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Metformin administration increases the survival rate of doxorubicin-treated mice

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Background: The chemotherapeutic agent doxorubicin (DOX), an anthracycline broadly used to treat different types of cancers, induces several side effects, including cardiotoxicity, hepatotoxicity, and nephrotoxicity, in a time- and dose-dependent manner. Metformin (MET) is an antidiabetic drug used as a first-line treatment for type-2 diabetes, and is reported to work against various various drug-induced toxicities. This study aimed to investigate whether the administration of MET prophylactically suppresses DOX-induced toxicity, and prolongs the survival following DOX treatment. *Methods:* Fifty mice were divided into four groups, and each group received different treatments. The animals in the control group received a single injection of saline. The animals in the DOX group received a single dose of DOX (25 mg/kg). The animals in the MET group received MET on a daily basis. The animals in the DOX+MET group received only a single dose of DOX and daily doses of MET. The animals were observed on a daily basis for determining their body weight and evaluating the survival rate of the four study groups. *Results:* DOX accelerated the mortality rate of the animals in the DOX-treated group. Co-administration of MET and DOX increased the survival rate of the mice. *Conclusion:* The results of this study demonstrated that the administration of MET can reduce DOX-induced toxicity and increase the survival rate among chemotherapy-treated mice.

1. Introduction

Doxorubicin (DOX) is an anticancer drug used to treat several types of cancers (Thorn et al. 2011). The use of DOX and other anthracyclines is limited by their adverse effects, which includes cardiotoxicity, hepatotoxicity, and nephrotoxicity (Injac et al. 2009; Ayla et al. 2011). The exact mechanisms underlying DOX-induced cardiotoxicity are not fully understood (Thorn et al. 2011). One of the mechanisms of DOX-induced toxicity involves the increased generation of reactive free radicals, including superoxide anions and hydroxyl radicals (Keeney et al. 2018). Several attempts have been made to reduce the toxic effects of DOX by combining it with different medications. For instance, antioxidant agents are clinically used to decrease the harmful effects of free radicals and chemotherapy-induced toxicity.

Numerous studies conducted in the recent times have revealed that apart from cardiotoxicity, treatment with DOX causes various biological alterations, including oxidative stress, mitochondrial deregulation, lipid peroxidation, DNA damage, and apoptosis. One of the major side effects of DOX treatment is oxidative stress, which is a crucial effector responsible for causing myocardial damage (Songbo et al. 2019). DOX increases the production of free radicals and reactive oxygen species (ROS) resulting in mitochondrial dysfunction and cell damage (Yan et al. 2017). Therefore, the inhibition of uncontrolled oxidative stress could prevent DOX-induced cardiotoxicity and improve the survival rate.

Antioxidants such as dexrazoxane have been found to reduce the DOX-induced generation of free radicals; however, the use of dexrazoxane is limited by its side effects Seifert et al. 1994). Therefore, the identification of new drug combinations for reducing DOX-induced toxicity can improve the quality of life of the patients and enhance the effectiveness of chemotherapy. Previous studies have reported that metformin (MET) can provide some protection against chemotherapy-related toxicity (Mao-Ying et al. 2014; Zhou et al. 2016). MET can alter the intracellular signaling pathways that enhance insulin receptor sensitivity, however, the other mechanisms of action of MET are yet to be fully understood. The present study aimed to examine whether pretreatment with MET can provide protection against the toxic effects of anthracyclines in a murine model of DOX-induced death. It also aimed to identify a new drug that can protect against DOX-induced toxicity. At the end of the study, the number of animals that survived DOX treatment was compared with the number of surviving, MET-pretreated animals.

2. Investigations and results

2.1. MET usage improved the overall survival rate

A parallel mouse model was developed for a better understanding of whether MET and its signaling pathway had an effect on increasing the survival rate of the DOX-treated animals, which was studied using the Kaplan–Meier test. The survival analysis of the DOX-treated and DOX+MET-treated mce revealed that treatment with DOX+MET significantly increased the survival rate in comparison to the group treated with DOX alone ($p = 0.01$). The protective effect of MET began from the fifth day of treatment. The study thus revealed that combining MET with DOX can potentially reduce the toxic effects of DOX. The slopes of the survival rates of the control group and the MET-treated group were flat, as depicted in Fig. 1.

2.2. MET prevents the loss of body weight

As depicted in Fig. 2, the body weights of the mice in the DOX group and the DOX+MET group were significantly lower than those of the mice in the MET group and the saline-injected control group ($p < 0.05$). The body weights of the control groups signifi-

