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Research Article
Role of Carnitine on Hematological Parameters and Attenuation of Cardiac (Pro)renin Receptor and Caspase-3 Expression in Hypoglycemia-induced Cardiac Hypertrophy

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Abstract

Background and Objective: In diabetes management, hypoglycemia unawareness (HU) syndrome is the result of frequent incidences of hypoglycemic episodes due to intensive glycemic control. The HU leads to a longer duration of hypoglycemia due to the absence of alerting symptoms, causing several complications in the cardiovascular system, such as hypertension and cardiac injury. In the current study, the role of carnitine was evaluated in the improvement of hematological parameters and prevention of cardiac PRR and caspase-3 expression in hypoglycemia-induced cardiac hypertrophy. **Materials and Methods:** A group of male Wistar albino rats (n = 15) was divided into 5 groups (n = 3) and treated with insulin glargine (InG) at doses 0, 10, 15, 20 and 25 U/kg/day for finding the optimal dose to provide sustained hypoglycemic condition. Second group (n = 20) was divided into 4 groups (n = 5) and treated with saline, InG 20 U/kg/day, InG+D-carnitine (InG+DC) for carnitine depletion and InG+acetyl-L-carnitine (InG+ALCAR) for carnitine supplementation. **Results:** The present study demonstrated that hypoglycemia-induced leukopenia was exaggerated with carnitine deficiency, whereas, white blood cells decreased from $6.93 \pm 1.58 \times 10^3$ in InG group to $5.47 \pm 0.77 \times 10^3$ in InG+DC group as all compared with control group ($12.62 \pm 1.45 \times 10^3$). The ALCAR ameliorated the hematological parameter and treated hypoglycemia-induced leukopenia. In the heart, (pro)renin receptor (PRR) and caspase-3 expression were upregulated during hypoglycemia and exaggerated with carnitine deficiency leading to cardiac hypertrophy. The ALCAR prevented hypoglycemia-induced cardiac hypertrophy might be through the down regulation of PRR and caspase-3 expression. **Conclusion:** Carnitine has an important role in the prevention of hypoglycemic complications, such as leukopenia and cardiac hypertrophy, through the regulation of PRR and caspase-3 in cardiac tissues.

Key words: Hypoglycemia, carnitine, cardiac hypertrophy, (pro)renin receptor, caspase-3

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Hypoglycemia (plasma glucose <70 mg dL^{-1}) is the most common side effect of the management of hyperglycemia[1]. It is primarily related to the intensive control of diabetes due to a restricted diet or treatment[2]. Most patients with diabetes suffer from repetitive hypoglycemic episodes (up to 10/week) which induce neuronal and cardiovascular symptoms to alert patients to take actions[2]. In type 1 diabetes (T1D), around 85% of patients have at least one hypoglycemic episode a month and they annually have at least one severe hypoglycemic attack[3]. In a previous epidemiological study by the UK hypoglycemia Study Group, it was found that 22% of insulin-treated patients with diabetes had severe hypoglycemia during their first 5 years of the disease, which increased to 46% after 15 years[4]. Hypoglycemia is less common in T2D (43% of these patients have at least one episode per month), but its incidence rate increases with disease duration and taking insulin analogs or oral hypoglycemic agents that mimic insulin release, such as sulfonylurea[3,5,6].

During hypoglycemia, the brain activates a counter-regulatory response against hypoglycemic attack called hypoglycemia awareness, which is a group of symptoms that include anxiety, sweating and shaking, to alert the hypoglycemic patient to take corrective actions[7]. These neuroglycopenic signs are induced due to the importance of continuous glucose supply to the brain to avoid harmful consequences of hypoglycemia that might lead to a coma, cardiac arrest and death[7]. Moreover, the brain promotes various hormonal actions for the suppression of insulin release, the induction of glucagon production and catecholamine release to accelerate blood flow to the brain and other fetal organs, decrease peripheral glucose metabolism and induce hepatic gluconeogenesis[8]. The long-term effects of repetitive hypoglycemic events include the defection of the counter-regulatory mechanisms leading to an acquired syndrome called hypoglycemia unawareness (HU). Patients with HU are unable to promote the alerting symptoms to attenuate the lethal consequences of hypoglycemia, such as neuronal degenerative and cardiovascular disease (CVD).

