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The effect of different digoxin concentrations on heart tissue and antioxidant status in iron-overloaded rats

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Original Article

Abstract

BACKGROUND: Thalassaemia is a hereditary disorder and has an economic burden on patients and the government. The most prevalent complication in these patients is iron overload which is followed by cardiomyopathy. Digoxin is considered as a treatment against heart failure in thalassaemia. The present study evaluated the effect of two digoxin concentrations on iron content and antioxidative defense in cardiac tissue of iron-overloaded rats.

METHODS: The study was conducted on 48 rats which were divided into 6 groups. Group 1 was the control group and did not receive any treatment and group 2 was the iron overload group. In addition groups 3 and 4 were the digoxin control groups which received 1 and 5 mg/kg/day of digoxin, respectively. Groups 5 and 6 received 1 and 5 mg/kg/day of digoxin plus iron-dextran, respectively. After 1 month, malondialdehyde (MDA), superoxide dismutase (SOD), glutathione peroxidase (GPX), and total antioxidant status (TAS) were assessed in cardiac tissues.

RESULTS: Co-administration of iron-dextran and digoxin (1 and 5 mg/kg/day) significantly increased SOD and TAS levels ($P < 0.0010$) and reduced MDA ($P < 0.0010$) in heart tissue compared to control and iron overload groups. GPX levels significantly reduced in groups 5 and 6 (iron + digoxin 1 ($P < 0.0500$) and iron + digoxin 5) ($P < 0.0010$) compared to the iron control group.

CONCLUSION: Digoxin remarkably facilitates iron uptake by cardiomyocytes by affecting other channels such as L-type and T-type Ca^{2+} channels (LTCC and TTCC). Digoxin administration in the iron-overloaded rat model deteriorated antioxidative parameters and increased iron entry into heart tissue at higher doses. Therefore, in patients with beta thalassaemia major, digoxin must be administered with great care and serum iron and ferritin must be regularly monitored.

Keywords: Digoxin, Iron Overload, Superoxide Dismutase, Glutathione Peroxidase

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Introduction

Thalassaemia is a common hereditary disorder in which the synthesis of haemoglobin subunits is disturbed. There are about 15 million patients with thalassaemia all over the world and there are 240 million beta thalassaemiacarriers.[1] Iran is placed on the thalassaemia belt, and it has been reported that the prevalence of thalassaemia in Iran is about 3.6% and that there is a high population of carriers in Iran (about 4% of the population).[2] Iron is considered as an essential element and it contributes to the structure and function of proteins. Iron is required for hemoglobin and myoglobin production. Iron also has an important role in the formation of reactive oxygen species (ROS), including hydroxyl radical, superoxide radical, and hydrogen peroxide, which are toxic and cause profound damage to DNA, proteins, and lipids.[3-5] Since Iron has an important role in ROS generation and the human body has no mechanism for iron removal, iron accumulation in the body causes devastating damage to biological pathways and critical organs such as the heart, liver, bone marrow, and pancreas. Therefore, the chance of progression toward diseases such as diabetes, heart failure, atherosclerosis, and metabolic syndrome increases

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in patients with thalassaemia.[5-8]

Heart disease is still the leading cause of mortality and morbidity in patients with thalassaemia. Previous studies have reported the prevalence of death in thalassaemia due to cardiomyopathy as 63-71%.[8-12]

Cardiac glycosides are naturally derived compounds and their core structure is steroidal. Digoxin is a member of this family and binds and inhibits Na^+/K^+-ATPase (NKA) activity. NKA inhibition by digoxin induces the efflux of potassium and increases sodium entrance into cardiomyocytes. This sodium accumulation in cardiomyocytes promotes Na^+/Ca^{2+} exchangers (NCX) which results in free calcium elevation. This phenomenon explains the inotropic action of digoxin on cardiac muscle.[13,14] Data about iron entrance into cardiac cells is controversial and has indicated that L-type Ca^{2+} channels (LTCCs), T-type Ca^{2+} channels (TTCCs), divalent metal transporter1 (DMT1), and transferrin receptor protein 1 (TfR1) are involved in iron uptake into cardiomyocytes.[15-20] LTCCs transport Ca^{2+}, but have also been reported to be capable of transporting other divalent cations including Fe^{2+}, Zn^{2+}, and Mn^{2+} into cardiomyocytes. It seems that digoxin may indirectly activate LTCCs by limiting NKA activity, so other divalent cations can be taken up by cardiomyocytes. In our previous study, we showed that digoxin significantly increased the cardiac iron content of iron-overloaded rats.[21] In the present study, we evaluated the antioxidant status of heart tissue in iron-overloaded rats under different concentrations of digoxin to assess the effects of digoxin on iron entry into heart tissue and also its effects on antioxidative defense in cardiomyocytes in iron overload.

