Pioglitazone ameliorates doxorubicin-induced hypothyroidism and cardiotoxicity in rat models

A.H. ALHOWAIL

Department of Pharmacology and Toxicology, College of Pharmacy, Qassim University, Buraydah, Al Qassim, Saudi Arabia

Abstract. - OBJECTIVE: The anticancer drug doxorubicin (DOX) is effective but is associated with complications such as hypothyroidism and cardiotoxicity. Pioglitazone (PIO), which is used to treat diabetes mellitus, has shown potential for treating hypothyroidism and cardiac dysfunction. Therefore, this study explores whether PIO can also ameliorate DOX-induced hypothyroidism and cardiotoxicity.

MATERIALS AND METHODS: Forty female Wistar rats were separated into control and three treated groups (DOX, PIO, and DOX+PIO), and their blood samples were examined for the thyroid hormones, including thyroid-stimulating hormone (TSH), thyroxine in total and free forms (T4 and FT4, respectively), and triiodothyronine in total and free forms (T3 and FT3, respectively), and the cardiotoxicity biomarkers [troponin I, creatine kinase (CK), and creatine kinase-myocardial band (CK-MB)].

RESULTS: The control and PIO groups did not exhibit significant alterations in any of the examined hormones and markers. In contrast, in the DOX group, T4, FT4, T3, and FT3 levels decreased significantly, whereas troponin I, CK, and CK-MB levels increased significantly, but no significant changes were detected in TSH levels. PIO co-treatment ameliorated these effects of DOX significantly in FT4, FT3, and troponin I.

CONCLUSIONS: PIO may provide protection against hypothyroidism and cardiotoxicity caused by DOX treatment, by significant reversal of FT4, FT3, and troponin I levels.

Key Words: Chemotherapy, Doxorubicin, Pioglitazone, Hypothyroidism, Cardiotoxicity, Heart disease.

Introduction

Doxorubicin (DOX) is an efficient anticancer medication utilized to treat multiple malignancies, including breast and prostate cancer. The main mechanisms of DOX are a decrease in DNA synthesis induced via topoisomerase II inhibition, an increase in the reactive oxygen species (ROS) formation, and the disruption of mitochondrial function. However, despite its efficacy against cancer growth, it has several limitations, including cardiotoxicity, nephrotoxicity, hepatotoxicity, and hypothyroidism. Furthermore, recent stud-
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Cardiotoxicity can cause blood flow deficiency to the rest of the body, including the brain, and this can result in cerebral hypoxia. Impaired brain oxygenation alters general brain function and also affects the functionality of other organs. Accordingly, it has been reported that among patients on DOX treatment who developed cardiac deficiencies, 25-70% also developed cognitive problems. Similarly, hypothyroidism may also have adverse effects on the brain, as neuroimaging research has demonstrated alterations in the hippocampal structure and function of patients with hypothyroidism. In addition, hypothyroidism is characterized by abnormal cardiac function by decreased cardiac contractility. Few investigations have focused on the mechanism and etiology of DOX-induced hypothyroidism and cardiotoxicity, or agents that can be employed to protect patients from these toxicities. Therefore, additional research is required to develop new compounds that can reduce the effect of DOX on the thyroid gland and cardiac functions, potentially by improving thyroid hormone release and cardiac outputs.

Pioglitazone (PIO) is a derivative of thiazolidinedione, an anti-diabetic medication, that is used as a selective agonist for peroxisome proliferator-activated receptors (PPARs). Agonism of PPAR improves insulin sensitivity, glucose uptake, and fat metabolism. PIO is currently approved for the therapy of diabetes mellitus, which is associated with insulin resistance. Furthermore, PIO has been reported to be a candidate drug for the curing of neurodegeneration such as Alzheimer’s and Parkinson’s disease, and has also been shown to enhance thyroid hormone receptor activation and, thereby, improve thyroid hormone function. In a rat model of hypothyroidism, PIO administration was found to alleviate oxidative stress and memory function. Some studies have also reported that PIO can reverse cardiomyopathy induced by type 1 diabetes mellitus. However, one study by Zhong et al. has reported the possibility of PIO-inducing cardiomyopathy. Further studies are essential to understand more clearly the impact of PIO on DOX-induced hypothyroidism and cardiotoxicity and evaluate it as a candidate protective agent against the adverse effects of DOX.

Our prior study assessed the neurotoxic effects of DOX therapy on the function of the brain and investigated the potential ameliorative effect of combining PIO with DOX. The DOX-treated rats exhibited neurotoxicity and cognitive impairment, increased mortality rate, reduced body weight, and elevated neuroinflammation (as indicated by increased IL-1β, TNF-α, and IL-6 levels), and these effects, except for the reduced body weight, were prevented by co-treatment with PIO. These findings demonstrate the beneficial effects of PIO in attenuating DOX-induced neurotoxicity and lay the basis for the present study. Accordingly, the high incidence of hypothyroidism and cardiotoxicity in breast cancer patients and DOX therapy use in breast cancer regimens, limited is identified about DOX effects on female rodents in the development of hypothyroidism and cardiotoxicity after DOX therapy and protection. Therefore, this study examined female rats’ DOX-induced hypothyroidism via assessing thyroid hormone levels and cardiotoxicity via assessing cardiotoxic biomarkers as well as possible PIO protection in female rodents.

