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Co-administration of imipramine and doxorubicin reduces the survival rate and body weight of mice

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Abstract. – OBJECTIVE: Doxorubicin (DOX) is a chemotherapeutic agent widely used to treat cancers, particularly breast cancer. DOX has side effects, including cardiotoxicity, hepatotoxicity, and nephrotoxicity. Imipramine is an antidepressant that increases the release of neurotransmitters. This study aimed to investigate the effect of co-administration of imipramine and DOX on DOX-induced toxicity.

MATERIALS AND METHODS: Forty female mice (10-12-weeks-old, 30-38 g) were divided into four groups (n = 10 per group). The animals in the control group received a single-dose saline injection. The animals in the DOX group received a single dose of DOX (20 mg/kg) by intraperitoneal (i.p.) injection. The animals in the imipramine group received the drug daily in their drinking water (0.13 mg/mL) for 9 days, starting 1 day before the DOX injection received by the DOX group. The animals in the combination group (DOX+imipramine) received a single dose of DOX (20 mg/kg, i.p. injection), and a daily dose of imipramine in their drinking water (0.13 mg/mL) for 9 days starting 1 day before the DOX injection. The animals were observed daily to record mortality, and their body weights were recorded every alternate day.

RESULTS: DOX treatment increased the rate of mortality compared with that for control animals. Imipramine co-administration with DOX increased the rate of mortality significantly ($p < 0.05$) compared with DOX treatment alone. The mortality rate in both the control and imipramine-treatment groups was zero. DOX co-administered with imipramine resulted in significantly reduced body weight compared with control animals.

CONCLUSIONS: The combination of DOX and imipramine reduced the survival rate of female mice, suggesting that imipramine increases the toxic effects of DOX.

Key Words:
Doxorubicin, Imipramine, Survival rate, Body weight.

Introduction

The survival rate is commonly used to evaluate toxicity of a drug treatment in animals or humans over time[1]. In this type of study, mortality is typically measured from the first day of treatment for a defined period of time; a comparison is then made between treated and untreated subjects to understand the effect of the treatment. The survival time is defined as the time from the first day of treatment until death occurs[2]. The Kaplan-Meier survival curve is frequently used to analyze the effects of interventions, such as drugs, on the survival rate of animals[3,4].

Doxorubicin (DOX) belongs to the anthracycline class of chemotherapeutic agents, which are used to treat many types of cancer, such as breast, prostate, lung, and colon cancers[5]. DOX has many mechanisms of action[6]. Topoisomerase II is important for DNA repair and acts by relaxing positive and negative supercoils, thus reducing the growth of the replication fork in DNA[7,8]. DOX acts by binding to topoisomerase II in cancer cells, which leads to the disruption of DNA transcription and causes DNA degradation and cell death[6]. In addition, DOX is reported to increase the generation of free radicals that cause damage to cellular membranes, DNA and proteins[9].

Imipramine is a tricyclic antidepressant (TCA). TCAs exert their effects by selectively blocking serotonin (5-hydroxytryptamine) and norepi-

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nephrine reuptake[10]. It has been shown that the hippocampus can induce significant neurogenesis in response to chronic imipramine treatment[11,12]. Although the mechanism is not well understood, studies have shown that imipramine and other antidepressants stimulate neurogenesis by upregulating brain-derived neurotrophic factor (BDNF), vascular endothelial growth factor, and fibroblast growth factor 2, and their receptors[13-15]. Conversely, stress has been reported to lead to BDNF downregulation, which is reversible with imipramine treatment[16]. DOX treatment has also been reported to downregulate the expression of BDNF[17].

Previous studies examining the combination of imipramine with chemotherapeutic agents have reported that it prevents toxicity associated with temozolomide[18] and enhances the efficacy of DOX[19]. However, these studies did not indicate the effect of the combinations of these drugs on survival rate. This study evaluated the effect of imipramine on the survival rate of mice when given in combination with DOX.

Materials and Methods

Chemicals

DOX (ADRIM®) was obtained from Fresenius Kabi Oncology Ltd. (Haryana, India). Imipramine hydrochloride (crystalline powder, Alfa Aesar) was purchased from Thermo Fisher Scientific (Waltham, MA, USA).

