RESEARCH ARTICLE

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Could Amifostine Prevent Experimental Radiotherapy-Induced Acute Pericarditis?

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Abstract

Background: Amifostine is a powerful antioxidant that is one of the documented three chemo-radio prototectants recommended for clinical use. There is no data exploring amifostine in prevention of acute pericardial damage. We aimed to investigate whether amifostine has protective effect against acute pericardial injury due to radiotherapy in an experimental rat model. **Methods:** Twenty-four rats were divided into four groups: control group, radiotherapy-only group, amifostine-only group, radiotherapy+amifostine group. In groups receiving radiotherapy, hearts were irradiated with a Co 60 teletherapy device at a distance of 80 cm and 20 Gy at a depth of 2 cm. Thirty minutes before interventions, 200 mg/kg amifostine or same volume 0.9% NaCl were administered intraperitoneally. Subjects were sacrificed 24 hours after the procedure. Pericardial histopathological changes were investigated by light microscopy. **Results:** There was focal inflammation of >= 50% in all rats exposed-to-radiotherapy. All groups receiving radiotherapy revealed a significant increase in pericardial inflammation compared to the groups that did not receive irradiation ($p<0.05$). There was no difference between the radiotherapy-only group and amifostine+radiotherapy group for pericardial inflammatory response ($p>0.05$). **Conclusion:** Acute pericarditis was detected in all rats receiving radiotherapy. There was no positive effect of amifostine administration before radiotherapy on acute pericardial inflammation.

Keywords: Amifostine- prevention- radiotherapy- toxicity- pericarditis

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Introduction

Amifostine (AMI) is a powerful antioxidant and free radical scavenger. It is a pro-drug. It can not pass through the cell membrane as the thiol group is phosphorylated. Amifostine should be dephosphorylated to be converted to the active metabolite WR-1065 (Lindegaard JC et al., 2000). WR-1065 has radioprotector effect with disulfide and free thiol group in molecular structure (Castiglione et al., 2000). The radioprotective activity of amifostine is mostly due to cleavage of free radicals in the environment. However, it is also known to induce repair by giving a hydrogen atom to the damaged molecule (Kataoka et al., 2002). It is also believed that by inactivation of the enzyme topoisomerase 2 by catalysis, it is possible to save time for repair of DNA damage (Symon et al., 2001). Amifostine has a radioprotective effect in normal cells but not in tumor cells because the alkaline phosphatase enzyme activating amifostine is lower in the tumor tissue (Castiglione et al., 2000). In addition, normal cells carry the active metabolite of amifostine WR-1065 into the cell by active transport while it is by the passive transport in tumor cells. As a result, if radiotherapy (RT) is administered shortly after the infusion of amifostine, WR-1065 enters the tumor cell less. Thus, larger amount of WR-1065 passes into the normal cells for protection compared to the tumor cells (Castiglione et al., 2000). Preclinical and clinical investigations have shown that amifostine protects normal tissue, including the heart, from oxidative damage of various chemotherapeutic agents (Hensley et al., 2009; Herman et al., 2000; Jahnukainen et al., 2001). There are also a number of studies that show that amifostine is protective against the radiotherapy-induced toxicity. Amifostine shows radioprotection, especially in the salivary gland, rectum, and bone (Antonadou et al., 2002; Ben-Josef et al., 2002; Damron et al., 2001; Rudat et al., 2000; Wasserman et al., 2000).

The heart is in the field of radiotherapy area in common malignancies according to its location, such as lung cancer, breast cancer, Hodgkin lymphoma. There are only a few preclinical studies that investigate the protective effect of amifostine from radiotherapy-induced cardiotoxicity (Kruse et al., 2003; Tokatli et al., 2004; Trajković et al., 2007). These studies all investigated the protective effect

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of amifostine on the myocardial structure and function in the middle and long term.

Pericardial disease is a common manifestation of radiation-induced heart disease and is usually involved. In an autopsy study of radiation-induced heart disease, radiation-induced pericardial disease was recorded in 70% (Veinot et al., 1996). It has been demonstrated by both clinical and experimental investigations that pericardium is affected both in the early and late phase depending on radiotherapy fraction dose, total dose, and cardiac volume covered by irradiated area (Stewart JR et al., 1968; Veinot et al., 1996). To the best of our knowledge there is no study investigating the effect of amifostine on radiotherapy-related acute pericardial injury. In this study, we aimed to investigate whether amifostine has protective effect against acute pericardial injury due to radiotherapy in an experimental rat model. As a secondary output, the effect of amifostine on acute myocardial injury was also studied.

Materials and Methods

Experimental design and irradiation

A total of 24 Sprague-Dawley rats were included. They were kept in normal laboratory conditions at room temperature (22°4/-2°C) using a 12/12-hour light/dark cycle and provided with commercially available rat chow and tap water ad libitum.

