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Effects of glucocorticoid on cardiac chronotropic responsiveness in cirrhotic rats: A possible role for dopamine receptors

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ABSTRACT

Objective(s): Cirrhosis causes chronotropic dysfunction by weakening the β-adrenergic receptor (β-AR) signaling pathway in cirrhotic cardiomyopathy (CCM). Downstream signaling of glucocorticoids and dopamine receptors influences the β-AR pathway. Thus, the effects of glucocorticoids on chronotropic incompetence and the possible involved pathway were investigated in this experiment.

Materials and Methods: Bile duct ligation (BDL) surgery was performed on Wistar rats to induce cirrhosis. Four weeks after BDL or sham surgery, the subjects were given an intramuscular injection of either saline (NS) or dexamethasone (dexa) (2.2 mg/kg/day) for three consecutive days. *In vivo*, chronotropic responsiveness to isoproterenol and QTc interval were evaluated by electrocardiogram (ECG). Real-time polymerase chain reaction (RT-PCR) and immunohistochemistry (IHC) were performed to determine the effectiveness of dexa on dopamine D1, D2 receptors, and GNAL mRNA expression. Moreover, the tumor necrosis factor-alpha (TNF-α) and interleukin-1beta (IL-1β) levels in rats' hearts were assessed.

Results: Dexa treatment reduced the prolonged QT intervals in cirrhosis. It also dedcreasd spleen weight, as well as TNF-α levels which are increased in cirrhosis. Moreover, dexa increased D1 protein expression in IHC.

Conclusion: Dexa effectively improved cirrotic heart by improving QT intervales and increasing spleen weight, reducing a pro-inflammatory cytokine, and up-regulateing D1 receptor protein expression.

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Introduction

Cirrhosis may result in cirrhotic cardiomyopathy (CCM), a chronic cardiac condition with chronotropic dysfunction (1). CCM is recognized by impaired cardiac contractility to stress, prolonged QT interval, and systolic and diastolic dysfunctions. Moreover, cirrhosis may lead to hyperdynamic circulation with peripheral arterial vasodilation and increased cardiac output and blood volume. In CCM, a reduction in cardiomyocyte contractility occurs due to persistent overload on the heart (1, 2). There are several mechanisms for CCM, i.e., abnormalities in the beta-adrenergic receptor (β-AR) signaling pathway, which were due to the reductions of β-ARs density, cyclic adenosine monophosphate (cAMP) production, G-protein and adenylate cyclase (AC) levels, and alteration in plasma membrane fluidity (1).

Glucocorticoids are naturally secreted by the adrenal cortex from cholesterol under the hypothalamic-pituitary-adrenal (HPA) axis control, as one of the main neuroendocrine systems in organisms (3). Glucocorticoids are not only endogenous hormones, however, they are administered exogenously as an anti-inflammatory and immunosuppressant for their long-term favorable and lifesaving outcomes. Due to their potent anti-inflammatory effect and ability to reduce the cytokines, they are administered as lifesaving steroids (4). Glucocorticoids are steroids that have demonstrated cardioprotective qualities (5, 6). They make catecholamine more available at receptor sites by reducing catecholamine metabolism and inhibiting catecholamine reuptake at neuromuscular junctions. Glucocorticoids also increase the sensitivity of the cardiovascular system to catecholamine by raising the binding affinity of β-ARs and increasing the receptor-G protein coupling and cAMP production. Furthermore, glucocorticoids enhance the β-AR mRNA level in some other tissues (7).

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Glucocorticoids can influence dopamine receptors (8, 9). Notably, dopamine neurotransmitter in addition to affecting the central nervous system (CNS), affects the cardiovascular system. Moreover, dopamine receptors amplify the β-AR's signaling pathway and intracellular calcium (Ca^{2+}) concentration, which is essential for cardiac contractility (10, 11). G-proteins and their receptors are widely expressed in the cardiovascular system and are involved in the pathophysiology of cardiovascular diseases (12). Various G-proteins, Gαs, Gαi, Gαq, and Gα12/13, activate several downstream diverse signal transduction pathways (13, 14). The G protein subunit Gαolf gene (GNAL) is a member of the Gαs family and is identified in different body parts including the heart (13). The Gaolf plays a role in dopamine D1 receptors signaling, adenylyl cyclase (AC) activation, and stimulation of cAMP production (15, 16). Dopamine D1 and D2 receptors are expressed in the cardiovascular system. D1 receptors play an important role in the induction of AC and stimulate the cAMP generation. In addition, the D2 receptors inhibit the enzyme AC (10), and the generation of cAMP in renal and mesenteric arteries (17). Unlike D1 receptors which have a stimulatory effect on the release of catecholamine, D2 receptors suppress the release of catecholamine in chromaffin (18).