Animals

Forty female albino mice (10-12-weeks-old, 30-38 g) were housed individually in a 12-h light-dark cycle (lights on at 7:00 am). The animals had free access to food and water at all times. Animal studies were undertaken after obtaining Institutional Ethical Approval from the research unit at the College of Pharmacy, Qassim University (Approval No. 2020 - CP - 6).

Experimental Design

The mice were separated into four groups (n = 10 per group): (1) a control group; (2) a DOX-treatment group; (3) an imipramine-treatment group; and (4) a group with DOX and imipramine. The mice in the control group received a single intraperitoneal (i.p.) injection of saline on day 1 of the study and normal drinking water. Mice in the DOX group received a single i.p. injection of 20 mg/kg DOX on day 1 of the study and normal drinking water. Mice in the imipramine group received imipramine (0.13 mg/mL) daily in their drinking water for 9 days, beginning 1 day before the DOX injection received by mice in the DOX group. Mice in the combination treatment group (DOX+imipramine) received a single dose of DOX (20 mg/kg, i.p. injection) and daily administration of imipramine (0.13 mg/mL) in their drinking water for 9 days, beginning 1 day before the DOX injection. The mice were observed daily to record mortality and their body weights were recorded every alternate day.

Statistical Analysis

All data were analyzed using one-way analysis of variance and are represented as the mean ± standard error of the mean. The data were subjected to Tukey's test to evaluate the parameters, and $p < 0.05$ was considered statistically significant. Survival was assessed using Kaplan–Meier survival curves.

Results

Effect of DOX and Imipramine on the Survival Rate of Mice

A matching mouse model was developed to better understand the effect of imipramine on the survival rate of mice treated concurrently with DOX. DOX treatment increased the rate of mortality compared with that for control animals. Co-treatment of mice with DOX+imipramine significantly ($p < 0.05$) decreased the survival rate of mice compared with DOX treatment alone. No mortality was recorded in the control group or the imipramine-treatment group during the study period (Figure 1).

Effect of DOX and Imipramine on the Body Weight of Mice

The average body weights of mice in the DOX, imipramine, and DOX+imipramine groups were significantly lower than the average body weight of control mice at the end of the study period ($p < 0.05$) (Figure 2).

Discussion

The primary objective of the present study[20] was to determine the impact of imipramine on the survival rate of DOX-treated mice using Kaplan-Meier curves, which are a statistical ap-

