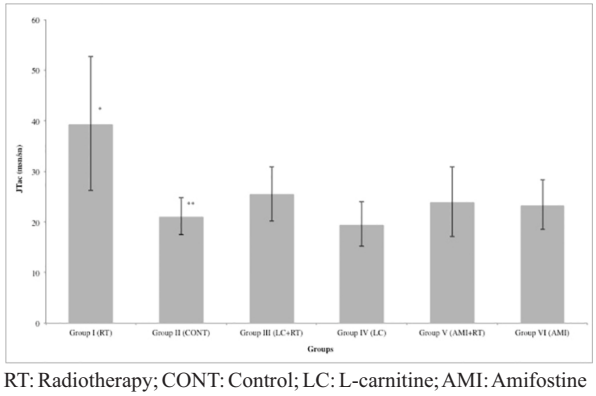
Figure 3. JTa intervals of different groups and standart deviation



RT: Radiotherapy; CONT: Control; LC: L-carnitine; AMI: Amifostine
$\chi 2$=19.674, p=0,00 1* RT-CONT, p=0.001; RT-LC+RT, p=0.004; RT-AMI+RT, p=0.008

For ventricular fibrosis, definite cases of fibrosis were observed in the RT group with the majority being grade 2 fibrosis. There were significant differences between AMI+RT and RT, LC+RT and RT, and AMI+RT and LC+RT (p=0.009, p=0.006, p=0.032, respectively) (Table 4). AMI was more effective in preventing ventricular fibrosis. On the other hand, in the LC and AMI groups, a large amount of grade 0 ventricular fibrosis similar to the CONT group were observed, while grade 1 ventricular fibrosis was observed only in one case in the LC group and in two cases in the AMI Group.

Figure 4. JTac intervals of different groups and standart deviation



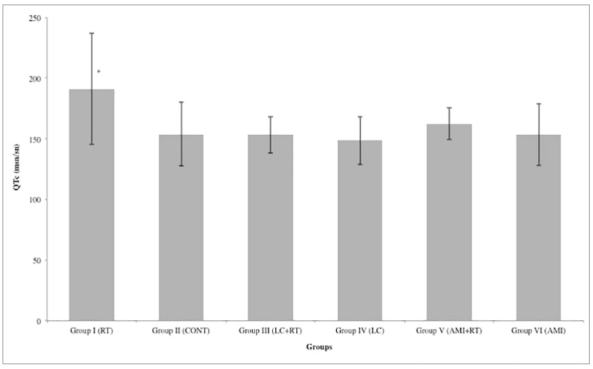
RT: Radiotherapy; CONT: Control; LC: L-carnitine; AMI: Amifostine
$\chi 2$=23.417, p<0.001

*RT-CONT, p<0.001; RT-LC+RT, p=0,007; RT-AMI+RT, p=0,007
**CONT-LC+RT, p=0.055

For atrial fibrosis, in the RT group connective tissue increase on epicardial, myocardial, and endocardial surfaces was determined. There was a significant difference between AMI+RT and RT (p=0.026). However, results were similar between AMI+RT and CONT (p=0.759), LC+RT and RT (p=0.579), LC+RT and CONT

(p=0.186), and AMI+RT and LC+RT (p=0.928). Although upon adding LC to RT an increase in the percentage of lower grades was observed in comparison to the RT group, grade 2 atrial fibrosis was also observed at a rate of 33%. It can be said that adding LC to RT has a partial protective effect that does not attain histopathological statistical significance (Table 5).

Figure 5. QTc interval of different groups and standart deviation



RT: Radiotherapy; CONT: Control; LC: L-carnitine; AMI: Amifostine χ^2=12.595, p=0,027.

* RT-CONT, p=0,027; RT-LC+RT, p=0,004; RT-AMI+RT, p=0,045.

Electrocardiography

A significant difference in terms of the heart rate (RR interval) was determined (χ^2=14.644, p=0.012) between the groups. Although there is a statistical tendency pointing to the effect of slowing the heart rate of RT, a statistical significance could not be found (RT-CONT, p=0.055). Furthermore, the addition of AMI and LC did not create any difference. The parameters that were corrected according to the heart rate due to the differences in heart rates were assessed to be QTc, JTc, JTa, TaTec.

The evaluation of ECG parameters revealed a significant difference in JTa, JTac and QTc between the CONT and RT groups (p<0.03). The addition of LC and AMI to the radiotherapy obviously prevented the prolongation of JTa, JTac and QTc intervals (p<0.05; Figure 3-5).