When the β_1-ARs are stimulated by an agonist-like isoproterenol, the β_1-ARs-ligand complex stimulates AC through interaction with G proteins. The stimulated AC causes the generation of cAMP, which induces the protein kinase A (PKA) activation. Activated PKA phosphorylates different proteins like phospholamban to induce cell contraction. PKA-phosphorylated phospholamban increases the Ca^{2+} pumping activity, which results in an increase of Ca^{2+} reuptake in the sarcoplasmic reticulum (SR), which leads to the stimulation of cell contraction (19, 20). Consequently, any defect in the β_1-ARs signaling pathway reduces the chronotropic responsiveness to adrenergic agonists (21).

In vascular endothelial cells of cirrhotic patients and animal models, the increased production of tumor necrosis factor-α (TNF-α) causes cardiac depression and contractile function suppression (19). We hypothesized that dopamine receptors, downstream regulation of GNAL, and pro-inflammatory cytokines affect chronotropic responsiveness through the mentioned pathway. To the best of our knowledge, for the first time, we investigated the effect of glucocorticoids on CCM and whether this influence may be related to dopamine receptors, their downstream GNAL, and pro-inflammatory cytokines.

Materials and Methods

Animals and experimental grouping

Male Wistar albino rats (weight 250–280 g) were purchased from the Department of Pharmacology, Comparative Biology Unit, Tehran University of Medical Sciences (TUMS). The rats were kept in a 12-hr light/dark cycle at a temperature of 22 °C with free access to regular nutrition and drinking water. The principles of medical ethics (Guidelines for the Care and Use of Laboratory Animals (NIH US publication No. 85-23, revised 1985)) were followed strictly during the experimentation. All experiments performed on animals were approved according to the ethical principles of the institute.

A total of 24 rats were randomly selected and divided into two groups: Sham (sham-operated), bile duct ligation (BDL) cirrhotic groups, with 12 rats per group. In addition, the sham group was subdivided into the sham/NS (normal saline-treated control) group and the sham/dexa (dexa-treated control) group. Similarly, the BDL group was subdivided into the BDL/NS (normal saline-treated cirrhotic) and BDL/dexa (dexa-treated cirrhotic), with six animals per group. *In vivo* chronotropic study and QTc interval analysis, *In vitro* immunohistochemistry (IHC), dopamine receptors, and GNAL expression, and TNF-α and interleukin-1β (IL-1β) levels were studied for each group.

Induction of cirrhosis: Bile duct ligation (BDL)

The rats were anesthetized by administering a mixture of ketamine (100 mg/kg) and xylazine (8 mg/kg) (22). For BDL, an abdominal midline incision was made to expose the common bile duct. Next, the common bile duct was doubly ligated with 4-0 silk material and the common bile duct between the ligatures cut. After the operation, the abdominal layers were closed independently with 3-0 silk sutures. The laparotomy procedure was the same for the Sham-operated rats except the common bile duct was not ligated and cut (23). Signs of cirrhosis were developed in rats 28 days after the common BDL (22). The respective treatments were done on days 26-28 after Sham and BDL surgeries.

Treatments

The sham/dexamethasone (dexa) and BDL/dexa groups received a daily dose of 2.2 mg/kg dexa intramuscularly for the last three days of the 4th week after BDL (short-term treatment) to prevent complications such as vascular disorders and insulin resistance, and give an adequate anti-inflammatory blood level without the side effects (24). The sham/NS and BDL/NS groups received an equal volume of normal saline intramuscularly at the same time. The consecutive studies were performed either on day 29 after surgery or day 4 after treatment.

The use of short-term dexa in our experiment had pharmacological justification that patients with heart failure symptoms usually present to the hospital in the decompensated stage of CCM (25). Previous studies have shown up-regulation of beta receptors with short-term use of corticosteroids and treatment with corticosteroids, similar to beta-agonists, may be considered during exacerbation of heart failure and cannot be taken during remission stage related to chronic unwanted side effects. In addition, chronic use of corticosteroids can cause functional tolerance. Notably, metabolic and systemic side effects are rare with short-course use (26).

Chronotropic study

The rats were anesthetized with a mixture of ketamine and diazepam (100 mg/kg and 0.4 mg/ kg, IP, respectively). Under anesthesia, the electrocardiogram (ECG) was recorded with three stainless steel electrodes placed subcutaneously. The electrodes were connected on the other end to a bioamplifier (ADInstruments, Bella Vista, Australia) of the PowerLab system. The ECG was recorded after intraperitoneal injection (IP) of different doses of isoproterenol (0 mg/ml (4 min before isoproterenol administration), 0.5, 1, and 2 mg/ml) at four-minute intervals (27). The PowerLab analog/digital converter was used to digit the amplified signals (sampling rate: 10 kHz) 4 min after administration of the last dose of isoproterenol. The heart rate data were

displayed on LabChart 5 software (ADInstruments) and analyzed. The QT interval, the interval started from the Q wave till the end of the T wave, was presented as corrected QT (QTc), and calculated through Bazett's formula, i.e., QTc = QT/√R-R (22).