The CVD is the leading cause of disability and death in diabetic patients. Hyperglycemia and hypoglycemia promote pathological pathways causing hypertension, heart failure and cardiac arrhythmias[9-11]. The renin angiotensin-II system (RAS) has been associated with the induction of diabetic cardiomyopathy[12]. The over-activation of RAS is promoted through various mechanisms related to hyperglycemia, hypoglycemia and oxidative stress[9,13]. The overproduction of RAS components leads to the elevation of angiotensin-II (Ang-II) levels, the most potent endogenous vasoconstrictor, causing sustained vasoconstriction-induced renal and CVD[14].

Recently, the (pro)renin receptor (PRR) has been determined as a new member of RAS[15]. The PRR is a first single-transmembrane receptor for prorenin and renin as an initial step for Ang-II production in different tissues[15]. The PRR has been detected in the heart, kidneys, brain and pancreas for local Ang-II synthesis[15]. Recent studies have shown that PRR has multifaceted functions in the body dependent and independent on Ang-II production[15]. High PRR expression showed ~four-fold increase in the transformation of angiotensinogen to Ang-I and stimulation of ERK/mitogen-activated protein kinase (MAPK) in a blood pressure-independent manner[16]. The PRR-independent RAS mechanism has been extensively investigated to understand the etiologies of various CVDs, such as hypertension and heart disease[17,18]. The PRR activated cytokine signaling cascades independently on AT1R causing hypertension in the spontaneously hypertensive rat model[18]. In a recent study, PRR has been implicated in the activation of autophagy and the Wnt/β-catenin pathway, leading to cardiorenal end-organ damage[19]. In diabetes, PRR has been identified in the activation of the inflammatory response, cardiac apoptosis, collagen formation and transforming growth factor release inducing diabetic cardiomyopathy[20,21]. However, PRR has an essential role in causing CVDs in patients with hypertension and diabetes. Still, the link between hypoglycemia and induced hypertension and heart disease in patients with diabetes is not fully understood.

Carnitine (β-hydroxy-γ-N-trimethylaminobutyric acid) is an endogenous amino acid that presents in various cells, mainly cardiac and muscle cells, for fatty acid oxidation and energy production[22,23]. Carnitine is available in several compounds, such as L-carnitine, propionyl-L-carnitine and acetyl-L-carnitine, (ALCAR) for the supplementation of carnitine in different diseases related to carnitine insufficiency[22,23]. Recently, carnitine deficiency has been identified in various diseases, such as hypertension, diabetes and heart disease[22,24,25]. Carnitine supplements attenuate the complications of disease-induced carnitine deficiency, such as hypertension, cardiac hypertrophy, diabetic neuropathy, retinopathy and cardiomyopathy[24,25]. However, carnitine has an essential role in providing cardiovascular protection against CVDs and diabetic complications. However, the role of carnitine in the modulation of cardiovascular complications of hypoglycemia has not yet been fully identified. Therefore, the current study assessed the changes in hematological

parameters during chronic hypoglycemia in the presence or absence of carnitine. In addition, this study approved the function of carnitine in the modulation of cardiac PRR and caspase-3 expression and the prevention of hypoglycemia-induced cardiac hypertrophy.

MATERIALS AND METHODS

The study was carried out at Department of Pharmacology and Toxicology, College of Pharmacy, King Saud University, Riyadh, Saudi Arabia. The animal study and experiments were conducted from January, 2019 until January, 2020.

Animal study: A group of male Wistar albino rats (wt = 180-200 g) (n = 37) were fed a standard chow pellet diet and had free access to water under controlled conditions (25°C and a 12 h light/dark cycle) (Animal Care Center, College of Pharmacy, King Saud University, Riyadh, Saudi Arabia).

Rats were divided into 2 groups, first group (n = 15) received daily subcutaneous (s.c.) injections of insulin glargine (InG, Lantus SoloSTAR®) for ten days at doses of 0, 10, 15, 20 and 25 U/kg/day (n = 3) to identify the optimal daily dose of InG to produce a sustained hypoglycemic rat model.

Second group (n = 24) was used to assess the role of carnitine in the cardiovascular system during sustained hypoglycemia. This group was divided into 4 groups (n = 5). The 1st group was treated with saline (control) (0.5 mL 200 g/body weight) for 15 successive days. The 2nd group was received the same dose of saline for 5 days and then treated with InG (20 U/kg/day, s.c.) for 10 days. The 3rd and 4th groups were treated for the first 5 days with D-carnitine (DC, Sigma, St Louis, MO, USA) (500 mg/kg/day, i.p.) and acetyl-L-carnitine (ALCAR, Sigma, St Louis, MO, USA) (300 mg/kg/day, i.p.) alone and then received InG+DC and InG+ALCAR for the next 10 days, respectively (Table 1). The DC and ALCAR were used for the induction of carnitine deficiency and carnitine supplementation, respectively[26,27]. Blood glucose levels were measured in all treated rats using glucometer (ACCU-Chek Performa).