In each study group, there were four groups composed of six rats

Control group (CG)

After the rats were anesthetized with ether, 0.9% saline was injected intraperitoneally. After stabilization with body mask for cardiac radiotherapy field detection, simulation was performed for RT. However, they did not underwent RT and formed the control group.

Radiotherapy only group (RG)

After the rats were anesthetized with ether, 0.9% saline was injected intraperitoneally. After stabilization with body mask for cardiac radiotherapy field detection, simulation was performed for RT. After 30 minutes of injection, the heart was treated with 20 Gy of RT.

Radiotherapy plus amifostine group (RT+AMI)

After the rats were anesthetized with ether, 200 mg/kg amifostine was injected intraperitoneally. After stabilization with body mask for cardiac radiotherapy field detection, simulation was performed for RT. After 30 minutes of injection, the heart was irradiated at a single dose of 20 Gy.

Amifostine only group (AMI-G)

After the rats were anesthetized with ether, 200 mg/kg amifostine was injected intraperitoneally. After stabilization with body mask for cardiac radiotherapy field detection, simulation was performed for RT. However, they were not irradiated.

Irradiation procedures were performed as described by Dalloz et al. (Dalloz et al., 1999). Briefly, 5x5cm single field was planned. A 7 cm thick lead plate was produced with a 1.5 cm hole in the middle. Thus, the heart was taken into the target area and the other tissues were preserved. The rats were irradiated with Co-60 teletherapy device (Alcyon, GE) at a distance of 80 cm and a depth of 2 cm.

Position and fixation of the rat for simulation and/or irradiation are presented in Figure 1. Rats were sacrificed 24 hours after the procedure.

Assesment of cardiac damage

Functional studies and subsequent histopathological evaluation were performed 24 hours after the procedures. Myocardial functions i.e. heart rate, mean systolic and diastolic pressures were measured at rest and after isoproterenol stimulation with the Langendorff perfusion rat heart method. Consequently, half of the heart, including the pericardium, was placed in 10% formaldehyde for light microscopic evaluation. The material was passed through routine follow-up, embedded in paraffin blocks. Sections of 4-5 microns in thickness were prepared and stained. Pericardium was evaluated by light microscopy with hematoxylene eosin and mason trichrome stains for inflammation, edema and fibrosis. Myocardial histopathology was examined by light and electron microscopy. The other half of myocardium was frozen quickly and myocardial malondialdehyde levels were measured by Mihara's method (Mihara et al., 1978).

Statistical analysis

Numerical variables were given as mean ± standard deviation. Categorical variables are shown as frequencies. Two groups were compared with Mann Whitney U test. Fisher's exact test were used for 2X2 contingency tables for non-numerical data. Comparisons in the more than two groups were made by Kruskal Wallis-H analysis of variance. p values less than 0.05 were accepted as significant. SPSS (statistical package for social sciences) for Windows 15.0 program was used for statistical analysis.

Results

The weights of the rat hearts were 247 ±51 mg in control group, 253±29 mg in radiotherapy only group,

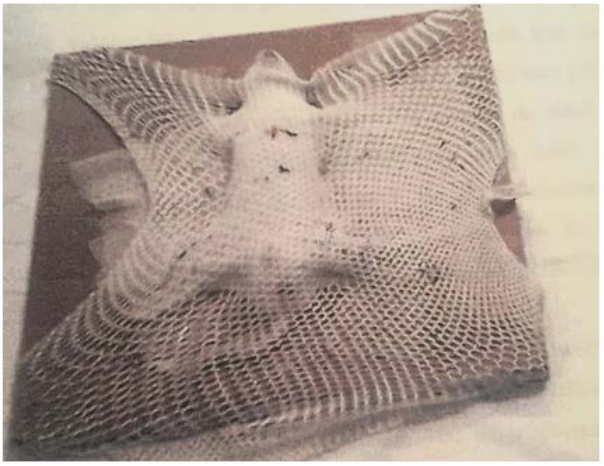

Figure 1. Position and Fixation of the Rat for Simulation and/or Irradiation.

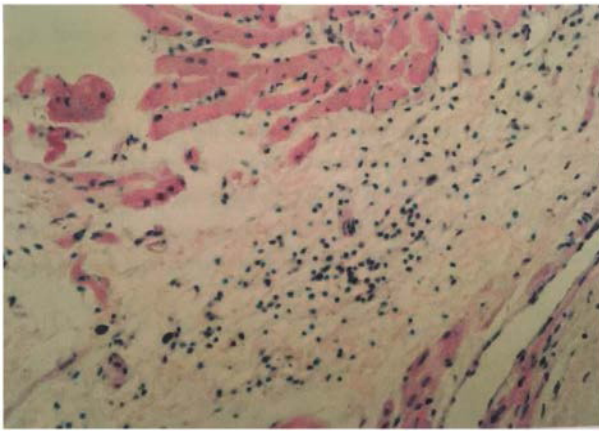

Figure 2. Lympho-plasmocytic Inflammatory Cell Infiltration on Pericardium in Radiotherapy+amifostine Group, Haematoxylin-Eosin x 200

248 ±16 mg in radiotherapy plus amifostine group, 252 ±3 mg in amifostine only group. There was no difference between the groups in terms of the heart weights ($p>0.05$).