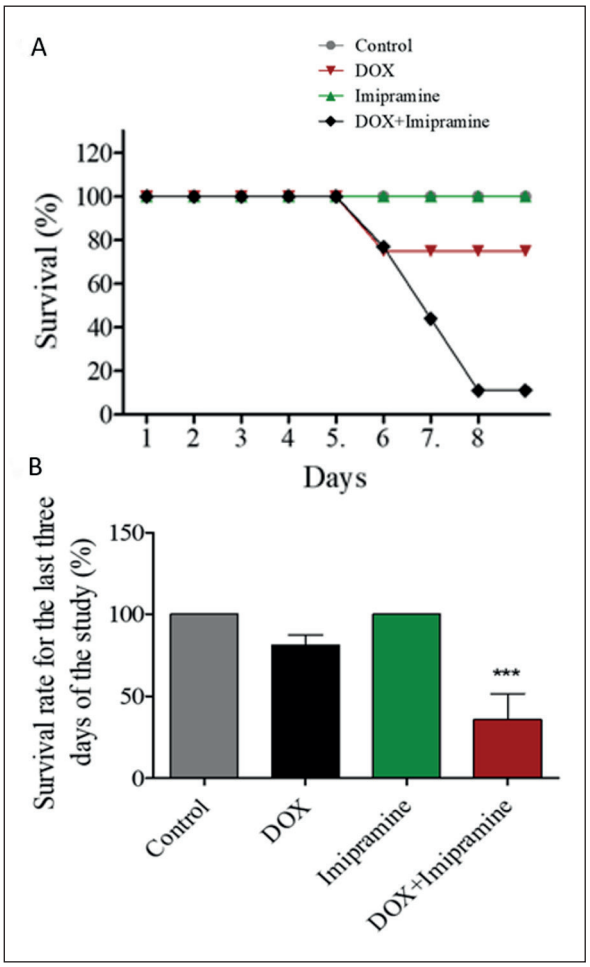

Figure 1. Effects of doxorubicin (DOX), imipramine, and DOX+imipramine treatment on the survival rate of experimental mice. **A**, Survival rate of mice over a period of 9 days. **B**, Average survival rate of mice in the last 3 days of the study. Data are presented as the mean ± SEM (n = 10 per group).

proach widely used to analyze time-to-event data. This approach is well-established for use in survival analysis. In particular, it is widely used in pharmaceutical and epidemiological studies[21] to assess the survival of subjects over time in cases where two or more of the studied groups are receiving the same or different medications[4].

The dosage protocols used in this study for DOX and imipramine were selected to be clinically relevant and were based on previous studies[22,23]. The survival rate of mice was measured every day throughout the 9-day study period. The percentage survival of DOX-treated mice was reduced to 75% after 5 days, and this was then maintained until the end of the study period. The survival rate of DOX+imipramine-treated mice was 80% on day 5, 50% on day 6, and 10% on day 9. This result revealed that the toxicity of DOX was increased by co-administration of imipramine. Further studies are required to determine the mechanism of this synergistic toxic effect.

Imipramine is a TCA. Our observations in this study indicated that chronic administration of imipramine significantly decreased the survival rate of mice co-treated with DOX compared with control mice or mice treated with imipramine alone (Figure 1B). Our study also showed a reduction in the average body weight of mice treated with DOX+imipramine compared with control and imipramine-treated mice, and compared with DOX-treated mice during the last 3 days of the study. Previous scholars[24] have indi-

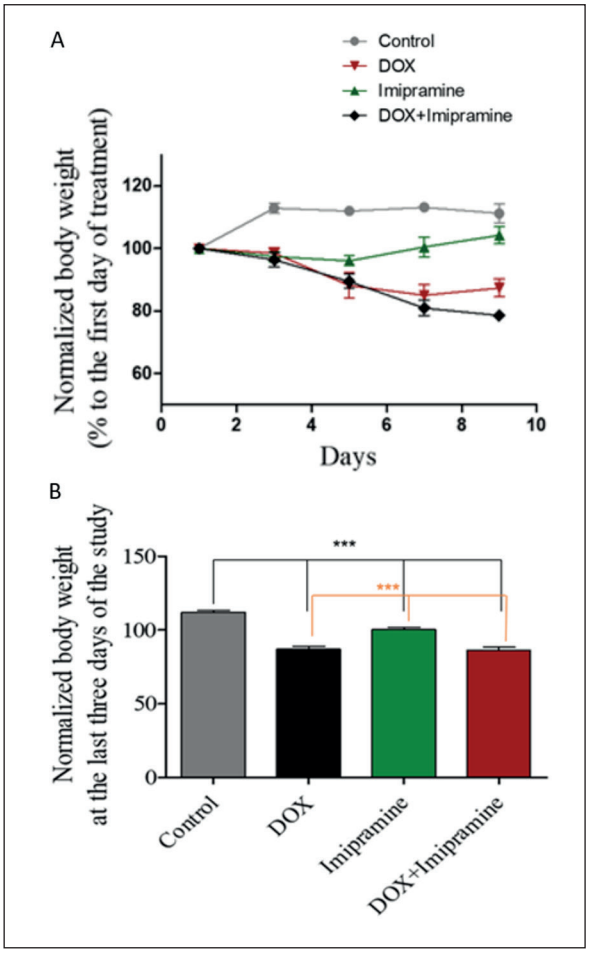

Figure 2. Effects of DOX, imipramine, and DOX+imipramine treatment on the body weight of experimental mice. **A**, Body weight change (%) in mice over a period of 9 days compared with the first day of treatment. **B**, Average body weight change (%) in mice during the last 3 days of the study compared with the first day of treatment. Data are presented as the mean ± SEM (n = 10 per group).

cated that imipramine has the ability to improve the functioning of mitochondria in glioblastoma multiforme cells. Some chemotherapeutic agents, such as trastuzumab and sunitinib, can cause reversible mitochondrial impairment, while others, such as DOX, can cause irreversible and lethal mitochondrial damage[25]. Imipramine could potentially improve mitochondrial function in the case of reversible damage caused by chemotherapeutic agents. This highlights the need for further research to elucidate the exact effects of imipramine on mitochondrial function.