Discussion

Although it is generally believed that the heart is quite resilient against any damage on the basis of RT, the risk of developing a cardiovascular disease as a result of RT administered to the thorax is a serious problem.[7] In this study it has been observed that 15 Gy cardiac RT causes in rats vascular damage, atrial fibrosis, myocardial degeneration, and ventricular fibrosis; that AMI protects the heart against atrial fibrosis, myocardial degeneration, and ventricular fibrosis; that the addition of LC reveals better results in comparison to the RT administered group, but where myocardial degeneration and ventricular damage are concerned, the protective effect in comparison to the control group was not at the desired level; that although it does not have a statistically significant protective effect against vascular damage and atrial fibrosis, it has been shown to reduce the RT linked heart damage histopathologically; that although AMI and LC were found statistically similar in terms of atrial fibrosis, myocardial degeneration, and vascular damage, the protective effect of AMI against myocardial degeneration, and ventricular fibrosis were determined to be stronger than the protective effect of LC; that AMI displayed a protective effect against atrial fibrosis, against which LC had no effect; and that RT administered to the heart caused the extension of JTa, QTc and JTac while LC and AMI prevented any extension of JTa, QTc, JTac intervals with similar effects.

It must be emphasized here that the clinical side effects observed in the heart following RT cannot be fully understood in terms of radiobiology. The reason for this could be the fact that the heart in comparison to the other organs contains different radiosensitive and vital structures, such as pericardium, myocardium, conduction system and vascular structures.[25] In various animal models the histopathological response of the heart following RT has been researched.[26,27] The majority of information on rats is on myocardium. Pathological changes in the heart following RT were characterized by small myocardial degeneration focal points, perivascular, and interstitial fibrosis as reported by Fajardo and Steward, Lauk et al., and Schultz-Hector.[26,28,29] Schultz-Hector determined myocardial degeneration in rats and rabbits after 70 days following a single dose 20 Gy X radiation (300 kV) administration while Lauk observed a similar effect after 120 days of administering 15 Gy single fraction x-ray.[29,30] When the linear quadratic model is used, it can be seen that the single dose of 15 to 20 Gy applied to the heart is the bio-equivalent of 40-60 Gy total clinical RT regime administered as 2 Gy of 20-30 daily fractions. In this calculation the alpha/beta ratio for myocardium has been assumed as 3.7 Gy.[31] For clinical symptoms to surface following low doses, 300 to 400 days must elapse.[31] In our study, the radiation was given by means of a linear accelerator, using a single anterior area of no angle. The administered dose of 15 Gy was in compliance with the literature and in the histopathological examination held after 180 days it was determined that RT caused vascular degeneration, atrial fibrosis, myocardial degeneration, and ventricular fibrosis.

The most frequently researched and popular radio-protector agent is AMI. AMI due to its high in vivo activity and medium level of toxicity is used as a radio-protector that reinforces the therapeutic effect of radiation. [32,33] In empirical studies it has been shown that the toxic effect of many chemotherapeutic agents including doxorubicin was reduced by AMI. [34] It has been reported that both AMI and WR-1065 (dephosphoryl metabolite of AMI) increase the viability of cardiac myocytes in rats that were subjected to doxorubicine.[13] Ohnishi et al. showed by means of the mitochondria preparations taken from the rat hearts that the cardiac protective effect of AMI is associated with antioxidant activity and the AMI reduced OFR that occurs due to doxorubicine.[35] Dobric et al. on the other hand determined that cardiac damage caused by doxorubicine is reduced by AMI (300 mg/kg).[14] Herman et al. administered for a period of 12 weeks 200 mg/kg i.p. AMI half an hour prior to a weekly does of doxorubicine 1 mg/kg, IV and determined that the use of AMI reduced myocardial degeneration.[36] In our study, 200 mg/kg AMI was administered before a single dose of RT in rats.

In the literature that could be accessed there are two studies showing the protective effect of AMI against heart irradiation.[19,20] Kruse et al. administered single fraction of 0, 15, 20, 22.5 Gy thorax RT to 12-week-old 250-300 g female Spraque-Dawley rats, by using 250 kV X ray and 15-20 minutes prior to this application also administered a single dose AMI (160 mg/kg) to the rats.[19] In the histopathological examination conducted after six months ventricular changes, atrial fibrosis and vascular damage were assessed. Definite pathological changes linked to RT were evident only in groups that received 20 Gy or higher doses of RT. After administration of 20 Gy RT, structural changes characterized by interstitial fibrosis and perivascular fibrosis were observed mostly in the sub-endochondrial areas and the apex of the left ventricle. The histopathological changes that occur after the administration of AMI have been determined to be qualitatively similar but less dense in nature. The animals in the control group displayed normal myocardial morphology. The studies have determined that 20 to 22.5 Gy of RT causes cardiac damage and that AMI histopathologically helps reduce the score of the damage. In the group that received AMI and 15 Gy RT, the histopathological scores obtained were better than the group that received only 15 Gy RT; however, a p value as a result of a statistical analysis was not provided and it was stated that qualitatively 15 Gy of RT did not cause cardiac damage. Consequently, Kruse et al. reported that following a heart RT, reduction in histopathological changes in the AMI branch of the study were observed.[19]