Immunohistochemistry (IHC) assay

Under deep anesthesia, the rat hearts were excised and then the ventricle was separated and preserved in formalin (22). The formalin-fixed heart samples were embedded in paraffin and were sectioned into 5-µm thickness by microtome (RM2235 Rotary Microtome, Leica Microsystem, US). Following deparaffinization in xylene and rehydration in different ethanol concentrations, the sections were kept in 0.03 % hydrogen peroxide solution (diluted in methanol) for 10 min to block the activity of endogenous peroxidase enzyme. The sections were immersed in an antigen retrieval solution to improve staining quality. The primary antibody (diluted as 1 in 100 parts in PBS) was added to the sections and incubated overnight at 2 to 8 °C temperature. Next, after washing sections with PBS, a 100 µl linker was added, and the sections were incubated for 20 min. Then, after adding polymer to the sections and incubating them for 30 min, diaminobenzidine (DAB) buffer was added and incubated for 5 min. In the last step, after washing the sections, they were placed in Hematoxylin for one minute to counterstain and were observed under a light microscope (22).

Enzyme-linked immunosorbent assay (ELISA)

After removing the hearts from anesthetized rats, the ventricle was separated and transferred to liquid nitrogen. TNF-α and IL-1β levels were quantified in the hearts through ELISA kits (Rat TNF-α ELISA Kit, MyBioSource, USA, Rat IL-1 beta ELISA Kit, MyBioSource, USA) as per the manufacturer's instructions. In short, 100 µl of each appropriate sample dilution and standard were added to the wells, and then the sealed plates were incubated at room temperature for 2 hr for TNF-α, and 37 °C for 90 min for IL-1β. After incubation, 100 µl of biotin-antibody (1x) was added to each well and incubated at 37 °C temperature for 60 min. After washing, 100 µl of streptavidin-horseradish peroxidase (1x) was added to each well and incubated at 37° C for 1 hr for TNF-α and 45 min for IL-1β. After the second washing, 100 µl of 3, 3', 5, 5'-Tetramethylbenzidine (TMB) substrate solution was added to each well, and the plates were incubated at 37° C in the dark for 30 min. Finally, 50 µl and 100 µl of stop solution were added to the wells for evaluation of TNF-α and IL-1β levels, respectively. The optical density (O.D.) absorbance was read at 450 nm through a microplate reader and calculated the levels of TNF-α and IL-1β in the samples by comparing them to the standard curve (28).

Real-time polymerase chain reaction (RT- PCR)

Quantitative RT-PCR is an assessment of dopamine D1 receptor, D2 receptor, and GNAL gene expression (29). The hearts were excised under anesthesia; then the atria were separated and placed in liquid nitrogen. After extracting total RNA by tripure isolation reagent, genomic DNA was removed by DNase I. PrimeScript reverse transcription (RT) reagent kit (TaKaRa, Japan) was used to synthesize complementary DNA (cDNA). The dNTP mixture, random hexamer (p(dN)6), and oligo (dT) primers were combined with DNase I-treated RNA. After heating at 65 °C temperature for 5 min, the samples were placed on ice and RT enzyme, buffer, ribonuclease inhibitors, and ribonuclease free water were added. The tubes were set at 25 °C temperature for 10 min and then at 42 °C temperature for 60 min in a thermocycler to make the RT enzyme active. To inactivate reversed transcriptase's destruction and prevent cDNA, the samples were placed at 85 °C for 5 min and then stored at -20 °C. The primer sequences of mRNAs used in the current study were designed as follows.

Rat dopamine D1 receptor (product size: 92 bp), Forward: 5′-GCTAAGCCTGGTCAAGAAC-3′; Reverse: 5′-CGGCCTCATCCATGGTAGAA -3′.

Rat dopamine D2 receptor (product size: 124 bp), Forward: 5′- CTGATCTTCTGGTGGCCACA -3′; Reverse: 5′- GCTGTGCACATCATGACATC -3′.

Rat GNAL (product size: 109 bp), Forward: 5′-GAGGAAAGTAAGCCGGGGTA -3′; Reverse: 5′-AGTGCTTTTCCCGGACTCA -3′.

Real-time PCR was conducted by adding 1 µl of cDNA, 10 µl of SYBR Premix Ex Taq II master mix (2x) (TaKaRa, Japan), 7 µl of deionized water, and five pmol each of forward and reverse oligonucleotide primers. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as an internal control gene to normalize the RT-PCR data. The thermal cycling protocol used was consists of denaturation, performed at 95 °C temperature for 10 min; annealing of 40 cycles, carried out at 95 °C temperature for 20 sec, at 60 °C temperature for 25 sec and 72°C for 15 sec. Then extension/elongation step was performed at 72 °C for 7 min. The Melting temperatures (TM) of the GAPDH, GNAL, D1 and D2 receptors genes were 84, 86, 85, and 82 °C, respectively. Finally, the competitive critical threshold (ΔΔCT) method was used to analyze the data (22, 29).

