Effects of digoxin on cardiac iron content in rat model of iron overload

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Original Article

Abstract

BACKGROUND: Plasma iron excess can lead to iron accumulation in heart, kidney and liver. Heart failure is a clinical widespread syndrome. In thalassemia, iron overload cardiomyopathy is caused by iron accumulation in the heart that leads to cardiac damage and heart failure. Digoxin increases the intracellular sodium concentration by inhibition of Na^+/K^+-ATPase that affects Na^+/Ca^{2+} exchanger (NCX), which raises intracellular calcium and thus attenuates heart failure. The mechanism of iron uptake into cardiomyocytes is not exactly understood.

METHODS: We assessed the effect of different concentrations of digoxin on cardiac iron content in rat model of iron overload. Digoxin had been administrated intraperitoneally (IP) for one week before main study began to assure increased digoxin levels. Group 1 received four IP injections of iron-dextran (12.5mg/100g body weight) every 5 days evenly distributed over 20 days. Groups 2-4 received 0.5, 1 and 5 mg/kg/day IP digoxin, respectively. Last three groups 5-7 received iron-dextran as group 1 and digoxin concentrations 0.5, 1 and 5 mg/kg/day, respectively.

RESULTS: Cardiac iron contents were significantly higher in iron overload groups that received different concentrations (0.5, 1 and 5 mg/kg/day) of digoxin than their counterparts in control groups and this pattern was also observed in pathology assessment.

CONCLUSION: It seems that digoxin plays an important role in iron transport into heart in iron overload state but exact mechanism of this phenomenon is not clear. L-type Ca^{2+} channels are good candidates that probably could be involved in iron accumulation in cardiomyocytes. Thus it would be better to reconsider digoxin administration in thalassemia and iron overload conditions.

Keywords: Iron Overload, Digoxin, Iron Dextran Complex, Cardiac Iron Content

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Introduction

Iron is an essential trace element that has many biological and biochemical functions. Iron is an important component of hemoglobin, myoglobin, cytochrome p450 system and many other proteins. Iron levels are precisely regulated under normal physiological conditions via complex mechanisms.[1] In some clinical conditions such as hemochromatosis, bone marrow failure and massive transfusion, there is an intensive load of iron in serum and subsequent accumulation in tissues.[1] In such conditions, iron metabolism is disturbed and results in increased mortality. Iron plays a pivotal role in generation of reactive oxygen species (ROS) and therefore causes many disorders such as ischemia-reperfusion injury, atherosclerosis and problems in other tissues.[2]

Iron accumulation in heart and liver damages these tissues.[3] Iron overload cardiomyopathy results from increased cardiac iron deposits. Iron accumulation in cardiac muscle cells is the leading cause of heart failure in iron overload conditions which increases mortality in affected patients.[4] Patients with thalassemia, especially thalassemia major need massive transfusions. Hence, they are predisposed to iron overload and there is no physiological mechanism to get rid of iron load in this group. Cardiomyopathy is common in patients with thalassemia and leads to left ventricle dysfunction, heart failure and death.[5,6]

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Digoxin increases the intracellular sodium concentration by interfering with Na^+/K^+-ATPase (NKA) activity. Intracellular sodium increment affects Na^+/Ca^{2+} exchangers (NCX) that subsequently leads to an increase in intracellular calcium concentration.[7,8] The exact mechanism of iron uptake into cardiomyocytes is still not fully and clearly understood. Many studies suggest that L-type Ca^{2+} channels (LTCCs) are likely to be involved in iron uptake into cardiomyocytes.[7,9,10] These channels primarily transport Ca^{2+} and also other cations such as Fe^{2+}, Zn^{2+}, Co^{2+}, Ba^{2+} and Mn^{2+} into cardiac muscle cells.[9,11,12] Digoxin inhibits NKA activity, and that probably activates LTCCs involved in divalent cations transport, therefore digoxin indirectly activate LTCCs. According to these information, we designed this study to investigate the effect of digoxin administration on iron transport into cardiomyocytes in iron overloaded rat model.