Different to the study of Kruse et al., after a period of six months following the administration of 15 Gy RT, statistically significant levels of atrial fibrosis, myocardial degeneration, ventricular fibrosis and vascular degeneration were observed only in the RT administered group.[19] The reason for this could be the difference in the energy levels of the x-ray used. Moreover, a decrease in the histopathological changes were observed with the addition of AMI to RT, but in our study a statistically significant difference in atrial fibrosis and ventricular fibrosis was observed in a manner supporting the addition of AMI. Furthermore, a decrease in histopathological changes in regards to vascular damage was observed in line with the other study. In our study, a histopathological evaluation of atrial fibrosis, ventricular fibrosis and vascular degeneration was carried out on the basis of the scale used by Kruse et al., and as such we are of the belief that a comparison of data would prove useful.[19]

Tokatli et al., in the rat study, administered a single fraction of 15 Gy RT to the heart by using a Co-60 device and prior to the RT they administered 200 mg/kg of AMI. In the evaluation carried out after 100 days, they determined a nominal myocardial generation at the rate of 33% in the RT branch.[20] And they stated that this rate was statistically significant when compared to AMI-RT and the control group (p=0.042). Myocardial degeneration was not determined in the AMI-RT and control groups. In our study, it was determined that AMI has a protective effect against myocardial degeneration. This result was in keeping with conclusions drawn in the study of Tokatli et al.[20] In our study, a histopathological evaluation was conducted after six months (180 days) and as such more severe scores in terms of myocardial were obtained in comparison to the study of Tokatli et al., while the addition of AMI to RT produced lower degeneration scores.[20] For this reason it can be said that the damage in the heart due to RT may surface at a later stage and that AMI has a protective effect against myocardial degeneration.

The radio-protective effect of carnitine in some tissues has been shown in various experiments conducted on animals. In the study of Dokmeci et al. LC was administered to adult rats at a dose of 50 mg/kg by gavage for a period of 15 days and following that, 8 Gy whole body irradiation in single fraction was conducted. In this study, the increase in the levels of plasma and liver malondialdehyde as a result of the effect of LC with RT has been shown to prevent the decrease in the level of glutathione in the liver, as well as the decreases in superoxide dismutase and catalase levels of erythrocytes and the negative effects on the bone marrow. [21] Sezen et al. showed that brain damage due to radiation is prevented in 8-12-week-old rats that were administered 200 mg/kg i.p. LC followed by 15 Gy whole brain RT of single fraction.[37] In another study of the same group it was shown that the formation of cataract was reduced after a whole brain RT was administered following an injection of 100 mg/kg i.p. LC.[38] In vivo and in vitro studies have demonstrated that LC has a protective effect on myocardium where cardiotoxcity based on adriamycin is concerned. Muhammed et al. divided 18 mg/kg cumulative dose of adriamycin into 6 to be administered every two weeks in doses of 3 mg/kg i.p. with 250 mg/kg LC applied half an hour before.[39] They demonstrated that LC can be used as a clinical protective agent without disturbing the anti-tumor effect of adriamycin in cardiomyopathy that is induced by adriamycin.

Consequently, whether LC that is known to have a protective effect on heart tissue and proven to prevent many late stage RT-related side effects on various tissues other than heart, is effective in preventing cardiac damage due to RT has been investigated for the first time in this study. By analyzing the RT and chemotherapy studies that use LC, 300 mg/kg i.p. LC was applied as a single dose half an hour before RT for purposes of comparing it with AMI. Although it is not at the desired level when compared to the CONT group, the protective effect of LC against myocardial degeneration and ventricular fibrosis caused by RT has been determined. And although it is not statistically significant, it has also been shown to reduce the grade of atrial fibrosis and vascular damage.