Statistical analysis

The data were expressed as mean ± SEM. Two-way analysis of variance (ANOVA) was used for chronotropic study data, and one-way ANOVA was used for analyzing other parameters. *Post hoc Tukey*'s test was applied to find the significant differences among the groups. The *P*-value<0.05 was considered statistically significant. The data was analyzed through GraphPad Prism 8 (GraphPad Software, Inc., San Diego, CA, USA).

Results

The spleen weight

Two-way ANOVA analysis indicated a significant difference in terms of spleen weight (F (3, 20) = 55/13; *P*<0.0001) between all the experimental groups. In the NS-treated groups, BDL was able to increase the spleen weight of the cirrhotic rats significantly compared to Sham (BDL/NS (mean ± SEM= 2.9 ± 0.19) vs Sham-operated/NS (1.2 ± 0.11); *P*<0.0001). Moreover, this increase in spleen weight of the cirrhotic group could be significantly reduced by treatment with dexa (BDL/dexa (1.6 ± 0.09) vs BDL/NS; *P*<0.0001) and there is no significant difference between BDL/dexa and sham-operated/NS, as shown in Figure 1.

The QT interval analysis

The QT intervals were measured for all the experimental groups. Two-way ANOVA analysis showed a significant difference in terms of QT intervals (F (3, 15) = 6/068; *P*=0.0065) between all experimental groups (Figure 2). The QT interval of the cirrhotic group was significantly higher than the control group (BDL/NS rats (0.29 ± 0.01) compared

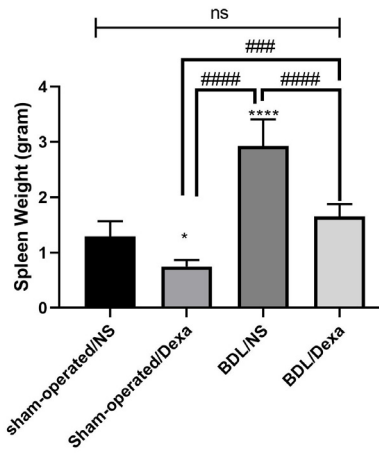

Figure 1. Average spleen weights of Sham (sham-operated) and BDL (cirrhotic) rats treated with saline or dexa (2.2 mg/kg/day)
The data are shown after treatment with respective agents for 3 consecutive days (short-term treatment). The data were analyzed using two-way ANOVA with *post hoc* Tukey's test and presented as mean ± SEM, (n = 6). *P<0.05, ****P<0.0001 Sham/dexa and BDL/NS compared to Sham/NS; ###P<0.001, ####P<0.0001 BDL/dexa compared to BDL/NS.
BDL: Bile duct ligation; NS: Saline

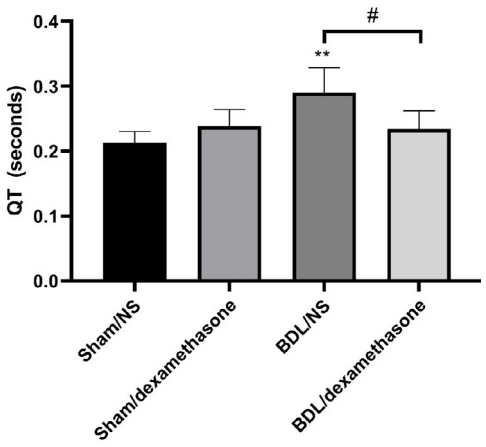

Figure 2. QTc interval of Sham (sham-operated) and BDL (cirrhotic) rats were calculated from ECG
The data are shown after treatment with saline or dexa (2.2 mg/kg/day) for 3 consecutive days (short-term treatment). The data were analyzed by two-way ANOVA with post hoc Tukey's test and presented as mean ± SEM, (n= 6). **P<0.01 BDL/NS compared to Sham/NS; #P<0.05 BDL/dexa compared to BDL/NS.
BDL: Bile duct ligation; NS: Saline

to Sham-operated/NS rats (0.212 ± 0.007); P<0.01). Dexa treatment significantly lessened the QT interval in BDL/dexagroup (0.234 ± 0.012) compared to BDL/NS group; P<0.05 and there is no significant difference between BDL/dexa and Sham-operated/NS, as shown in Figure 2.