The mice in the control group showed a continuous increase in body weight throughout the experimental period. Mice treated only with imipramine maintained approximately the same weight throughout the study. Mice in the DOX and DOX+imipramine groups showed continuous declines in body weight until the end of the study period. The average body weights of DOX-, imipramine-, and DOX+imipramine-treated mice were all significantly reduced compared with the control group. Furthermore, DOX and DOX+imipramine treatment resulted in significant decreases in body weight compared with mice treated only with imipramine. This result shows that imipramine alone does not significantly reduce body weight but DOX does, consistent with the findings of other studies[26].

This study had certain strengths and limitations. It was conducted using normal mice that were pathogen free to evaluate the drug-drug interaction without interference from other factors. It used mice of the same age and strain to avoid any genetic effects or differences caused by age. In addition, the dosage protocol for each experimental group was kept constant (i.e., dosage and time of administration) and the mice were housed in identical conditions. However, a limitation of the study was that the mice were free of cancer and DOX is usually administered when cancer has developed in humans or animals; thus, cancer and its associated symptoms, such as inflammation, could potentially interfere with the outcomes of this study.

Conclusions

There are no previous studies evaluating the effect of combined imipramine and doxorubicin intervention on survival rate. In this work, we showed that the combination of DOX and imipramine resulted in a significant reduction in the survival rate of mice compared with treatment with DOX alone or imipramine alone. DOX treatment decreased the survival rate of mice compared with control mice, while imipramine treatment did not have a significant effect on survival. In addition, DOX and DOX+imipramine treatment resulted in significant reductions in body weight compared with control or imipramine-treated mice. Our findings suggest that co-treatment with imipramine and DOX may produce an additive effect leading to cytotoxicity and increased mortality in mice. Further research is required to understand the precise effect of the combination of DOX and imipramine on the metabolism and physiology of treated subjects.

Conflict of Interest

The Authors declare that they have no conflict of interests.

Acknowledgements

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References

1) Singh R, Mukhopadhyay K. Survival analysis in clinical trials: basics and must know areas. Perspect Clin Res 2011; 2: 145-148.
2) Lee YH, Kung PT, Wang YH, Kuo WY, Kao SL, Tsai WC. Effect of length of time from diagnosis to treatment on colorectal cancer survival: a population-based study. PLoS One 2019; 14: e0210465.
3) Dudley WN, Wickham R, Coombs N. An introduction to survival statistics: Kaplan-Meier analysis. J Adv Pract Oncol 2016; 7: 91-100.
4) Kishore J, Goel M, Khanna P. Understanding survival analysis: Kaplan-Meier estimate. Int J Ayurveda Res 2010; 1: 274-278.
5) Thorn CF, Oshiro C, Marsh S, Hernandez-Boussard T, McLeod H, Klein TE, Altman RB. Doxorubicin pathways: pharmacodynamics and adverse effects. Pharmacogenet Genomics 2011; 21: 440-446.
6) Nitiss JL. Targeting DNA topoisomerase II in cancer chemotherapy. Nat Rev Cancer 2009; 9: 338-350.
7) Lucas I. Topoisomerase II can unlink replicating DNA by precatenane removal. EMBO J 2001; 20: 6509-6519.
8) Watt PM, Hickson ID. Structure and function of type II DNA topoisomerases. Biochem J 1994; 303: 681-695.
9) Kocsis JH, Frances AJ, Voss C, Mann JJ, Mason BJ, Sweeney J. Imipramine treatment for chronic depression. Arch Gen Psychiatry 1988; 45: 253-257.