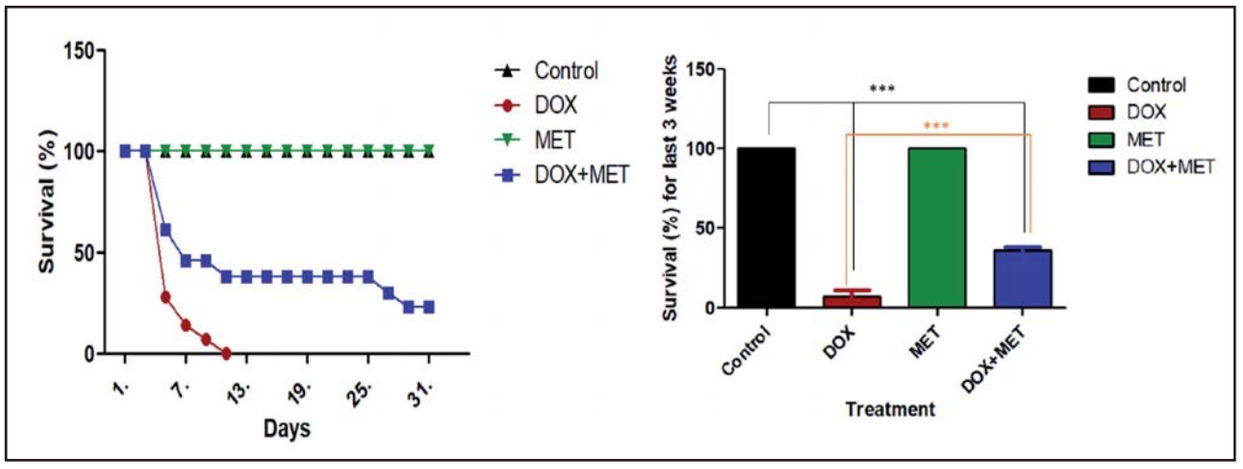

Fig. 1: The effect of MET on survival was determined in a mouse model. MET improved the survival rate of the DOX-treated mice. The mice were treated with a single dose of DOX (25 mg/kg), and a continuous oral administration of MET dissolved in drinking water.

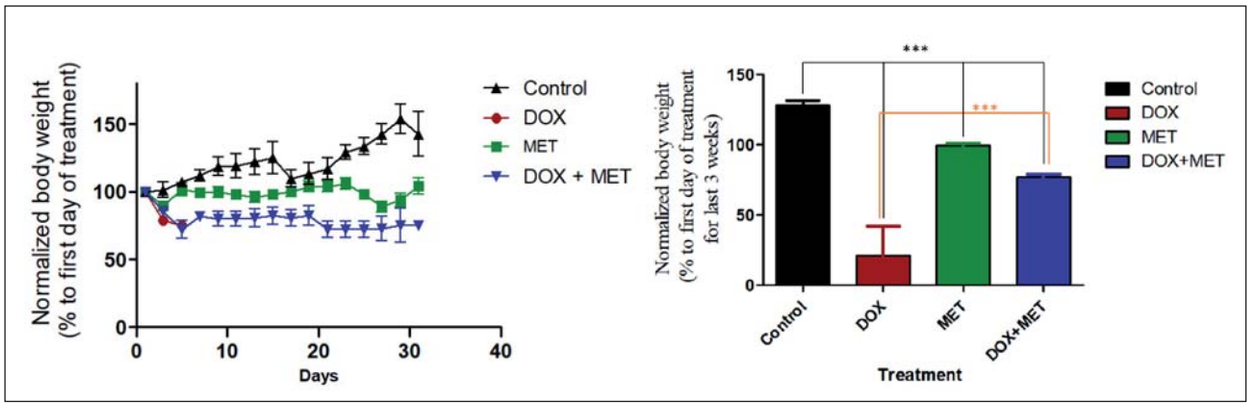

Fig. 2: The effects of DOX and MET on the body weights of the animals in the four experimental groups. The effects of treatment with DOX, MET, and DOX+MET on the body weights of the animals are graphically represented. The mice in the DOX group received a single i.p. injection of DOX at a dose of 25 mg/kg (solid red rectangle). Their body weights were monitored every other day. The data are represented as the mean ± SEM (n = 10–13), and were normalized to the average body weight on day 1 (that is, the first day of DOX injection).

cantly differed from those of the DOX, MET and DOX+MET groups. However, the body weights of the MET groups did not significantly differ from DOX+MET groups.

3. Discussion

The inability to achieve the optimal chemotherapeutic dose and exposure can result in the reoccurrence or progression of cancer, drug resistance, and death. DOX is widely used to treat various types of cancers. However, the development of side effects limits its overall use in chemotherapy (Thorn et al. 2011). The use of anthracyclines has been associated with increased oxidative stress, along with other undesirable effects, including cardiotoxicity and mortality (McGowan et al. 2017; Gouspillou et al. 2015). Several lines of evidence have demonstrated that chemotherapy reduces endogenous antioxidants (Kaya et al. 2005). Various strategies and hypotheses, including the co-administration of antioxidants, have been investigated for reducing the adverse effects of chemotherapy-induced cognitive impairment without affecting the anticancer efficacy of the chemotherapeutic agent (Kang et al. 2015; Singh et al. 2018). Therefore, new drugs must be identified and developed for reducing the side effects of chemotherapy.

In this study, we developed a murine model of chemotherapy for examining the hypothesis that MET protects against DOX-induced toxicity. Towards the end of the study, 50 mice were randomly assigned into four groups: the control group, MET group, DOX group, and the DOX+MET group. The findings indicated that the animals in the DOX group treated with DOX alone, had a lower survival rate than the animals in the DOX+MET group, whereas the animals in the control and MET groups remained alive throughout the duration of the study.