Materials and Methods

Study was performed on 56 male Sprague Dawley rats obtained from Kerman Physiology Research Center, Kerman, Iran (weight = 200-230 g). Animals were kept in standard condition and were provided with rat chow and water ad libitum. Iron content of serum and heart tissue were measured by iron assay kit (BioVision, Catalog #K390-100) and digoxin levels of serum and heart tissue were measured by digoxin assay enzyme-linked immunosorbent assay (ELISA) kit (Digoxin AccuBind ELISA Kits, 925-300). Iron-dextran (Sigma, D8517) and digoxin were also prepared from Sigma-Aldrich.

This study was approved by the Ethic Committee, Kerman University of Medical Sciences. Iron overload was induced by iron-dextran. Fifty six male Sprague-Dawley rats were randomly divided in 7 groups as below. At first digoxin had been administrated intraperitoneally (IP) daily for a 7-days period before main study began to assure high digoxin levels in groups receiving digoxin in main study. Digoxin was administrated daily and iron dextran was administrated every 5 days.

Group 1 (iron overload): received 12.5 mg/100g body weight iron-dextran every 5 days.

Group 2 (digoxin control 0.5): received 0.5 mg/kg/day digoxin.

Group 3 (digoxin control 1): received 1 mg/kg/day digoxin.

Group 4 (digoxin control 5): received 5 mg/kg/day digoxin.

Group 5 (iron + digoxin 0.5): received 12.5 mg/100g body weight iron-dextran every 5days + 0.5 mg/kg/day digoxin.

Group 6 (iron + digoxin 1): received 12.5 mg/100g body weight iron-dextran every 5 days + 1 mg/kg/day digoxin.

Group 7 (iron + digoxin 5): received 12.5 mg/100g body weight iron-dextran every 5 days + 5 mg/kg/day digoxin.

Group 1 received four IP injections of iron-dextran (12.5 mg/100g body weight) every 5 days evenly distributed over a period of 30 days. Groups 2-4 received 0.5, 1 and 5 mg/kg/day IP digoxin injections, respectively. Last three groups (5-7) received iron-dextran as group 1 and also 0.5, 1 and 5 mg/kg/day IP digoxin injections.[13,14]

At the end of the study, animals were anesthetized by ether and sacrificed, blood sample were collected and serum were separated. Then, after an abdominal incision, the heart was removed and rinsed with 0.9% NaCl to remove excess blood. A sample of the heart tissue was collected for iron and digoxin assessment. Tissues were homogenized in cold sample buffer by Hielscher homogenizer and centrifuged at 15000 rpm for 15 minutes. Supernatant was used for further evaluations. A small sample of heart was fixed immediately in 10% formalin for histological processing. Sections (4 mm) were cut and stained for histopathological evaluation. Prepared slides after Prussian blue staining were evaluated and scored 0-4 based on severity by two pathologists that were blinded to animal grouping.[15]

Statistical analyses were performed via SPSS software for Windows (version 16.0, SPSS Inc., Chicago, IL, USA). All the results are presented as mean ± standard deviation (SD). Group differences were examined for significance using one-way analysis of variance (ANOVA) followed by the Tukey's post hoc test.

Results

Data is shown in table 1. Serum digoxin levels in groups 3-4 and 6-7 were significantly higher than group 1. Serum digoxin levels in groups that receive 1 and 5 mg/kg/day digoxin were significantly higher than digoxin 0.5 control group.

Heart digoxin levels were significantly lower in iron control group compared to all other groups.

Heart iron levels were significantly higher in groups that received combination of digoxin and iron (groups 5-7) than their counterpart control groups (groups 2-4); this pattern was also observed in pathology assessment (Figure 1, A-G).