In vivo chronotropic study

Heart rates were measured after injection of different doses of isoproterenol. The highest response, twenty sec before the injection of the higher dose, was considered a chronotropic response to isoproterenol in all experimental groups. There was a significant difference in heart rate (F (3, 64) = 36/3, P<0.0001) between all groups. Cirrhosis significantly caused a drop in the isoproterenol-stimulated heart rate in BDL/NS rats (308/2 ± 16/45) compared to Sham-operated/NS rats (387/2 ± 16/19); P<0.0001. Treatment of cirrhosis with dexa significantly enhanced the chronotropic responsiveness (BDL/dexa (344/2 ± 18/31) vs BDL/NS; P=0.0422). According to the results, the maximum response of the heart to isoproterenol occurred after administration of 0.5 mg/ml of isoproterenol, as shown in Figure 3.

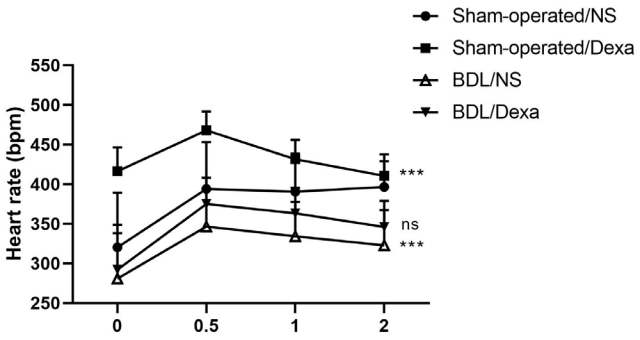

Figure 3. Average heart rates of Sham (sham-operated) and BDL (cirrhotic) rats were measured using the PowerLab system
The data are shown after treatment with saline or dexa (2.2 mg/kg/day) for 3 consecutive days (short-term treatment). The data were analyzed by two-way ANOVA with *post hoc* Tukey's test and presented as mean ± SEM, (n = 6). ***P<0.001 BDL/NS compared to Sham/NS.
BDL: Bile duct ligation; NS: Saline; Dexa: Dexamethasone

Dopamine D1 & D2 receptors expression

The mRNA expression level of the dopamine D1 receptor gene (DRD1), dopamine D2 receptor gene (DRD2), and GNAL in rat brain were measured. Our results demonstrated that there was a significant difference between the experimental groups in terms of DRD2 (F (3, 12) = 20.12; P<0.0001, GNAL (F (3, 8) = 38.25; P<0.0001) (Figure 4). D1 gene expression did not change significantly by either cirrhosis or its treatment with dexa as mentioned in Figure 4A. BDL significantly decreased mRNA expression of DRD2 in cirrhotic rats compared to control rats, both among the dexa-treated groups and among the saline-treated group (BDL/NS (0.152 ± 0.043) vs Sham-operated/NS (1/72 ±

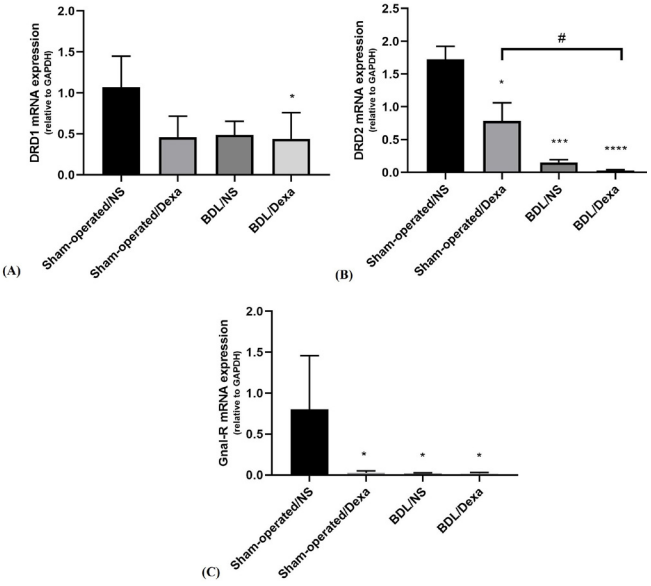

Figure 4. Expression of dopamine D1, D2, and GNAL receptors expression of Sham (sham-operated), and BDL (cirrhotic) rats was quantified through RT-PCR
The data are shown after treatment with saline or dexa (2.2 mg/kg/day) for 3 consecutive days (short-term treatment). The data were analyzed by two-way ANOVA with *post hoc* Tukey's test and presented as mean ± SEM, (n = 6). 4B: *P<0.05, ***P<0.001, ****P<0.0001 Sham/dexa and BDL/NS group compared to Sham/NS.
GNAL: Gaolf gene; BDL: Bile duct ligation; NS: Saline; RT-PCR: Real-time polymerase chain reaction; Dexa: Dexamethasone

