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Research Article
Effects of Metformin on the Survival Rate of CMF (Cyclophosphamide, Methotrexate and 5-fluorouracil)-treated Rats

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Abstract

Background and Objective: Metformin (MET) has been shown to reduce the toxicity of chemotherapy and thereby improve patient survival rates. The present study aimed to determine the effects of MET treatment on survival of rats receiving the CMF chemotherapy protocol consisting of cyclophosphamide (CYP), methotrexate (MTX) and 5-fluorouracil (5-FU). **Materials and Methods:** Forty male rats were divided into 4 groups (n = 10 per group): the control group, which received a single dose of saline, the CMF group, which received a single dose of 37 mg kg^{-1} MTX, 75 mg kg^{-1} CYP and 40 mg kg^{-1} 5-FU, the MET group, which received MET in drinking water (3 mg mL^{-1}) every day and the CMF+MET group, which received MET in drinking water every day and a single dose of CMF (37 mg kg^{-1} MTX, 75 mg kg^{-1} CYP and 40 mg kg^{-1} 5-FU). The survival of the animals was evaluated and their body weights were measured daily. **Results:** Rats that received the CMF and CMF+MET treatments showed much lower survival rates than those in the MET and control groups. The toxic effect of CMF+MET appeared to be greater than that of CMF alone, all rats in the CMF+MET treatment group died within 1 week and all rats in the CMF-treatment group died within 10 days. While rats in the control and MET groups showed an increase in body weight after initiation of treatment, those in the CMF and CMF+MET groups still living showed significant reductions in body weight. **Conclusion:** Administration of a single dose of CMF reduced the survival rate of rats. The addition of MET to the CMF protocol increased the toxic effect. Thus, MET increases the toxicity and body weight loss associated with CMF treatment.

Key words: Metformin, chemotherapy, survival rate, drug interaction, drug toxicity, mortality rate

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Metformin (MET) belongs to the biguanide class of drugs and is used to treat hyperglycemia caused by type 2 diabetes mellitus[1]. The mechanism of action underlying the blood glucose-lowering effect of MET is not yet fully understood, however, it is known to exert its effects by reducing hepatic glucose production as well as increasing insulin sensitivity. MET is also reported to directly activate adenosine monophosphate (AMP)-activated protein kinase (AMPK), which is usually activated in the AMP-rich state and hypoxia[2-4]. AMPK activation is known to phosphorylate mechanistic targets of the mammalian target of rapamycin (mTOR) pathway, which is involved in cell function regulation, leading to inactivation of mTOR. mTOR also plays an important role in the regulation of cell proliferation[5].

Previous studies using animal models have shown that chemotherapy can lead to side effects including hepatotoxicity, nephrotoxicity, cardiotoxicity and impairment of cognitive function[6,7]. For instance, in a study involving a water maze and swim latency task, Briones and Woods[8] reported that cyclophosphamide (CYP), methotrexate (MTX) and 5-fluorouracil (5-FU) administered to rats intraperitoneally (i.p.) disrupted learning and memory processes. In addition, our previous study and others have demonstrated that CYP and/or doxorubicin impaired memory function in rodent models and increased toxicity leading to apoptosis in both *in vitro* and *in vivo* models[9-11]. It has been reported that chemotherapy-induced cognitive impairment is not consistent across animal models[12]. Several mechanisms have been proposed to underlie chemotherapy-induced hepatotoxicity, nephrotoxicity and cardiotoxicity[13-15]. For example, experimental studies have shown that doxorubicin administered systemically can inhibit mTOR protein expression, which can potentially cause cytotoxicity[16].

The objective of this study was to determine the effects of MET on survival of rats treated with a chemotherapeutic protocol called CMF, which is a combination of CYP, MTX and 5-FU. On the basis of the available evidence, it was hypothesized that MET would be associated with an increased survival rate for CMF-treated rats.

MATERIALS AND METHODS

Study area: The research study was carried out in Department of Pharmacology and Toxicology, College of Pharmacy, Qassim University, Kingdom of Saudi Arabia from 6/1/2020 to 28/1/2020.