Table 1. Biochemical and pathological parameters in studied groups

Groups	Iron	Digoxin 0.5	Digoxin 1	Digoxin 5	Iron + digoxin 0.5	Digoxin 5	Iron + digoxin 5
Serum digoxin (ng/ml)	4.8 ± 2.0‡§	10.8±4.3§	50.5±30.0*†‡§	140.0 ± 30.0*†‡	5.5 ± 1.7‡§	140.0 ± 30.0*†‡	125.0±37.0*†‡
Heart digoxin (ng/mg) tissue	0.2 ± 0.0§	24.4±9.2§	1,427.0±1,401.0§	51,140.0±36,211.0*†‡	10.6.6±85.0§	51,140.0±36,211.0*†‡	43,123.0±24,025.0*†‡
Serum iron (nmolar)	18.6±3.0†‡§	6.5 ± 1.6*	7.0 ± 1.5*	7.8 ± 2.4*	8.8 ± 2.0*	7.8 ± 2.4*	11.2 ± 2.0*†‡
Heart iron (nM/mg) tissue	0.2 ± 0.1	0.3 ± 0.1	0.4 ± 0.1	0.4 ± 0.8	0.7±0.2*†‡§	0.4 ± 0.8	1.0 ± 0.1*†‡§
Pathology score	3.2±0.4†‡§	0.0 ± 0.0*	0.5 ± 0.5*	0.4 ± 0.5*	3.2±0.4†‡§	0.4 ± 0.5*	3.9 ± 0.5†‡§

Values are mean ± SD; n = 8 rats/group. data analyzed by one-way ANOVA
* Statistically significant compared to iron group (P < 0.05)
† Statistically significant compared to digoxin 0.5 group (P < 0.05)
‡ Statistically significant compared to digoxin 1 group (P < 0.05)
§ Statistically significant compared to digoxin 5 group (P < 0.05)

Discussion

Massive and long term transfusion in patients with thalassemia or bone marrow failure is considered as a life protecting therapy.[1] Following transfusions, iron levels rise in the body and iron overload condition occurs. Iron overload leads to some complications such as ROS generation and also iron deposition in many tissues including heart and liver.[2] Cardiac cellular damage and heart failure by iron known as iron overload cardiomyopathy occurs under iron overload condition and is the main cause of death in thalassemia major patients.[5,6] Digoxin has an indirect effect on intracellular calcium levels. Digoxin therapy to maintain cardiac function leads to increased intracellular sodium concentration that subsequently increases the intracellular calcium concentration by affecting NCX.[7,8] However, it is suggested that LTCCs also play a part in divalent cations transport into cardiomyocytes.[10] We have shown that digoxin administration in digoxin control groups (groups 2-4) and iron overloaded groups (groups 5-7) caused iron accumulation in the heart tissue. There was a dose dependent increase in iron content of cardiomyocytes by different concentrations of digoxin.

Several findings support the role of LTCCs in myocardial iron transport. Tsushima et al.[10] showed that myocardial iron uptake was driven by LTCCs and suggested that LTCCs blockers could be a useful treatment in iron overload condition. Compared to our data, it seems reasonable to account LTCCs as a major player in iron transport into cardiomyocytes.