Materials and Methods

Iron was evaluated using an Iron assay kit (BioVision; Catalog #K390-100) and digoxin was assayed using the Digoxin enzyme-linked immunosorbent assay (ELISA) kit (Digoxin AccuBind ELISA Kits; code 925-300). Iron-dextran (Sigma; D8517) and digoxin were purchased from Sigma-Aldrich Corporation (St. Louis, MO, USA). The measurement of superoxide dismutase (SOD), glutathione peroxidase (GPX), and total antioxidant status (TAS) in cardiac tissue was conducted using specific kits supplied by Randox laboratories Ltd. (Crumlin, UK) (TAS: Cat. No. NX2332; SOD: Cat. No. SD125; and GPX: Cat. No. RS505). Malondialdehyde (MDA), as representative of lipid peroxidation, was measured as a thiobarbituric acid-reactive substance (TBARS) at 534 nm, and the calibration graph was obtained using different concentrations of 1,1,3,3-tetramethoxypropane.[21,22]

The animals were purchased from Kerman Physiology Research Center, Iran, and after acclimatization, the animals were kept under controlled conditions (24 ± 1 °C, 12-hour light-dark cycle, and free access to rat chow and water). In the present study, 48 male Sprague Dawley rats weighing 200-230 g were selected and randomly divided into 6 groups. The study was approved by the ethic committee of Kerman University of Medical Sciences, Iran. In order to develop iron overload in rats, iron-dextran was used. Before beginning the main study, digoxin was administrated by intraperitoneal injection for a week to ensure high digoxin levels in the corresponding groups.

Group1 (control group): untreated group

Group2 (iron overload): received 12.5 mg/100g body weight iron-dextran every 5 days

Group3 (digoxin control 1): received 1 mg/kg/day digoxin

Group4 (digoxin control 5): received 5 mg/kg/day digoxin

Group5 (iron+ digoxin 1): received 12.5 mg/100g body weight iron dextran every 5 days + 1 mg/kg/day digoxin

Group6 (iron+ digoxin 5): received 12.5 mg/100g body weight iron dextran every 5 days + 5 mg/kg/day digoxin

At the end of the study (day 30), the animals were sacrificed under ether anesthesia. Subsequently, the abdominal part of the animal's body was incised, and the heart tissue was removed and quickly placed in cold saline to extract the remaining blood from tissues. Heart tissues were collected for the assessment of iron and other antioxidants; therefore, the tissues were placed in cold lysis buffer, and then, homogenized using an ultrasonic processor (UP200H, Hielscher Ultrasonics, Germany) on ice to reduce heat generation during sonication process. Finally, the homogenates were placed in new tubes and centrifuged at 15000 rpm and 4 °C for 15 minutes. The supernatants were collected and aliquoted in new collecting tubes and kept at -80 °C for further examinations.[21]

Statistical analyses were performed in SPSS software (version 21, IBM Corporation, Armonk, NY, USA). The results were presented as mean ± standard error of mean (SEM). One-way analysis of variance (ANOVA) followed by Tukey's post hoc test were performed for analysis and pair-wise group comparison. All P values < 0.0500 were considered statistically significant.

Results

The results showed that digoxin administration remarkably elevated heart iron content in the group that received a combination of iron-dextran and digoxin compared to control and digoxin control groups (P > 0.0001) (Figure 1).

