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Radiopharmaceutical model using ^{99m}Tc-MIBI to evaluate amifostine protection against doxorubicin cardiotoxicity in rats

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The aim of our study was to use an *in vivo* radiopharmaceutical model to investigate the cytoprotective effect of amifostine against doxorubicin-induced cardiotoxicity. Male Wistar rats were randomly divided into four groups (n = 6): 1) Saline (control); 2) Doxorubicin (DOX; 10 mg/kg^{-1} intraperitoneally); 3) Amifostine (AMI; 200 mg/kg^{-1} intraperitoneally); 4) Doxorubicin plus amifostine (DOX + AMI). Amifostine was injected 30 minutes before doxorubicin in Group 4. ^{99m}Tc-MIBI, 20 MBq/0.2 ml^{-1}, was injected through the tail vein 72 hours after the drug administration. Rats were killed and samples of myocardium were removed by dissection 60 minutes after the injection of radiopharmaceutical. Radioactivity in each organ sample was counted using a Cd(Te) detector equipped with RAD 501 single-channel analyzer. The percent radioactivity was expressed as a percentage of the injected dose per gram of tissue (%ID/g^{-1}). The %ID/g^{-1} activity was calculated by dividing the activity in each sample by the total activity injected and mass of each organ. ^{99m}Tc-MIBI uptake as %ID/g^{-1} was 1.194 ± 0.502 and 0.980 ± 0.199 in the control and AMI groups, respectively. Doxorubicin administration resulted in a significant increase in %ID/g^{-1} (3.285 ± 0.839) ($p < 0.05$). Amifostine administration 30 minutes before doxorubicin injection resulted a significant decrease in %ID/g^{-1} (2.160 ± 0.791) ($p < 0.05$) compared with doxorubicin alone. The results showed that amifostine significantly attenuated doxorubicin-induced cardiotoxicity.

Key words: radiopharmaceutical, ^{99m}Tc-MIBI, amifostine, doxorubicin, cardiotoxicity

INTRODUCTION

DOXORUBICIN, an anthracycline antineoplastic antibiotic is one of the most potent and widely used drugs in clinical oncology. It is used in the treatment of a wide variety of solid tumors and hematological malignancies including breast and gastrointestinal tumors, lymphomas and leukemias. Doxorubicin intercalates between adjacent DNA base pairs and inhibits topoisomerase II enzymes. In addition, it can undergo one electron reduction to form free radicals.[1] However, doxorubicin has several toxic effects such as myelosuppression, nausea, vomiting, alopecia, and cardiotoxicity. Cardiotoxicity is the most serious and dose-limiting side effect. The most accepted hypothesis of the mechanism of doxorubicin induced cardiotoxicity is the formation of free radicals and superoxides.[2,3] Based on the free radical hypothesis, many animal and human studies have been carried out to prevent the anthracycline-induced cardiotoxicity by using free radical scavengers and antioxidants without interfering with the antitumoral activity. Cardioprotective effects of intracellular iron chelating agent dexrazoxane, free radical scavenger *N*-acetylcysteine and antioxidants vitamin C, vitamin E and alpha-tocopherol were demonstrated. But, human studies have not confirmed the results except those of dexrazoxane.[4–6]

Amifostine (AMI, WR-2721, Ethyol) is a pro-drug that forms an activated free thiol (WR-1065) through dephosphorylation by membrane-bound alkaline phosphatase in

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normal cells.[7] AMI selectively protects normal cells from the toxic effects of antineoplastic agents by scavenging free radicals, donating hydrogen ions and binding to active metabolites of alkylating and platinum agents.[8] There are a growing number of studies reporting its marked clinical efficacy as a cytoprotective agent against the toxicities associated with chemotherapy and radiotherapy.[9] However, arguments continue about its clinical use.[10]

Radiopharmaceuticals are radioactive compounds which consist of a radionuclide and a nonradioactive compound used for diagnosis and therapy of diseases. Nuclear medicine has been using radiopharmaceuticals for diagnostic and therapeutic purposes for more than a half century. Radiopharmaceuticals are usually administered in trace quantities and exhibit no pharmacologic effect. Their pharmacokinetics and selective organ localization are the basis of their design and clinical use. Differences in their biodistribution in pathological conditions or diseases provide the diagnostic data. Considerable evidence has been accumulated that the biodistribution or pharmacokinetics of radiopharmaceuticals might be altered by a variety of drugs.[11,12] The alteration of the biodistribution of the radiopharmaceutical may be induced as a result of drug-induced toxicity.[13]

^{99m}Tc-MIBI (methoxyisobutyl isonitrile) is a synthetic, monovalent cation that is used as a myocardial perfusion agent.[14] The cellular uptake of ^{99m}Tc-MIBI is related to the cell membrane potential, and passage through the membrane is thought to involve passive diffusion[15,16] and is localized mostly inside mitochondria probably due to the high negative mitochondrial membrane potential compared with other intracellular organelles.[17]

The introduction of cytoprotective agents into clinical use is important in terms of prevention against side effects and improvement of therapeutical potential of available and investigational antineoplastic agents. In this study, we used an *in vivo* radiopharmaceutical model using ^{99m}Tc-MIBI to evaluate the cytoprotective effect of amifostine against doxorubicin-induced cardiotoxicity. Since there are many proposed cytoprotective compounds and it is difficult to demonstrate the efficacy of an agent, this simple *in vivo* experimental model may serve as a tool for first-line evaluation and comparison of the cytoprotective effects of these agents.