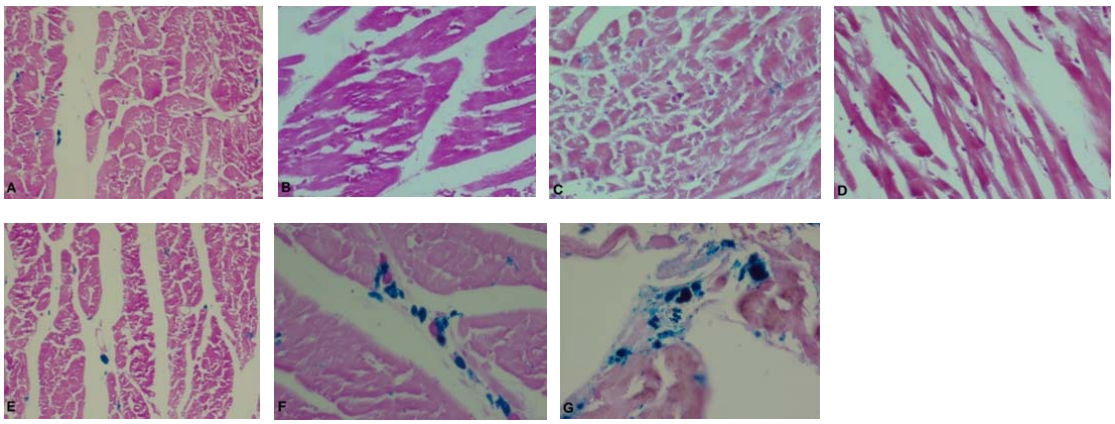

Figure 1. Prussian blue staining of heart. Groups respectively received A. Iron-dextran (12.5 mg/100g body weight), B. 0.5 mg/kg digoxin, C. 1 mg/kg digoxin, D. 5 mg/kg digoxin, E. Iron-dextran as group A and 0.5 mg/kg digoxin, F. Iron-dextran as group A and 1mg/kg digoxin, G. Iron-dextran as group A and 5 mg/kg digoxin

Efonidipine is a T-type Ca^{2+} channels (TTCCs) and LTCCs blocker but it blocks TTCCs greater than LTCCs.[16] It has been reported that efonidipine prevented iron uptake into cultured thalassemic cardiomyocytes in iron overload conditions.[17] On the other hand, verapamil (a LTCCs blocker) could not prevent iron uptake but efonidipine prevented iron uptake. These findings from previous studies suggested that TTCCs also play a significant role in iron uptake into cardiomyocytes.[18] A rat heart in iron loaded condition showed that iron uptake was increased by the LTCCs agonist, Bay K 8644, and iron uptake was inhibited by the LTCCs blocker, nifedipine.[10] In other study, Oudit and colleagues[18] have demonstrated that treatment with LTCCs blockers such as amlodipine and verapamil led to LTCCs inhibition in cardiomyocytes; hence reduced myocardial iron accumulation.

Xu and colleagues[7] showed that NKA inhibition by ouabain-induced Ca^{2+} influx. They also provided direct evidence that KB-R7943 (NCX blocker) and nifedipine both could halt ouabain-induced Ca^{2+} influx, indicating that both LTCCs and NCX contributed to the rise of intracellular Ca^{2+} levels. The exact mechanism of iron transport into cardiomyocytes is not fully and properly understood but according to Xu and colleagues' observations, it seems that LTCCs along with NCX are involved in this phenomenon especially in iron overload conditions.[7] But digoxin role in this process is not investigated and needs to be clarified. Digoxin elevates intracellular Ca^{2+} levels and probably maximizes its effect on Ca^{2+} elevation by elevating LTCCs activity that could increase iron transport into cardiomyocytes.[8,18]

LTCC activity is increased by iron elevation under iron overload condition.[9] In our groups that received same iron concentrations (groups 5-7), there was a digoxin dependent iron transport into the cardiomyocytes. Findings from previous studies are controversial and are challenging about LTCCs probable role in iron transport into cardiomyocytes. We showed that digoxin in digoxin control groups (groups 2-4) raised iron content in cardiomyocytes but in iron overloaded group (group 1) despite presence of iron overload condition, there was lower iron content in heart tissue compared to other groups. Considering digoxin role in Ca^{2+} (and probably other divalent cations such as Fe^{2+}) transport by LTCCs into cardiomyocytes,[7,9,10] our findings support this hypothesis that digoxin has a pivotal role in activation of LTCCs and helps iron entrance into cardiomyocytes in iron overload condition. On the other hand, digoxin also has an iron overload independent effect on LTCCs to enter iron into cardiomyocytes.

Conclusion

If we accept that digoxin increases the flow of ions (other divalent cations except Ca^{2+}) through LTCCs, as Xu and colleagues have shown in their study,[7] it seems that it has an important role in iron entrance into cardiomyocytes especially in thalassemic and other iron overload conditions, and digoxin administration in these patients must be reconsidered or must be administrated with caution. Also the direct effect of digoxin needs to be further investigated in animal models and it is necessary to administer digoxin chronically in iron overload models and investigate gene expression and protein levels of LTCCs in heart tissue in order to prove the effect of digoxin on these channels.

Acknowledgments

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Conflict of Interests

Authors have no conflict of interests.

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