10) Han X, Tong J, Zhang J, Farahvar A, Wang E, Yang J, Samadani U, Smith DH, Huang JH. Imipramine treatment improves cognitive outcome associated with enhanced hippocampal neurogenesis after traumatic brain injury in mice. J Neurotrauma 2011; 28: 995-1007.

11) Pechnick RN, Zonis S, Wawrowsky K, Cosgayon R, Farrokhi C, Lacayo L, Chesnokova V. Antidepressants stimulate hippocampal neurogenesis by inhibiting p21 expression in the subgranular zone of the hippocampus. PLoS One 2011; 6: e27290.

12) Bachis A, Mallei A, Cruz MI, Wellstein A, Mocchetti I. Chronic antidepressant treatments increase basic fibroblast growth factor and fibroblast growth factor-binding protein in neurons. Neuropharmacology 2008; 55: 1114-1120.

13) Castren E, Voikar V, Rantamaki T. Role of neurotrophic factors in depression. Curr Opin Pharmacol 2007; 7: 18-21.

14) Rantamaki T, Hendolin P, Kankaanpää A, Mijatovic J, Piepponen P, Domenici E, Chao MV, Männistö PT, Castrén E. Pharmacologically diverse antidepressants rapidly activate brain-derived neurotrophic factor receptor TrkB and induce phospholipase-Cgamma signaling pathways in mouse brain. Neuropsychopharmacology 2007; 32: 2152-2162.

15) Tsankova NM, Berton O, Renthal W, Kumar A, Neve RL, Nestler EJ. Sustained hippocampal chromatin regulation in a mouse model of depression and antidepressant action. Nat Neurosci 2006; 9: 519-525.

16) Hang P, Zhao J, Sun L, Li M, Han Y, Du Z, Li Y. Brain-derived neurotrophic factor attenuates doxorubicin-induced cardiac dysfunction through activating Akt signalling in rats. J Cell Mol Med 2017; 21: 685-696.

17) Bielecka AM, Obuchowicz E. Antidepressant drugs can modify cytotoxic action of temozolomide. Eur J Cancer Care 2017; 26: e12551.

18) Munson JM, Fried L, Rowson SA, Bonner MY, Karumbaiah L, Diaz B, Courtneidge SA, Knaus UG, Brat DJ, Arbiser JL, Bellamkonda RV. Anti-invasive adjuvant therapy with imipramine blue enhances chemotherapeutic efficacy against glioma. Sci Transl Med 2012; 4: 127ra36.

19) Alhowail A, Almogbel Y. Metformin administration increases the survival rate of doxorubicin-treated mice. Pharmazie 2019; 74: 737-739.

20) Liu Z, Feng S, Li J, Cao H, Huang J, Fan F, Cheng L, Liu Z, Cheng Q. The epidemiological characteristics and prognostic factors of low-grade brainstem glioma: a real-world study of pediatric and adult patients. Front Oncol 2020; 10: 391.

21) Ferguson JE, Dodwell DJ, Seymour AM, Richards MA, Howell A. High dose, dose-intensive chemotherapy with doxorubicin and cyclophosphamide for the treatment of advanced breast cancer. Br J Cancer 1993; 67: 825-829.

22) Ramirez K, Sheridan JF. Antidepressant imipramine diminishes stress-induced inflammation in the periphery and central nervous system and related anxiety- and depressive- like behaviors. Brain Behav Immun 2016; 57: 293-303.

23) Bielecka-Wajdman AM, Ludyga T, Machnik G, Gołyszny M, Obuchowicz E. Tricyclic antidepressants modulate stressed mitochondria in glioblastoma multiforme cells. Cancer Control 2018; 25: 1073274818798594.

24) Gorini S, Angelis AD, Berrino L, Malara N, Rosano G, Ferraro E. Chemotherapeutic drugs and mitochondrial dysfunction: focus on doxorubicin, trastuzumab, and sunitinib. Oxid Med Cell Longev 2018; 2018: 7582730.

25) Alharbi I, Alharbi H, Almogbel Y, Alalwan A, Alhowail A. Effect of metformin on doxorubicin-induced memory dysfunction. Brain Sci 2020; 10: 152.