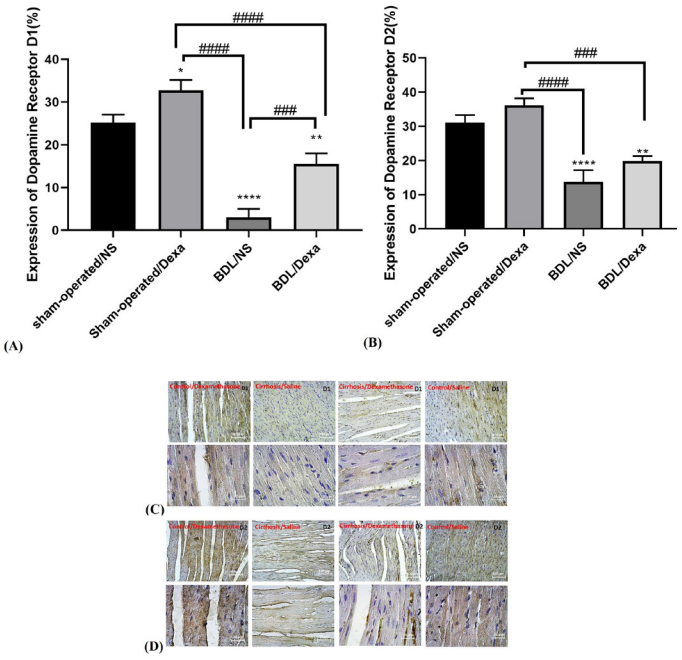

Figure 5. D1 and D2 receptors' expression levels (protein) in the hearts of Sham (sham-operated), and BDL (cirrhotic) rats treated with saline or dexa (2.2 mg/kg/day)

The respective agents were used for 3 consecutive days (short-term treatment). The receptors' levels were evaluated by IHC. The data were analyzed by two-way ANOVA and *post hoc* Tukey's test and presented as mean ± SEM, (n = 6). Figure 5A. ***P*<0.01, ****P*<0.001, *****P*<0.0001 Sham/dexa and BDL/NS compared to Sham/NS. ####*P*<0.0001 BDL/dexa compared to BDL/NS. and Figure 5B. ***P*<0.01, *****P*<0.0001 BDL/NS compared to Sham/NS. The IHC photomicrographs of the D1 and D2 receptors' expression in the hearts of Sham (sham-operated) and BDL (cirrhotic) rats treated with saline or dexa (2.2 mg/kg/day) (Figures 5C and 5D).

BDL: Bile duct ligation; NS: Saline; IHC: Immunohistochemistry; Dexa: Dexamethasone

0.2008); *P*=0.0002 and BDL/dexa (0.028 ± 0.012) vs Sham-operated/dexa (0.783 ± 0.277); *P*=0.0404) as mentioned in Figure 4B. BDL could down-regulate GNAL gene expression; considering the significant decrease in BDL/NS (0.08 ± 0.004) compared to Sham/NS (0.802 ± 0.327); *P*=0.0244, GNAL expression decreased by 1.33% in BDL/dexa (0.018 ± 0.006) compared to BDL/NS, but no significant difference was detected between them, as shown in Figure 4C.

IHC staining was performed to detect the protein expression level of D1 and D2 receptors. The data illustrated a significant difference in D1 (F (3, 8) = 105/2; *P*<0.0001) and D2 (F (3, 8) = 55/45; *P*<0.0001) receptors between all groups (Figure 5). Following BDL, the level of D1 receptors was significantly diminished in cirrhotic rats compared to Sham rats (BDL/NS (3/1 ± 1/1) vs Sham/NS (25/22 ± 1/049); *P*<0.0001. Furthermore, dexa treatment made a significant increase in the level of D1 receptors in both cirrhotic rats and sham rats (BDL/dexa vs BDL/NS; *P*=0.0005 and Sham-operated/dexa vs Sham-operated/NS; *P*=0.0116) as shown in Figure 5A. Moreover, the level of D2 receptors was markedly lessened in BDL rats compared to Sham rats (BDL/NS (13/73 ± 1/97) vs Sham-operated/NS (31/08 ± 1/25); *P*<0.0001 and BDL/dexa (19/86 ± 0.84) vs Sham/dexa (36/15 ± 1/18); *P*<0.001) Figure 5B.