**Materials and Methods**

**Chemicals**

DOX and pioglitazone hydrochloride (Glados®) were sourced from EBEWE Pharmaceutical Manufacturing located in Attersee, Austria, and Tabuk Pharmaceutical Company located in Tabuk, Saudi Arabia, respectively.

**Animals and Drug Administration**

Forty Wistar female rats (weighing 150-250 g) were housed individually in plastic cages. The animals were preserved in typical standard laboratory environments under a light and dark cycle for 12 hours each and at 25°C room temperature. They were given free admittance to food and water. The rats were separated into four rat groups (7 in each group): a control group and three different groups treated with DOX, PIO, and DOX+PIO. DOX was intraperitoneally administered at 5 mg/kg dose every three days for twelve days. Thus, the rats in the DOX groups received a complete dose of 20 mg/kg. In the PIO groups, a dose of 2 mg/kg was delivered by dissolving PIO in the drinking water provided to the rats on the same days that DOX was administered. In the control group, rats were administered with saline.

**Electrochemiluminescence Immunoassays**

On day 14, after the last drug dose was administered, the rats were sacrificed by cervical de-
capitation after induction of anesthesia with CO2. Blood was promptly collected into EDTA tubes from the control group, and treated with PIO, DOX, and DOX+PIO groups, and vials containing the blood samples were centrifuged at (12,000 × g) for 10 minutes. The separated plasma was transferred to 2 mL vials and stored at -80°C. Subsequently, the blood samples were analyzed using a fully automated analyzer that utilizes a patented electrochemiluminescence technology for immunoassay analysis of TSH, thyroxine (T4), triiodothyronine (T3), free thyroxine (FT4), free triiodothyronine (FT3), troponin I, creatine kinase (CK), and creatine kinase-myocardial band (CK-MB). The levels of troponin I, CK, and CK-MB are elevated following cardiac infarction, and cardiac toxicity and injury, and they are, therefore, considered as biomarkers of cardiotoxicity28,29. The ECL method employed the COBAS INTEGRA 400 plus system according to the manufacturer’s directions (Roche Diagnostics GmbH, Mannheim, Germany).

Statistical Analysis
The results of the existing study were analyzed via GraphPad Prism 10.0.0.153 (GraphPad, Boston, MA, USA) and were shown as SEM. The ECL results were statistically analyzed using a one-way analysis of variance. Data for each treatment group were compared against the control data. A $p < 0.05$ was counted as demonstrating statistical significance.

Results

TSH Levels Unaffected by DOX or PIO

The results of the ECL analysis indicated that the TSH levels did not significantly vary between the control and three treated groups. Thus, neither the DOX nor the PIO treatments impacted TSH levels (Figure 1).

PIO Reduces DOX Toxic Effects on T4 and FT4 Levels

ECL analysis revealed that the concentrations of T4 and FT4 were noticeably lower in the DOX-treated group compared to the control group. Co-treatment with PIO improved the T4 and FT4 levels, but not significantly (Figures 2A and 2B).

![Figure 1. Effects of DOX, PIO, and combination on TSH levels. The graph depicts no significant alterations between the four study rats’ groups. The data are presented as SEM for seven animals in each group, analyzed utilizing one-way ANOVA.](image1)

![Figure 2. Effects of DOX, PIO, and combination on T4 (A) and FT4 (B) levels. DOX treatment (20 mg/kg, intraperitoneal injection) caused a significant decrease in the T4 and FT4 levels, but PIO treatment did not affect the T4 and FT4 levels. The DOX+PIO group showed improved T4 and FT4 levels compared to the DOX group. Data are expressed as SEM for seven rats in each rat group. (*) $p < 0.01$ and (**) $p < 0.001$ related to control rats, and (#) $p < 0.05$ related to DOX rats.](image2)
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**PIO Reduces DOX Toxic Effects on T3 and FT3 Levels**

ECL analysis revealed that the concentrations of T3 and FT3 were considerably lower in the DOX group compared to the control group, but the PIO therapy alone did not seem to have a substantial impact on the T3 or FT3 levels, compared to the control group. PIO co-treatment appeared to rescue this effect of DOX, although this effect was not significant (Figures 3A and 3B).

**PIO Reduces DOX Toxic Effects on Troponin I Levels**

Comparing the DOX-treated rats to the control ones, the troponin I levels were noticeably higher in the DOX-treated rats (*p < 0.05) (Figure 4). PIO also appeared to induce an increase in troponin I related to the control rats, but the difference was not statistically significant. PIO+DOX co-treatment led to a reduction, although not significant, in the DOX-induced increase in troponin I.

**PIO Reduces DOX Toxic Effects on CK and CK-MB Levels**

Compared to the control rats, DOX therapy resulted in a considerable elevation in CK and CK-MB levels. PIO co-treatment restored their levels, but not to a significant extent (*p < 0.05) (Figures 5A and 5B).