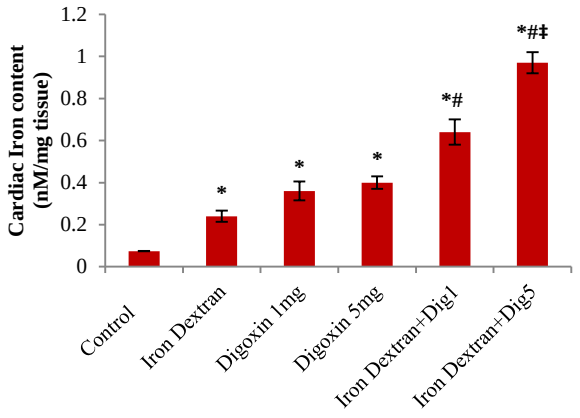

Figure 1. Cardiac iron contents in different studied groups.
Data is expressed as mean ± SEM.
SEM: Standard error of mean
* Statistically significant compared to control group, # Statistically significant compared to iron overload control group, ‡ statistically significant compared to iron-dextran + digoxin 1 group

Co-administration of iron-dextran and digoxin (1 and 5 mg/kg/day) significantly increased SOD and TAS levels (P < 0.0010), but reduced MDA levels (P < 0.0010) in the heart tissue compared to the control and iron overload control groups (Figures 2-4).

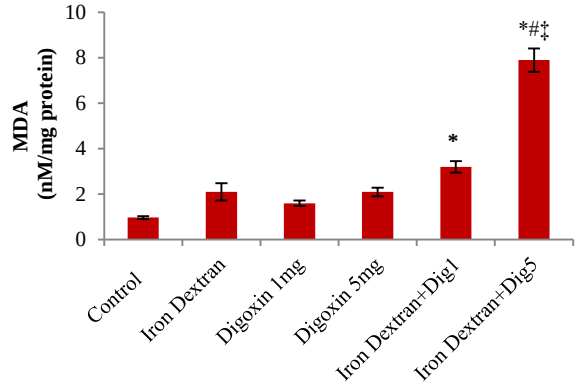

Figure 2. Malondialdehyde levels in heart tissue of rats in different studied groups
Data is expressed as mean ± SEM.
SEM: Standard error of mean
MDA: Malondialdehyde
* Statistically significant compared to control group, # Statistically significant compared to iron overload control group, ‡ statistically significant compared to iron-dextran + digoxin 1 group.

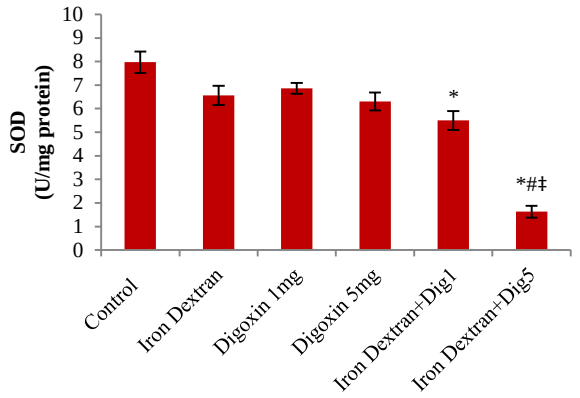

Figure 3. Superoxide dismutase levels in heart tissue of rats in different studied groups.
Data is expressed as mean ± SEM.
SEM: Standard error of mean
SOD: Superoxide dismutase
* Statistically significant compared to control group, # Statistically significant compared to iron overload control group, ‡ Statistically significant compared to iron-dextran + digoxin 1 group

GPX levels significantly reduced by iron + digoxin 1 (P < 0.0500) and iron + digoxin 5 (P < 0.0010) compared to the iron control group (Figure 5).

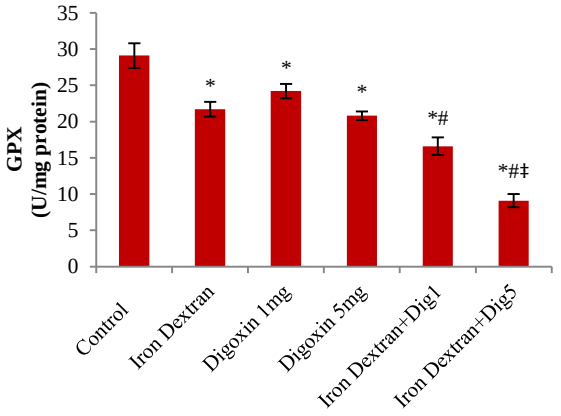

Figure 4. Glutathione peroxidase in heart tissue of rats in different studied groups
Data is expressed as mean ± SEM.
SEM: Standard error of mean
GPX: Glutathione peroxidase
* Statistically significant compared to control group, # Statistically significant compared to iron overload control group, ‡ Statistically significant compared to iron-dextran + digoxin 1 group

Significant changes were observed in total antioxidant capacity (TAC); TAC was significantly reduced in the group which received iron + digoxin 1 compared to the control group (P < 0.0010) (Figure 5).