The results of the present study also demonstrated that there was a higher incidence of death in the animals that received only DOX in comparison to the animals that received a combination of DOX and MET. It is well established that DOX induces cardiotoxicity, which could be a major cause of death of the experimental animals. MET is reported to have a cardioprotective effect that is mediated *via* the reduction of DOX-induced cardiotoxicity (Argun et al. 2016). The primary mechanism of action of MET involves the activation of AMP-activated protein kinase (AMPK), which participates in multiple signaling pathways, including those responsible for the activation of the protein kinase B (PKB/AKT) pathway (Han et al. 2018) and Mammalian target of rapamycin (mTOR) inhibition (Shaw 2009). The AKT pathway is a protective and pro-survival pathway, and could be one of the potential mechanisms behind the rescue effect against chemotherapy-induced cardiotoxicity, thus protecting the heart and prolonging the life span of the animals.

Apart from acting as an antidiabetic agent, MET also has antitumor activity (Saif et al. 2018). Previous studies have proposed that MET can affect mitochondrial function by reducing ATP production, which in turn reduces cell proliferation (Wheaton et al. 2014) and also inhibits the mTOR pathway that is involved in cell proliferation (Shaw 2009). The study by Anisimov et al. (2010) additionally revealed that MET significantly reduced tumor size in transgenic mice with adenocarcinoma. Therefore, co-adminis-

tration of MET and DOX could have a synergistic effect against cancer and could protect against chemotherapy-induced toxicity.

The body weights of the animals were measured on every alternate day during the study. The results of this study demonstrated that the DOX-treated animals survived only for nine days, and showed a continual reduction in body weight during this time, compared to that on the first day of DOX injection. On the contrary, the control group showed a continued gain in body weight during the study. The body weight of the MET group remained constant throughout the study, in comparison to that on the day of injection. However, the body weight of the MET group was significantly reduced in comparison to that of the control group. This was expected since MET can maintain glucose levels and control fat deposition even when mice are fed on a high-fat diet (Kanagasabapathy et al. 2013). Moreover, when DOX was combined with MET, the body weight of the animals reduced in the first five days of the study, but remained stable from the fifth day to the end of the study.

To the best of our knowledge this study is a first to demonstrate the treatment-related survival benefits and protective effect of MET in a murine chemotherapeutic model. The dose used in this study was clinically equivalent to the dose used in human cancer patients, so the results are comparatively similar to those observed in human subjects with cancer. The animal experiments were performed synchronously, and it was ensured that the animals in the different experimental groups belonged to the same strain and were of similar ages, for minimizing the effects of age and strain differences in the study outcomes. Moreover, the study used wild type, cancer-free mice for directly evaluating the effects of the treatments with DOX and MET, so as to eliminate any interference from the effects of cancer.

Chemotherapy is known to directly improve the survival rate of cancer patients. MET is known to reduce glucose levels, and has also been reported to protect against chemotherapy-induced hepatotoxicity and nephrotoxicity (Ling et al. 2017; Du et al. 2016; Hadi et al. 2012; Li et al. 2016). The present study investigated the protective effect of MET in prolonging the survival rate of mice treated with DOX. The results of the study revealed that MET had a protective effect against DOX-induced toxicity, and increased the survival rate in the murine DOX-treated model.

4. Experimental

4.1. Animals

Fifty 10–12-week-old mice were individually housed in an environment with 12 h light/dark cycle (lights were turned on at 7:00 am). The animals were allowed *ad libitum* access to food and water at all times. The animals were observed on a daily basis, and their body weights were measured every other day. The animals were divided into four groups, and each group received different treatments. The animals in the control group received a single injection of saline. The animals in the DOX group received a single dose of 25 mg/kg DOX. The animals in the MET group received MET on a daily basis. The animals in the DOX+MET group received a single dose of DOX and daily doses of MET. The animals were observed on a daily basis for the determination of their body weights and for evaluating the survival rate of the animals in all the four groups.

4.2. Drug administration

The mice were intraperitoneally (i.p.) injected with a single dose of DOX, administered at a dose of 25 mg/kg. MET hydrochloride was dissolved in drinking water at a concentration of 3 mg/ml. MET was administered on a daily basis, three days prior to DOX injection. The mortality rate of the animals was monitored on a daily basis; however, the body weight was measured every other day.

4.3. Statistics

All the data obtained from the *in vivo* study were analyzed by one-way ANOVA, and were represented as the mean±SEM. The data were subsequently individually compared by two-tailed Student's *t*-test, and $p < 0.05$ was considered to be statistically significant. The experiments were repeated for n=13-14.

Conflict of interest: There is not conflict of interest in the research article.

Ethical approval: The ethical and protocol of this research was approved by the research unit at College of Pharmacy at Qassim University.

Dedication: We are dedicating this study to examine the protective effect of metformin to reduce the toxicity effects following doxorubicin treatment.

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