Pericardium

Inflammation, edema and fibrosis were investigated by light microscopy. In all irradiated rats there was lympho-plasmocytic inflammatory cell infiltration >= 50-60 % in the pericardium. There was no difference in degree of inflammation and edema between the radiotherapy only group and radiotherapy plus amifostine group ($p>0.05$). In non-irradiated rats, the percentage of focal inflammation areas was between 0% and 20%, and there was no difference between control group and amifostine only group ($p>0.05$). There was a significant difference in percentage of pericardial inflammation foci between the non-irradiated groups and the irradiated groups ($p<0.05$). The light microsopic finding of lympho-plasmocytic inflammatory cell infiltration on pericardium in the radiotherapy plus amifostine group is given in Figure 2.

Myocardium

When myocardial functions were assessed; resting heart rate, resting mean systolic pressures, resting mean diastolic pressures, isoproterenol stimulated mean systolic pressures, and isoproterenol stimulated diastolic pressures were similar in all groups. There was a difference between radiotherapy only group and control group for isoproterenol stimulated heart rate, only. However, since there was no difference between amifostine only group and radiotherapy only group, it was evaluated that there was no difference between myocardial functions in all groups. Similarly, there was no difference between groups in terms of malondialdehyde levels. In all 4 groups, both the light and electron microscopic findings were normal and similar.

Discussion

Radiation-induced pericardial toxicity is one of the most common manifestations of cardiac irradiation (Madan et al., 2015). It may present as acute pericarditis, delayed pericarditis, pericardial effusion, and constrictive

Table1. Langendorff Perfused Rat Heart Parameter Values of CRG and CG

	RT	Control group	RT+AMI	AMI-G
Before Isoproterenol administration				
Hearth rate (/min)	212±35	240±42	208±5	164±44
Systolic BP (mmHg)	90±42	87±37	89±29	66±37
Diastolic BP (mmHg)	67±40	77±35	75±26	52±34
Before Isoproterenol administration				
Hearth rate (/min)	291±19	345±34	265±35	257±51
Systolic BP (mmHg)	85±39	66±11	74±22	58±31
Diastolic BP (mmHg)	61 ±28	56±9	57±18	42±23

pericarditis (Yusuf et al., 2011). Constrictive pericarditis is one of the major cardiac toxicities of radiation. Acute pericarditis may precede to late phase pericardial fibrosis and constrictive pericarditis. Chest pain, fever and ECG abnormalities are the most common clinical sign and symptoms of radiation-induced acute pericarditis. Contrast material–enhanced CT or MR imaging shows pericardial effusion with pericardial enhancement, a finding indicative of inflammation (Walker et al., 2013). Hooper et al. reported that in an emergency department 15.6% of 179 patients diagnosed with acute pericarditis received radiotherapy in the pericardial area (Hooper et al., 2013). It is well known that pericardial inflammation is frequently seen on the pericardium in the acute phase of post irradiation (Fajardo et al., 1970; Lauk et al., 1985; Schultz-Hector, 1992). In our study, there was widespread inflammation in the pericardium histopathologically in both the radiotherapy only group and radiotherapy+amifostine group. There was no significant pericardial inflammation in the groups without RT concurrent with the classical literature. The degree of inflammation in the radiotherapy given groups were not diffferent among the radiotherapy only group and radiotherapy plus amifostine group. Hence, our study demonstrated that amifostine failed to exert any protective effect against RT induced acute pericardial injury.

The reason why amifostine failed to protect against acute pericardial injury is not clear. Amifostine is somewhat considered as a standard radioprotector. It is one of the three chemo-radio prototectants according to the last guideline of American Society of Clinical Oncology (Hensley et al., 2009). However, it seems that amifostine has different radioprotective effect in different normal tissues. It has been reported that amifostine protected the intestinal mucosa during the intestinal irradiation. In a study by Ben-Josef et al. the effects of intrarectal application of amifostine were evaluated in the prevention of late rectal injury and amifostine administration of 1500-2500 mg was reported to reduce rectal injury (Ben-Josef et al., 2002). It was also shown that amifostine that is administered before fractionated bone irradiation protected the bone growth (Damron et al., 2001). Symon et al. demonstrated that amifostine protects hepatocytes from RT damage in liver irradiation (Symon et al., 2001). Nevertheless, amifostine could not be positioned for routine use in protection of these tissue injuries related to the RT. Amifostine, however, provides good