On the day of surgery, all rats were anesthetized with ketamine and xylazine (ketamine 100 mg kg^{-1} and xylazine 10 mg kg^{-1}, i.p.). Then, rat's chest was opened by aseptic and sharp surgical scissors and forceps. Blood samples were collected directly from the heart. Rats were euthanized by exsanguination from the heart. Blood samples were analyzed using an ERMA PCE-210 cell counter to evaluate hematological parameters, such as white blood cell (WBC), red blood cell (RBC), platelet (PT), hemoglobin, (Hb) and hematocrit (HCT) counts and for the measurement of NT-proB-type natriuretic peptide (NT-proBNP) and creatine kinase (CK-MB) as biomarkers of cardiac hypertrophy. Hearts were harvested and frozen for the measurement of cardiac PRR and caspase-3 expression on mRNA and protein levels.

Table 1: Type of treatment and duration

Groups	Type of treatment	Duration (days)	Type of treatment	Duration (days)
1	Saline	15		
2	Saline	5	InG+Saline	10
3	DC	5	InG+DC	10
4	ALCAR	5	InG+ALCAR	10

Table 2: Primers for real-time PCR experiments

Genes	Forward primers	Reverse primers
PRR	TGGGAAGCGTTATGGAGAAG	GGTTGTAGGGACTTTGGGTGT
Caspase-3	GAGTGCTCGCAGCTCATACCT	CCTCACGGCCTGGGATTT
β-actin	CCAGATCATGTTTGAGACCTTCAA	GTGGTACGACCAGAGGCATACA

Enzyme-linked immunosorbent assay (ELISA): After blood collection, the serum was immediately separated using a centrifuge and aliquoted in Eppendorf tubes to measure NT-proBNP and CK-MB. On the day of analysis, all samples were placed at room temperature (25°C) and analyzed using commercial sandwich ELISA kits (G-bioscience®, Geno Technology Inc., USA).

Real-time quantitative polymerase chain reaction (RT-PCR) assay: On the day of the experiment, the cardiac tissues were cut into small pieces under ice for total RNA extraction using TRIzol reagent (Invitrogen®, USA). After RNA collection, cDNA was produced using a High Capacity cDNA synthesis reverse transcription kit (Applied Biosystems®, USA) and RT-PCR was performed using SYBR® Green PCR MasterMix (Applied Biosystems®, USA), as per the manufacturer's instructions. The mRNA levels of PRR and caspase-3 were measured on an Applied Biosystems 7500 real-time PCR system (Applied Biosystems®, USA)[28]. The primers were designed using the PubMed database and were purchased from Integrated DNA Technologies (IDT, Coralville, IA) (Table 2). The data are shown as the fold-change in mRNA expression levels normalized to GAPDH as the loading control.

Western blot analysis: The heart tissues were cut into small pieces under ice and homogenized using ice-cold radioimmunoprecipitation assay (RIPA) lysis buffer (Thermo Scientific, USA) mixed with a Protease and Phosphatase Inhibitor Single-Use Cocktail (Thermo Scientific, USA). The mixture was then centrifuged at 14,000 rpm for 15 min and a Direct Detect® spectrometer (EMD Millipore, USA) was used to measure the concentration of protein in

the supernatants. Loading samples were prepared in 2x Laemmli Sample loading buffer (Bio-Rad, USA) containing β-mercaptoethanol (βME). In total, 40 µg of total protein was loaded into each lane, resolved on 10 or 12% SDS-PAGE gels and transferred to PVDF membranes using a Trans-Blot Turbo Transfer System (Bio-Rad, USA). Membranes were then blocked with 5% nonfat dry milk in Tris Buffered Saline (TBS) containing 0.1% Tween-20 at room temperature (25°C) for 1 h. After 1h, membranes were washed using TBST and separately incubated at 4°C overnight into anti-PRR primary antibody (1:1000) (Abcam, USA), anti-caspase-3 primary antibody (1:1000) (Santa Cruz Biotechnology Inc., USA) or anti-GAPDH primary antibody (1:1000) (Santa Cruz Biotechnology Inc., USA) as a protein loading control. Membranes were then washed and incubated with secondary goat anti-rabbit or goat anti-mouse IgG (1:10,000) for 1 h at room temperature (25°C). After extensive washing with TBST, bands were visualized with the ECL (Immobilon Western Chemiluminescent HRP Substrate, Millipore Sigma, USA) and a Bio-Rad ChemiDoc MP Imaging System was used to develop the immunoblots.