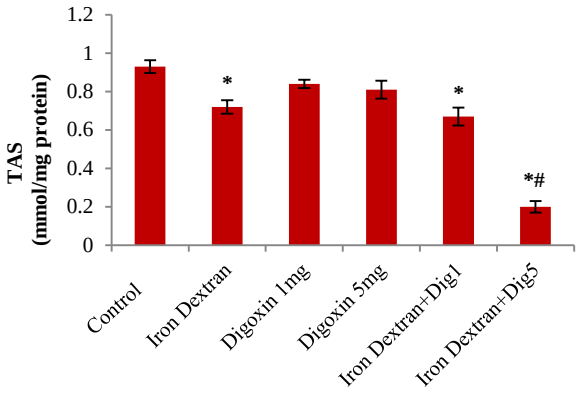

Figure 5. Total antioxidant status quantity in heart tissue of rats in different studied groups.
Data is expressed as mean ± SEM.
SEM: Standard error of mean
TAS: Total antioxidant status
* Statistically significant compared to control group,
# Statistically significant compared to iron overload control group

Moreover, a significant reduction was observed in TAC in the iron overload group and the group that received 5 mg of digoxin compared to the control and iron overload groups ($P < 0.0001$) (Figure 5).

Discussion

Thalassaemia is a group of inherited diseases in which globin chains which participates in haemoglobin formation are affected. In patients with thalassaemia, there is inadequate hematopoiesis, and patients with thalassemia major require regular transfusion. It has been shown that, in patients with thalassaemia, there is a high probability of hemochromatosis which results from repeated blood transfusion and entrance of high amounts of iron into their body.[6,12,23,24] Unfortunately, there is no known way for removal of excess iron from the body, so the entrance of iron into these patients' bodies cause a life threatening condition in these patients.[12,23] The main problem following transfusion and entry of large amounts of iron into the body of patients with thalassemia is iron deposition in critical organs such as the heart and liver, and bone marrow.[23,24] The most prevalent disease in patients with thalassemia is cardiomyopathy. This cardiomyopathy is the result of iron deposition and iron overload dependent ROS generation which is very harmful.[12] Thalassaemia is an expensive disease and its treatment places an economic burden on patients and the government.[25] Therefore, reduction of iron overload in these patients can reduce post-transfusion adverse effects.

The present study investigated lipid peroxidation and enzymatic antioxidative defense in the hearts of iron overloaded rats and effects of different concentrations of digoxin on these parameters. The iron contents of heart tissues were found to be significantly higher in digoxin and iron treated groups compared to the control group. These data demonstrated that serum iron and digoxin elevation increase iron uptake by cardiomyocytes. Iron uptake by cardiac muscle cells in groups 5-6, which received a combination of iron and digoxin, compared to the iron control group was significant. This shows that digoxin somehow increases iron uptake by cardiomyocytes. Furthermore, 5 mg compared to 1 mg digoxin showed more remarkable iron elevation in cardiomyocytes which showed the dose-dependent action of digoxin on iron uptake into cardiac muscle cells. Previously, it has been demonstrated that digoxin has an important role in iron entry into cardiomyocytes and deteriorates cardiac histological parameters.[21] Therefore, it can be concluded that digoxin administration in patients with thalassaemia must be reconsidered and performed with more care and serum iron levels of patients must be monitored. Digoxin administration in patients with thalassaemia helps them to recover their cardiac function and is vital for these patients to maintain their cardiac activity.[13,14] Nevertheless, it has been demonstrated that digoxin can indirectly affect other channels in cardiomyocytes such as LTCC and TTCC. These channels are involved in the uptake of Ca and other divalent cations by cardiac cells.[15,16,20]

In the present study, oxidative parameters of heart tissue of iron-overloaded rats were assessed after two different doses of digoxin administration in iron overload state. It was found that MDA, which is a lipid peroxidation marker, and cytotoxic aldehyde were significantly elevated and that 5 mg digoxin showed a much higher MDA elevation effect than 1 mg digoxin in the iron overload rat models. Administration of 1 mg digoxin elevates MDA only compared to the control group. Moreover, there were no differences between iron overload control and iron overload plus 1 mg digoxin in terms of MDA levels, which shows that 1 mg of digoxin is not as potent as 5 mg in increasing iron entry, and consequently, lipid peroxidation. The other factors evaluated included SOD and GPX in heart tissue. These two enzymes along with other antioxidants work together against

free radicals and protect cells from the destructive effects of these toxic agents. Another study reported that patients with thalassaemia are in an oxidative state.[4]