Chemicals: CYP (Endoxan®) was obtained from Baxter (Germany), MTX (Methotrexate®) was obtained from Hospira UK Ltd. (United Kingdom), FU (Utoral®) was from Korea United Pharm Inc. (South Korea) and MET hydrochloride (Metfor®) was obtained from Tabuk Pharmaceuticals (Saudi Arabia).

Animals and treatments: Forty male rats were individually housed in a pathogen-free room with a 12-12 h light/dark cycle (lights were turned on at 6:00 am). The rats were allowed access to food and water at all times. The animals were divided into 4 groups (n = 10 in each group). The control group, received a single dose of saline. The CMF group received a single dose of 37 mg kg^{-1} MTX, 75 mg kg^{-1} CYP and 40 mg kg^{-1} 5-FU i.p. The MET group received MET in drinking water every day at a concentration of 3 mg mL^{-1}. The CMF+MET group received MET in drinking water continuously started one d ay before CMF administration and a single dose of CMF (37 mg kg^{-1} MTX, 75 mg kg^{-1} CYP and 40 mg kg^{-1} 5-FU i.p.). The animals were observed daily for mortality and their body weight was measured.

Statistical analysis: All data were analyzed in GraphPad Prism 5 software using one-way analysis of variance, followed by the Tukey test and are presented as Mean±SEM values. Significance was set at $p<0.05$.

RESULTS

CMF and MET use reduced the survival rate: CMF and CMF+MET treatments decreased the survival rate of rats compared with that associated with MET treatment alone or control treatment. The toxic effect was greater in the CMF+MET-treated group than in the CMF group. The toxic effect of CMF began from the fourth day after treatment which reflected by death of the rats. In the CMF+MET-treated group, all rats were dead within 1 week. In the CMF-treated group, some rats survived until day 10 (Fig. 1).

CMF and MET resulted in reduced body weight: As shown in Fig. 2, the body weights of the rats in the CMF and CMF+MET groups significantly r educed compared with those in the MET and control groups ($p<0.05$). The body weights of rats in the MET and control groups increased compared with those on the 1st day of the respective treatments.

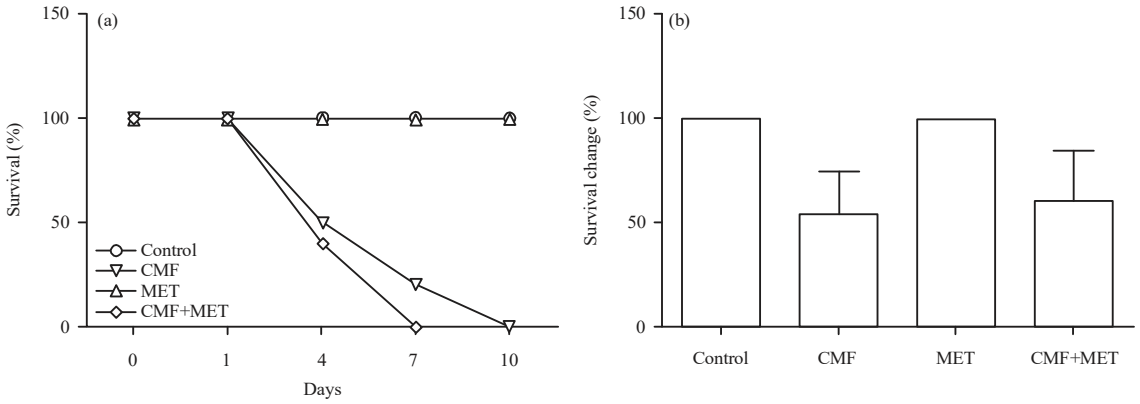

Fig. 1(a-b): (a-b) Comparison of survival rates among treatment groups (10 rats in each group)