protection, especially in the salivary gland, and reduces the xerostomia. Prophylactic use of amifostine during RT of head and neck cancer prevented the acute and late-onset RT toxicity i.e. the oral mucositis and xerostomia (Antonadou et al., 2002; Rudat et al., 2000; Wasserman et al., 2000). Accordingly, the American Society of Clinical Oncology (ASCO) recommends the use of amifostine to decrease acute and late xerostomia with fractionated radiation therapy alone for head and neck cancer on their last revised guideline (Hensley et al., 2009). In this study, amifostine did not protect the heart from the radiation-related acute pericardial damage. Pericardium is a passive biological membrane as is the duramater. It is known that amifostine can not pass the blood-brain barrier and therefore amifostine is reported to not be able to protect the central nervous system (Washburn et al., 1976). One can suggest that amifostine may not reach adequate concentration in the pericardial tissue. This may explain why it could not exert any protective effect in the acute pericardial injury. This point was beyond the scope of our study therefore we can not comment further. Future studies specifically designed on this issue may help to comment more. On the other hand, we examined the acute pericardial inflammation that is at the end of day 1. It is still possible that amifostine may exert radioprotective effects on mid-long term pericardial injury which might be apparent at the following days. We suggest that these points are area for future research.

There are limited studies investigating whether amifostine is a chemoprotector or radioprotector on the heart. Experimental studies reporting positive effects on anthracycline cardiotoxicity have been published (Herman et al., 2000; Jahnukainen et al., 2001). There are only a few studies investigating whether amifostine has a protective effect on cardiotoxicity in heart irradiation (Kruse et al., 2003; Tokatli et al., 2004; Trajković et al., 2007). All the published studies investigating the cardioprotective effect of amifostine focused on cardiomyopathic heart injury. After a single dose of 20 Gy radiation, myocardial dysfunction begins between the 50th and 80th days and reaches a maximum at the 100th day (Madan et al., 2015). Initial structural changes in the myocardium after radiotherapy begin with ultrastructural loss of alkaline phosphatase at the 25th day after radiation in Wistar rats. In Spraque-Dawley rats, this time extends to around 30 days because of higher preirradiation alkaline phosphatase levels (Schultz-Hector et al., 1994). In 2003, Kruse et al. investigated the cardiac toxicity in rat hearts after 6 months of single dose cardiac radiotherapy. They have shown that amifostine prevents myocardial functional deterioration and myocardial histopathologic changes such as interstitial and perivascular fibrosis (Kruse et al., 2003). Tokatlı et al. also investigated the role of amifostine on the prevention of radiotherapy-induced cardiotoxicity. They observed that myocardial degeneration due to irradiation was significantly less in the amifostine receiving group in the late phase (Tokatli et al., 2004). In their study investigating the radioprotective effects of fullerenol, Trajkovic et al.considered amifostine as a standard radioprotective agent and confirmed amifostine as an effective radioprotective agent for both degenerative and vascular damages by histopathological evaluation of myocardial and coronary arteries on days 7 and 28 after RT (Trajković et al., 2007). Studies on the cardioprotective effects of amifostine on radiation-related injury generally report favorable mid and long-term outcomes on radiation-related myocardial damage. Our study has examined acute myocardial radiotherapy-induced injury. In our study, myocardial functions, malondialdehyde levels, and structure were found to be normal at 24 hours after irradiation. It was also similar between both irradiated and non-irradiated groups. These results are consistent with published studies investigating early myocardial findings after irradiation (Schultz-Hector et al., 1994; Schultz-Hector et al., 1992).

In conclusion, we studied the radioprotective effect of amifostine on radiation induced acute pericardial and myocardial injury. This study represents the first report examining the radioprotective effect of amifostine on radiation induced acute pericardial injury. Amifostine failed to exert any protective effect against irradiation related acute pericardial injury. The reason why amifostine failed to protect against acute pericardial injury is not clear and is an area for future reseach. Accordingly, new studies are needed to develop possible radioprotectants against acute pericarditis that represents the most common complications of cardiac radiotherapy. We also confirmed that radiotherapy did not result in any acute effect on myocardium evaluated by light microscopy, electron microscopy and functional studies. This point is in accordance with the limited existing literature.

Author Contribution Statement

None.

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Abbreviations

AMI: amifostine
AMI-G: amifostine only group
CG: control group
RG: radiotherapy only group
RT+AMI: radiotherapy plus amifostine group
Gy: Gray
RT: Radiotherapy

Ethical Approval

Karadeniz Techical University Faculty of Medicine Ethic Council Sciences approved the study, approval No: 2002/04. This study is a part of an accepted master thesis “The effects of amifostine and melatonin against radiotherapy induced cardiac toxicity” that was ethically approved by also the faculty board.

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