MATERIALS AND METHODS

Male Wistar albino rats, weighing 190–260 g, were provided by Experimental Surgery and Research Laboratories of Ege University. The animals were housed in cages in a quiet room at 22°C and 50–60% humidity and 12-h light/dark cycle. They had free access to standard commercial food and tap water. The study protocol was approved by the Institutional Animal Review Committee of Ege University. Rats were randomly divided into four groups: 1) Saline (control) (n = 6); 2) Doxorubicin (DOX; 10 mg/kg^{-1} intraperitoneally) (n = 6); 3) Amifostine (AMI; 200 mg/kg^{-1} intraperitoneally) (n = 6); 4) Doxorubicin plus amifostine (DOX + AMI) (n = 6). Amifostine was injected 30 minutes before doxorubicin in Group 4.

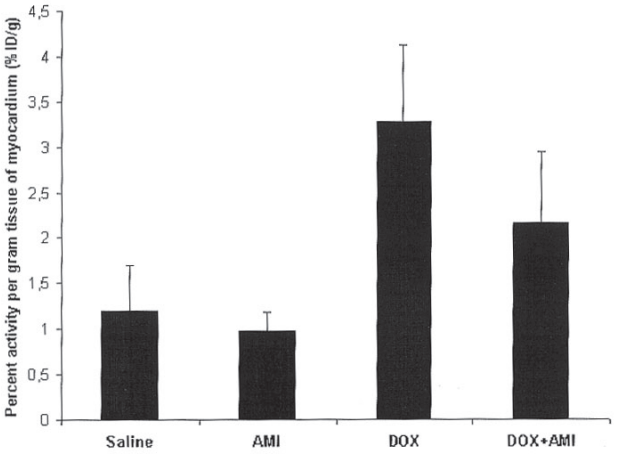

Fig. 1 The percentage of the injected activity per gram of myocardium (%ID/g^{-1}) in the saline (control), AMI (amifostine), DOX (doxorubicin) and DOX + AMI (doxorubicin plus amifostine) groups. Values are means ± SD.

Doxorubicin (Adriablastina, 10 mg, Pharmacia Carlo Erba) and amifostine (Ethyol, 500 mg, TR Erkim Ltd., Sti) were purchased commercially. Doxorubicin was reconstituted in 10 m*l* of 0.9% sodium chloride for injection. Amifostine was dissolved in 9.7 m*l* of double distilled water.

^{99m}Tc, as sodium pertechnetate, was milked from a ^{99}Mo/^{99m}Tc generator (Monrol A.S. Isranbul, Turkey) just before radiolabeling procedure. MIBI (Cardio-Spect, Medi-Radiopharma Ltd., Budapest) was purchased as a freeze dried commercial kit containing 0.12 mg methoxyisobutylisonitrile. ^{99m}Tc-MIBI was prepared by adding 740 MBq of ^{99m}Tc pertechnetate in 3 m*l* saline to the kit and boiled for 10 minutes. Quality control procedures were performed according to the manufacturer's instructions after cooling in room temperature. Labeling efficiency was greater than 95%.

^{99m}Tc-MIBI, 20 MBq/0.2 m*l*$^{-1}$, was injected through the tail vein 72 hours after the drug administration. Rats were killed by heart puncture under intense ether atmosphere, and the hearts removed by dissection 60 minutes after the injection of radiopharmaceutical. Samples of myocardium were weighed in pre-weighed containers. Radioactivity in each organ sample was counted using a Cd(Te) detector equipped with RAD 501 single-channel analyzer. The percent radioactivity was expressed as a percentage of the injected dose per gram of tissue (%ID/g^{-1}). The %ID/g^{-1} activity was calculated by dividing the activity in each sample by the total activity injected and mass of each heart.

Data were analyzed using a commercial software pack-

age (SPSS for Windows v.10, Chicago, USA). Differences among the %ID/g^{-1} activity of the hearts in four groups were analyzed by the nonparametric Kruskal-Wallis test. Data were presented as the mean ± SD. Mann-Whitney U test was used to compare two independent samples. All statistic test were 2-tailed and differences were evaluated at the 5% level of significance.