Ventricular TNF-α and IL-1β levels

The ELISA test was performed to determine the levels of TNF-α and IL-1β and the effect of dexa on their levels in all groups. There was a significant difference in TNF-α levels (F (3, 12) = 98/44; *P*<0.0001) and IL-1β (F (3, 12)

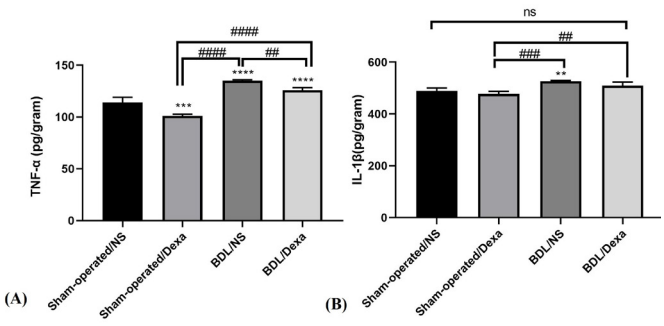

Figure 6. TNF-α and IL-1β levels of sham (sham-operated), and BDL (cirrhotic) rats were measured through ELISA

The data are shown after treatment with saline or dexa (2.2 mg/kg/day) for 3 consecutive days (short-term treatment). The data were analyzed by two-way ANOVA with *post hoc* Tukey's test and presented as mean ± SEM, (n = 6). 6A: ****P*<0.001 and *****P*<0.0001 sham/dexa and BDL/NS group compared to sham/NS; ##*P*<0.01 BDL/dexa group compared to BDL/NS. 6B: ***P*<0.01 BDL/NS group compared to sham/NS.

TNF-α: Tumor necrosis factor-alpha; IL-1β: Interleukin-1β; BDL: Bile duct ligation; NS: Saline; Dexa: Dexamethasone

= 17/83; *P*=0.0001) between all study groups (Figure 6). Cirrhosis significantly revealed a higher level of TNF-α in BDL rats compared to the Sham rats (BDL/NS (135/1 ± 0.417) vs Sham-operated/NS (114/1 ± 2/482); *P*<0.0001 and BDL/dexa (125/8 ± 1/26) vs Sham/dexa (101/1 ± 0.836); *P*<0.0001). Dexa treatment caused a significant decrease in the level of TNF-α in both cirrhotic rats and Sham rats (BDL/dexa vs BDL/NS; *P*=0.0039 and Sham-operated/dexa vs Sham-operated/NS; *P*=0.0002), Figure 6A. BDL also could increase IL-1β levels in BDL/NS rats compared to the Sham/NS group (BDL/NS (526/5 ± 1/535) vs Sham-operated/NS (489/5 ± 5/353); *P*=0.0013) as mentioned in Figure 6B.

Discussion

In this study, effects of a glucocorticoid, dexamethasone, on cardiac factors such as chronotropic responsiveness to isoproterenol and QTc interval were evaluated. In addition, the levels of pro-inflammatory cytokines, and expression of dopamine receptors (D1 and D2), and GNAL were measured in cirrhotic rats. Our findings, three weeks following the surgery, demonstrated apparent signs of biliary cirrhosis, including jaundice, ascites, and dark urine made the initial diagnosis of cirrhosis, and the observation of liver stiffness and a significant increase in the spleen weight further confirmed it, which is consistent with the development of portal hypertension (30). Aligned with previous studies, we found that cirrhosis significantly increased the spleen weight, which is associated with portal hypertension (18). In line with previous studies (1, 31, 32), our experiment showed cirrhosis caused CCM with the blunted chronotropic responses to isoproterenol and the prolonged QT interval, which is directly related to the degree of portal hypertension and inversely related to plasma Ca^{2+} concentration (33). The pathogenesis of the blunted chronotropic function is complicated (34), many studies have investigated the possible mechanism of pathogenesis involved in cardiac chronotropic dysfunction in cirrhotic rats (19). It has been shown that cardiomyocyte β-ARs density is notably reduced in cirrhotic rats (35).

On the other hand, based on our study, glucocorticoid treatment effectively reduced the spleen weight. It also significantly improved the prolonged QT interval in CCM

possibly by up-regulating D1 receptor protein expression and suppressing TNF-α level in the heart. Glucocorticoids increased the β-ARs and induced the cAMP response to isoproterenol in human lymphocyte cells in an *in vitro* study (36, 37). Recently, it has shown that exposure of immature rat heart to antenatal glucocorticoid led to cardiac proliferation. Antenatal dexa induced structural maturity accompanying cardiomyocyte proliferation in the premature fetal rat, and glycogen synthase kinase-3β (GSK-3β) and β-catenin are supposed to contribute to cardiac growth (38).

In line with our study, in an experiment including both male and female rats suffering from myocardial ischemia, dexa at a dose of 0.1 mg/kg could regulate the ultrastructure of the muscle (39). Likewise, glucocorticoid administration in day 17 of gestation of rats caused larger hearts with increased cardiomyocyte proliferative index, with enhanced increased cardiac cell generation (40). Similarly, dexa postnatal treatment from day 1 to 3, demonstrated increase in heart weight and heart to body weight ratio, in comparison to the control group in the glucocorticoid-exposed rat hearts (41).