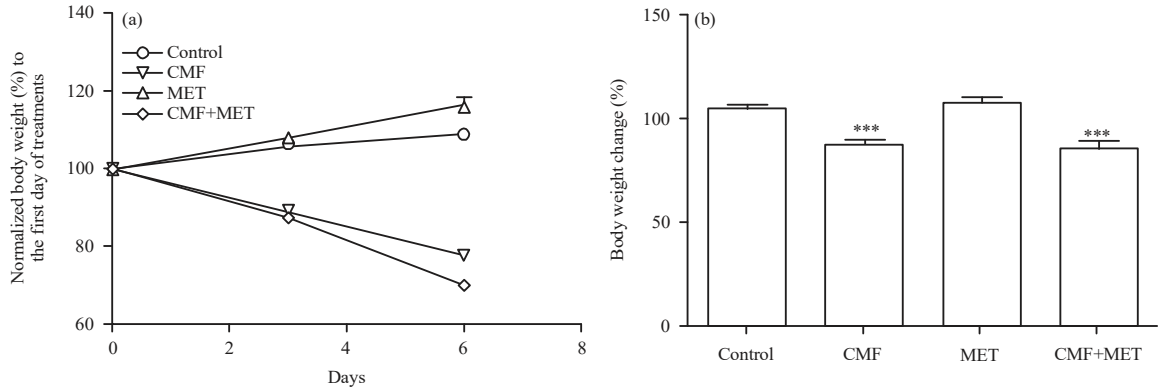

Fig. 2(a-b): (a-b) Changes in body weight of rats in treatment groups
***p<0.05

DISCUSSION

In the present study, the protective effect of the antidiabetic agent MET against toxicity induced by the CMF protocol [CYP (75 mg kg^{-1}), MTX (37 mg kg^{-1}) and 5-FU (40 mg kg^{-1})] was assessed in a rat model. MET has been reported to improve the quality of life of diabetes patients. It has also been reported to reduce the risk of Alzheimer's disease[17]. In addition, in previous studies, MET was found to reduce the toxicity of chemotherapeutic agents doxorubicin and cisplatin and thus increase the survival rate, reduce cardiotoxicity and improve cognitive function following chemotherapy[10,13,18]. Therefore, in the present study, it was hypothesized that MET would improve the survival rate of chemotherapy (CMF)-treated rats. However, the results showed that, in fact, MET had a negative synergistic effect with CMF and increased the mortality of the rats compared with that associated with CMF treatment alone.

Several studies have shown that MET enhances the effect of chemotherapy against cancer growth[19]. However, this study showed that MET has a synergistic effect against all cells, leading to animal death. In most *in vitro* studies using cancer cells, chemotherapy with co-administration of MET was reported to potentially increase the effectiveness of the chemotherapy[19]. However, the findings of this study suggest that MET increases both cancer and normal cell death that because it has an ability to inhibit mTOR protein, which is involved in growth and recovery mechanisms[20].

The current study has some strengths and limitations. To the best of our knowledge, this is the first study of the effect of MET when used in combination with CMF on survival rate in a rat model of chemotherapy. The dose used in this study was clinically relevant to the dose used in human cancer patients and the doses used in other experimental studies. Therefore, the results could be relevant to cancer patients. The animals used in this study were of same strain and age and all experiments were conducted simultaneously among the study groups to minimize the effect of confounders. Moreover, cancer-free rats were used to evaluate the direct effects of the CMF and MET treatments without interference from the effects of cancer.

CONCLUSION

The use of single-dose CMF at the specified concentrations led to reduced survival of rats. MET, when used in combination with CMF, increased the toxicity of CMF and ultimately mortality. Therefore, it is concluded that MET increased the toxicity of CMF treatment and/or impaired the animals' recovery, which was proven by the animals' increased mortality rate and body weight loss following the treatment.

SIGNIFICANCE STATEMENT

This study discovers the possible synergistic effect of metformin (MET) and the CMF chemotherapy protocol (consisting of cyclophosphamide, methotrexate and 5-fluorouracil). MET has previously been reported to reduce the toxic effects of chemotherapy and is frequently used to treat diabetes. Here, MET increased the toxicity of CMF treatment in a rat model. This study has implications for research into chemotherapy (and its toxicity) and clinical treatment of cancer.

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