RESULTS

The percentages of the injected dose per gram of myocardium (%ID/g^{-1}) in the control (saline), AMI, DOX and AMI + DOX groups were shown in Figure 1. ^{99m}Tc-MIBI uptakes as %ID/g^{-1} were 1.194 ± 0.502 and 0.980 ± 0.199 in the control and AMI groups, respectively. Doxorubicin led to a significant increase in myocardial uptake of ^{99m}Tc-MIBI ($p < 0.05$). In the doxorubicin group, %ID/g^{-1} was the highest, 3.285 ± 0.839, among all the groups ($p < 0.05$). Amifostine administration 30 minutes before doxorubicin injection resulted in a significant decrease in %ID/g^{-1} (2.160 ± 0.791) ($p < 0.05$). Although %ID/g^{-1} in the amifostine alone group was lower compared to the control group, the difference was not significant.

DISCUSSION

In this study, we used an *in vivo* radiopharmaceutical model to test the protective effect of amifostine against the acute cardiotoxicity of doxorubicin. This radiopharmaceutical model is based on using a radiopharmaceutical which is specific to the organ of interest to demonstrate the drug effect on the biodistribution of the radiopharmaceutical. It is well-known that biodistribution of the radiopharmaceuticals may be altered as a result of drug-induced toxicity.[13,18] The rat model that we used has been shown to produce acute doxorubicin cardiotoxicity with a single dose of 20 mg/kg^{-1}.[19,20] Our quantitative measurements of the percentage of radioactivity per gram tissue (%ID/g^{-1}) obtained by biodistribution studies of ^{99m}Tc-MIBI after administration of doxorubicin and amifostine revealed significant differences between groups ($p < 0.05$). The percentage of radioactivity of ^{99m}Tc-MIBI per gram tissue of the myocardium was significantly higher in the DOX group than those of others ($p < 0.05$). Amifostine exhibited an attenuation effect on the doxorubicin-induced deviation on the uptake of ^{99m}Tc-MIBI in the myocardium.

There are several studies that investigate the toxicological effects of chemotherapeuticals by using this model on the basis of alterations on the biodistribution of the radiopharmaceuticals.[21–23] In these studies, the authors observed significant alterations in the biodistribution of radiopharmaceuticals concordant with toxic effects of the administered drugs. They justified the alterations on the biodistribution of radiopharmaceuticals by the pharmacokinetic, pharmacologic and toxic effects of the drugs. It was reported that ^{201}Tl uptake was significantly higher in the hearts of doxorubicin-treated rats compared to the control rats, indicating a slow wash-out of ^{201}Tl from the myocardium.[24] In one study, the authors evaluated the kinetics of ^{99m}Tc-MIBI in doxorubicin-treated cultured chick heart cells.[25] Contrary to our findings, they observed decreased accumulation of ^{99m}Tc-MIBI in the cells. This disagreement may be attributable to the difference between *in vivo* and *ex vivo* kinetics of ^{99m}Tc-MIBI. The uptake of ^{99m}Tc-MIBI into the myocyte is related to the cell membrane potential and thought to involve passive diffusion and subsequently ^{99m}Tc-MIBI localizes mostly inside mitochondria because of negative membrane potential. We can not definitely explain the higher %ID/g^{-1} values in the myocardium of doxorubicin-treated rats. But it can be speculated that structural and functional changes in the sarcolemma as the consequence of doxorubicin-induced toxicity and alterations on intracellular pH and Na^+ concentrations that cause membrane potential alterations may lead to increased ^{99m}Tc-MIBI accumulation in the early period after injection. Increased washout rate and decreased retention of ^{99m}Tc-MIBI can be expected due to doxorubicin-induced mitochondrial dysfunction in the late measurements, e.g. three hours after the injection. Further *in vivo* and *in vitro* studies can clarify the situation and contribute to understanding of the mechanism of doxorubicin cardiotoxicity. The main goal of the present study was to test this radiopharmaceutical model for its ability to demonstrate the drug toxicity and protection against it. Doxorubicin caused a significant alteration in myocardial uptake of ^{99m}Tc-MIBI and amifostine attenuated this effect concordant with its cytoprotective effect.

Cytoprotective agents have been intensively studies recently, because the side effects of anticancer therapies remain a considerable obstacle to treatment success despite progress in the discovery of new antineoplastic agents. Life-threatening complications or irreversible tissue damage that severely affects patient quality of life is a daily challenge in clinical oncology. Moreover, dose-limiting toxicities prevent the application of appropriate therapeutic schedules and mask the curative potential of the available agents against cancer. Cytoprotective agents are important in terms of preventing the side effects and improving the therapeutical potential of the antineoplastic drugs. Amifostine is the most intensively studied agent and is licensed for the prevention of cisplatin-related toxicity and radiation-induced xerostomia and recommended in the American Society of Clinical Oncology clinical practice guidelines for the use of chemotherapy and radiotherapy protectants.[26] There are many other potential cytoprotective agents proposed which are mostly free radical scavengers and antioxidants and it is difficult and necessitates sophisticated techniques to demonstrate the efficacy of a proposed agent for cytoprotection. We suggest that this simple *in vivo* radiopharmaceutical

model may contribute to investigation of cytoprotective agents and demonstration and comparison of their efficacy.

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