Data about iron uptake by cardiomyocytes are controversial and many studies have reported that LTCC, TTCC, DMT1, and TfR1 are involved in iron uptake into cardiac cells.[16,17,20,26,27] Kumfu et al. (2011) showed that only TTCCs are involved in iron uptake in thalassaemic cardiomyocytes in culture media, and that the inhibition of other channels and receptors had no effects on iron uptake by thalassaemic cardiomyocytes which refuse their role in iron entrance into cardiac muscle cells.[26] In another study, it was shown that LTCC, TTCC, and DMT1 blockers attenuated cardiac MDA and cardiac iron content, and that TTCC blockers are more beneficial in the reduction of iron accumulation in the heart than LTCC blockers.[17] Moreover, it was demonstrated that the entrance of Fe^{3+} into thalassaemic cardiomyocytes is not conducted by TfR1, DMT1, LTCC, and TTCC. Therefore, they proposed that there must be another way by which Fe^{3+} is taken up in thalassaemic cardiac muscle cells.[27]

Recently, it has been reported that a dual LTCC and TCC blocker, efonidipine, showed as much beneficial effect as other commercially available iron chelators, and this blocker is a new and strong remedy for iron overload in thalassaemia.[20] Mishra and Tiwari (2013) demonstrated that most patients with beta thalassaemia major showed increased serum ferritin levels that result from insufficient chelation. Poor chelation therapy causes iron overload development which results in downstream problems in these patients including cardiac complications.[24]

Arispe et al. (2008) indicated that digitoxin was able to form channels in cell culture which pass Ca over the lipid bilayer membrane, and also the same channels were formed by digoxin.[28] They explained that the digoxin calcium channel can be considered as the main way of Ca entrance into cardiomyocytes and it could account for the mechanism of toxicity of these glycosides in the heart. They also showed that cardiac glycosides promote Ca uptake in a dose-dependent manner.[28] According to these findings, it can be concluded that digoxin, in addition to affecting other calcium channels such as LTCC and TTCC, increases iron uptake into cardiomyocytes by developing a digoxin calcium channel. The present study results showed that a high dose of digoxin is more potent to increase iron uptake than low doses. A high dose of digoxin probably forms more calcium channels over the cardiac muscle cells membrane and facilitates iron entry into cardiomyocytes. Deterioration of the antioxidant status and elevation of MDA in cardiac tissue of iron overloaded rats after digoxin administration also confirms the effect of digoxin on iron entrance into cardiomyocytes.

Remarkably facilitates iron uptake by cardiomyocytes probably via affecting other channels such as TTCC and LTCC, or by forming newly described digoxin calcium channels. In addition, iron caused ROS production in the heart that is an important mechanism by which cardiac damage occurs and results in apoptosis, and finally, heart failure.[8] Moreover, chronic iron overload diseases result in mitochondrial DNA (mtDNA) damage and respiratory disorders which in heart tissue could result in ischemic heart failure.[5] Previous studies have reported a relationship between iron content in the body and atherosclerosis; thus, in the iron overload state, in addition to the progress toward heart failure, there is also the chance of atherosclerosis and coronary artery diseases development.[7,29,30] In the present study, it was demonstrated that digoxin administration at high and low doses in iron overload model deteriorate antioxidative parameters and increase iron entry into heart tissue. Hence, in patients with beta thalassaemia major, digoxin must be administered with care and serum iron and ferritin must be regularly monitored.

Conclusion

Digoxin significantly facilitates iron entrance into cardiomyocytes by affecting other channels such as LTCC, or by forming newly described digoxin calcium channels. The present study results showed that a high dose of digoxin in the iron overload rat model deteriorated antioxidative status and extremely elevated iron entrance into the heart tissue; therefore, digoxin administration in conditions which result in the iron overload state must be undertaken with care and serum iron and ferritin must be regularly monitored.

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Conflict of Interests

Authors have no conflict of interests.

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