Regarding dopamine receptors, a clinical study indicated that D2 receptors' blockade gives rise to catecholamine release, following exercise (42). Therefore, D1 receptors augment the β-ARs signaling pathway, and eventually cardiac contraction and chronotropic responsiveness to adrenergic stimuli by increasing the AC enzyme and cAMP generation, but D2 receptors have the opposite effect on the mentioned pathway. BDL decreased Ca^{2+} current by reducing the L-type Ca^{2+} channel density in rat cells and caused a reduction in cardiac contractility (43, 44). D1 receptors increased Ca^{2+} currents by PKA activation, which causes an increase in Ca^{2+} currents by phosphorylation of L-type Ca^{2+}channels in rat striatal neurons (45), and in bovine chromaffin cells (46). Our results showed that D1 and D2 receptors (protein) levels were remarkably reduced by cirrhosis. Notably, cirrhosis significantly decreased both the mRNA and protein expressions of D2 receptors. Although dexa was able to increase protein expression of D1, it could not alter D2 expression.

Tobón *et al.* demonstrated that a specific part of the D1 receptors mRNA (1277 bp 3′ untranslated region) plays a crucial role in the post-transcriptional regulation of mice's catecholaminergic neuronal cell lines. They showed that microRNAs (small non-coding RNAs) suppress the mRNA's translation, and influence the stability of mRNA. Therefore, the miRNAs (miR-142-3p) inhibitors increased the protein expression of the D1 receptor (47). Although miRNA-15b inhibitors considerably enhance the protein expression of D1 receptors, remarkably decrease the D1 receptors' mRNA expression (48). Glucocorticoids can suppress microRNAs (49). Considering the D1 receptor's real-time PCR and IHC results, it seems that dexa increased the protein expression (translation) of D1 receptors by an inhibitory effect on microRNAs, but did not affect the mRNA expression. Based on our results, dexa improved cardiac complications possibly by elevation of D1 receptors level, which amplifies the β-adrenergic receptor signaling pathway. Nevertheless, dexa did not affect the level of the D2 receptors, which has an inhibitory effect on the mentioned pathway. Dexa could change pos-translational regulation and protein stability like autophagy inhibition or certain phosphorylation. It is suggested to investigate the correlation between the effect of glucocorticoids on D1 receptors gene expression and the function of microRNAs in future studies.

G proteins play a vital role in the β-ARs signaling pathway (50). According to the results, cirrhosis reduced the mRNA expression of G-protein (GNAL) in cirrhotic rats as in previous studies (19). In our results, dexa could not change GNAL in cirrhotic rats. We assumed that dexa treatment can increase the G-protein levels (protein expression) through the inhibition of microRNAs. Thus, evaluation of G-protein level is needed to perform through western blotting.

Cirrhosis increases the TNF-α and IL-1β levels in the rat's plasma and liver (51). Cirrhosis enhances the cardiac endocannabinoids, which is important for reducing the cardiac contractile responses to adrenergic stimuli in biliary cirrhotic rats (52). An increased level of TNF-α can contribute to the blunted cardiac contractility by inducing endocannabinoid activity and oxidative stress in mice with biliary cirrhosis. Inhibition of TNF-α can be considered an effective treatment for CCM (53). Our results are in agreement with the mentioned studies and cirrhosis elevated TNF-α and IL-1β levels in the heart tissue. However, dexa treatment only suppressed TNF-α level.

Conclusion

Dexa relieved the cardiac complications of cirrhosis by improving heart rate and QT intervals in CCM. Further, it reduced the increased spleen weight and the pro-inflammatory marker, TNF-α, caused by cirrhosis. Additionally, dexa could increase D1 receptor expression. As a consequence the protective effects of dexa may be partly mediated by D1 receptors. It seems that the short-term treatment with dexa may be considered a therapeutic agent to reduce cardiac complications in CCM. Future investigations are suggested to evaluate the effects of dexa on cardiac inotropic dysfunction, portal hypertension, microRNAs, and whether it correlates with D1 gene expression, G-proteins and dopamine receptors protein expression through western blot in CCM.

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Ethics Statement

The procedures and protocols used in the current study were reviewed and approved by the animal ethical committee of Tehran University of Medical Sciences, Tehran, Iran [Ethical Number: IR.TUMS.MEDICINE.REC.1395.1000].

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Authors' Contributions

Mo SZ and M SZ performed the experiment; M SZ performed the data analysis; Q N taught the methods and performed the experiment; SS SS designed part of the proposal; AR D interpreted the results; F J provided the

study design and concept, collected data, conducted the investigation and data analysis, drafted the article, and supervised the study.

Conflicts of Interest

The authors have no conflicts to disclose.

Declaration

The authors confirm that this manuscript is original, it has not been published previously, and it is not under consideration for publication elsewhere. All authors have reviewed and approved the final version of the manuscript. We also acknowledge the use of ChatGPT (OpenAI, 2025) for grammar checking and improving the fluency of the manuscript.

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