ECG changes arising in relation to RT applied to thorax tumors have been defined in various publications.[40,41] In a large series of T-wave change incidences applied to breast cancer treatment, this has been reported as 4% for the treatment of the right breast, whereas it has been reported as 70% for the treatment of the left breast. The relevance of this sign of perimyocardial damage has only been studied for small patient groups and only over short periods of time.[42]

In the study performed on 52 pediatric cancer patients treated with anthracycline and thorax RT, an extension trend was determined in the corrected QT interval.[43] In four cases where QTc is 0.44 seconds it was reported that QTc extends with exercise, and that this could be associated with severe ventricular arrhythmia and sudden cardiac death. In another study conducted with 134 cases of surviving pediatric cancer patients, who were treated with anthracycline and/or breast irradiation, ventricular tachycardia risk in the post-treatment period on average within 5 years (3 months – 21 years) was established associated with QTc extended with RT and doxorubicin. In this study, three (12.5%) of the 24 cases were treated with chest irradiation and their QTc reached 0.44 seconds. Supraventricular premature complexes (63%), supraventricular tachycardia (4%), ventricular premature complexes (50%), and ventricular tachycardia (4%) were only seen in a more significant manner in the group treated with RT, compared to control groups.[44]

Cardiac rhythm changes, which arise following RT, possibly develop due to ischemic fibrosis, which affects the conduction system.[45] Myocardial cell death signs usually emerge at a very late stage on ECG.[4,46] Karpova et al. irradiated the entire body of dogs as a single fraction with a Co-60 device.[47] Cardiac rhythm was observed as being stable during RT and in ECG examinations performed one month later; however, pronounced tachycardia was encountered in ECGs carried out later in animals to which RT was applied compared with those that were in the same age like in control group. Tachycardia was observed together with bradycardia in most of the cases. It was seen that findings connected with left ventricular overload occurred in 75% of the dogs 3.5 to 4.5 years later, and that P-pulmonale complex, which is a typical symptom of right atrium overload, occurred in 50% of the animals 6.5 to 7 years following that. It was proven that myocardial function deteriorated in a conspicuous manner in animals exposed to radiation with the changes occurring in II, III, and aVF derivations. In another study performed by Karpova, it was established that external (Co-60) and internal (inhalation-^{239}Pu) irradiation resulted in cardiac rhythm defects characterized by sinus arrhythmia, seen in proportionately increasing rates with radiation.[48] In the study of Tokatli et al., comparisons between RT administered rats and those in the control group, 24 hours and 100 days after such RT administration, did not show any significant difference in ECGs. This was considered to be due to the short follow-up.[20]

In our study, it was determined that RT statistically significant extends the JTa interval, JTac, and QTc, and that the addition of LC and AMI to RT prevents the extension of JTa interval, JTac, and QTc. It may be said that the addition of LC and AMI to RT may have a similar protective effect in the prevention of ventricular arrhythmia and sudden death, which may develop in thorax irradiations.

In conclusion, the effect of AMI, which has been previously shown to be effective in preventing cardiac damage associated with RT, has also been justified. Our results are comparable to that found in previous studies with significant protective effect of AMI especially in myocardial degeneration, atrial fibrosis, and ventricular fibrosis. Our study seems to be the first study that showed the radioprotector effect of LC in the prevention of cardiac damage associated with RT. When compared to the CONT group, although not at the desired level, LC nevertheless has a protective effect against myocardial degeneration and ventricular fibrosis generated by RT. The protective effect of AMI was superior to LC against myocardial degeneration and atrial fibrosis. AMI was found to be more effective than LC in protecting the heart from ventricular fibrosis.

According to ECG results obtained from our study, although a tendency for arrhythmia has been determined, both agents have been observed to protect the heart against rhythm disorders. We need further studies to find out many potential functional changes regarding cardiac damage associated with RT with the help of advanced cardiac evaluation techniques (ECHO, 24-hour ECG, effort test) by increasing the follow-up period. Furthermore, we think that the potential protective effect of LC may be increased at higher doses which may be tested in comparative studies in future.

The protective effect of LC against myocardial degeneration following RT was shown; however, when compared to the CONT group, the effect was not at the desired level. The protective effect of AMI against myocardial degeneration following RT was also observed. However, the comparison between radioprotective effects of the groups LC+RT and AMI+RT did not show any significant difference (p=0.347).

Although there was not a statistically significant effect of adding AMI and LC for the prevention of vascular damage following RT, better histopathological findings were obtained in comparison to RT group. We observed grade 0 and 1 vascular damage in the LC group, similar to the CONT group, while the grade 0 vascular damage rate was reduced in the AMI group, and the grade 1 vascular damage rate was increased even with grade 2 vascular damage observed in one of the cases.

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