**Discussion**

In this present study, the effect of DOX on thyroid and cardiac function was examined in DOX-treated rat models based on measurements of the plasma concentrations of TSH, T4, FT4, T3, FT3, troponin I, CK, and CK-MB. Further, the potential protective effect of PIO (which has been reported to improve thyroid and cardiac function) on DOX-induced hypothyroidism and cardiotoxicity was also investigated.

Previous studies have documented that DOX can alter thyroid and cardiac function clinically and experimentally. The results of the existing study are in agreement with these early results, as DOX was found to cause a significant increase in the markers of cardiac toxicity (that is, troponin I, CK, and CK-MB) and a significant decline in the levels of the thyroid hormones assayed. Thyroid hormone receptors are expressed abundantly in the brain and heart, and a reduction in thyroid hormones can affect the activities of these receptors and subsequently lead to altered heart and brain function. Accordingly, hypothyroidism has been reported to cause cardiac deficiency and mental problems. In addition, alterations in cardiac function have been found to reduce blood flow and affect the blood supply to the rest of the body, including the brain, and cardiac deficiency has also been associated with hypothyroidism. Thus, the pathways via which DOX affected cardiac and thyroid function may be related; therefore, they need to be investigated in more detail, as they may help identify potential therapeutic agents that can prevent or ameliorate these adverse effects of DOX. The current study investigated TSH, T4, FT4, T3, and FT3 levels after treatment with DOX, PIO, and a combination of DOX+PIO. Interestingly, the results indicated

![Figure 3](image-url)
no significant alterations in TSH levels following DOX or PIO treatments in all groups. Further, the T4, FT4, T3, and FT3 levels were notably decreased in the DOX-treated rats, but PIO did not cause any significant changes. That is, while PIO co-treatment was found to lead to an improvement in the circulating levels of T4, T3, FT4, and FT3, the observed effect was not significant in T4 and T, while there was a notable improvement in FT4 and FT3. A study on type 2 diabetes patients reported that PIO led to a notable decline in FT4 levels and a notable elevation in TSH levels. Thus, the present findings need to be explored further through more studies in order to confirm whether PIO does indeed have any ameliorative effect on DOX-induced thyroid toxicity.

The results of this study established the cardiotoxic effects of DOX based on significantly increased levels of troponin I, CK, and CK-MB related to the control group. Further, PIO was found to reverse these effects significantly in troponin I, but not to a significant extent in the CK and CK-MB levels. Nonetheless, similar beneficial effects of PIO have been reported previously. For example, one study in a mouse model showed that PIO could reverse DOX-induced cardiotoxicity. Further, a study on individuals with type 2 diabetes mellitus found that PIO could improve cardiac function. However, these findings need further verification, as PIO has also been reported to aggravate DOX-induced cardiomyopathy.

**Limitations**

The present findings are limited by its in vivo design. The findings need to be confirmed in in vitro experiments and clinical trials in the future. In addition, we were unable to examine the mechanistic details underlying the impacts of DOX and PIO on thyroid and cardiac function. For example, a previous study on a rat model reported that PIO could alleviate diabetic cardiomyopathy by suppressing cardiac CaMKII/NF-κB signaling and enhancing PPAR-γ expression. With regard to its effect on thyroid function, it would be fascinating to investigate the potential role of thyroid

![Figure 4. Effects of DOX, PIO, and combination on troponin I levels. DOX treatment (20 mg/kg, intraperitoneal injection) led to a noticeable elevation in troponin I levels related to control group. PIO treatment also led to an increase in troponin I levels, but it was not significant. DOX-PIO co-treatment counteracted the increase induced by DOX, but not significantly. The expression of data was as SEM for seven rats in each rat group. (p < 0.05) related to the control rats, and (p < 0.05) related to DOX rats.](image)

![Figure 5. Effects of DOX, PIO, and combination on CK (A) and CK-MB (B) levels. DOX treatment (20 mg/kg, intraperitoneal administration) led to a noticeable elevation in the CK and CK-MB levels that was reversed by DOX+PIO co-treatment (although not significantly). The expression of data was as SEM for seven in each rats group, and one-way ANOVA made comparisons with control rats. (*p < 0.05) related to control rats.](image)
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hormone receptors, particularly THRα1, which is principally found in the cardiac and skeletal muscle, and THRβ1, which is predominantly found in the hepatic, kidneys, and brain\(^1\).

**Conclusions**

Our results confirm previous findings that DOX treatment can induce hypothyroidism and cardiotoxicity and indicate that PIO may provide protection against these DOX-induced toxicities in rat models. These findings need to be verified through further studies that also investigate the related mechanisms, as they may hold the potential for future therapeutic strategies against DOX-induced toxicities.

**Conflict of Interest**
The authors declare that they have no conflict of interests.

**Informed Consent**
Not applicable.

**Funding**
This study was not funded by any external sources.

**Ethics Approval**
This research was permitted by the Institutional Animal Care of the College of Pharmacy and Scientific Research Deanship at Qassim University (Number Issue 22-16-02).

**Availability of Data and Materials**
The corresponding author can provide the study data upon request.

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