Statistical analysis: Data were expressed as Mean±SEM and analyzed by one-way analysis of variance (ANOVA) followed by Tukey-Kramer's or Dunnett's multiple comparison test when appropriate (GraphPad Prism 7). The $p<0.05$ was considered statistically significant.

RESULTS

Blood glucose levels: In InG-treated groups (10, 15, 20 and 25 U/kg/day), all rats had hypoglycemic conditions. At dose 10 and 15 U, rats had hypoglycemia gradually to reach 69 and 58 mg dL^{-1} at day 10th. At dose 20 and 25 U, rats had severe and sustained hypoglycemia for 10 days (Fig. 1). At dose 20 U, all rats had hypoglycemia from the first dose with lower mortality compared with 25 U. However, InG (20 U/kg/day) was selected for the next studies.

In the second study, all InG (20 U kg^{-1})-treated groups (InG+Saline, InG+DC and InG+ALCAR) had sustained hypoglycemia (blood glucose levels = 42-55 mg dL^{-1}) until the end of the treatment (Fig. 2).

Hematological parameters: The effect of hypoglycemia or hyperinsulinemia on blood cells was investigated in InG-treated rats at doses of 0, 10, 15, 20 and 25 U/kg/day. It was found that InG at a dose of 20 U kg^{-1} decreased the WBC significantly, with no noticed changes in RBC, Hb and HCT levels compared with the control group (Table 3).

In addition, the role of carnitine was assessed in the protection of blood cells during chronic hypoglycemia. The WBC counts significantly decreased (50% reduction) during InG (20 U kg^{-1}) and InG+DC treatment and ALCAR treatment maintained the WBC levels to within normal values during InG-induced hypoglycemia in the InG+ALCAR treated group,

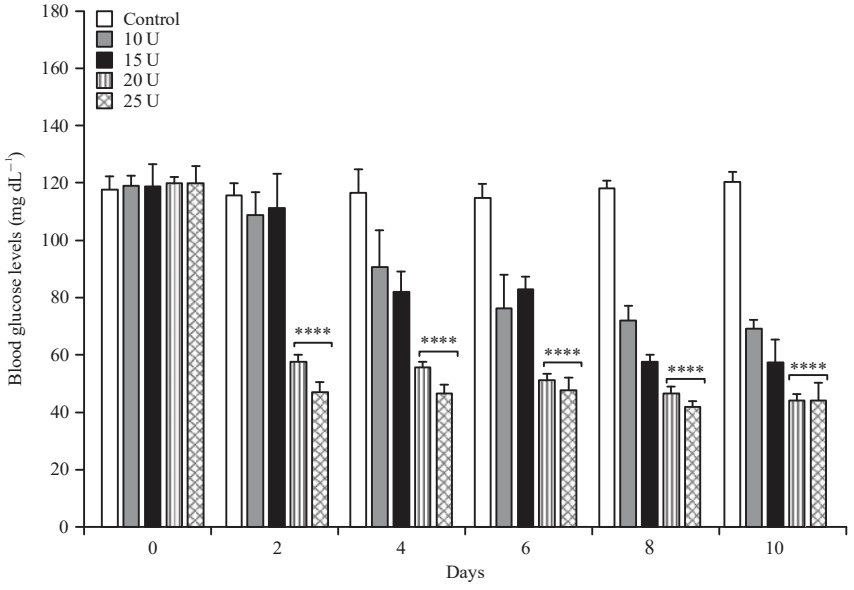

Fig. 1: Blood glucose levels and InG-treated animals

Measurement of blood glucose levels in InG (0, 10, 15, 20, 25 U/kg/day) treated groups at day 0, 2, 4, 6, 8 and 10, values are expressed as Mean±SEM (n = 3), ****$p<0.0001$ for control vs 20 and 25 U, analysis were performed using two-way ANOVA followed by Tukey's multiple comparisons test

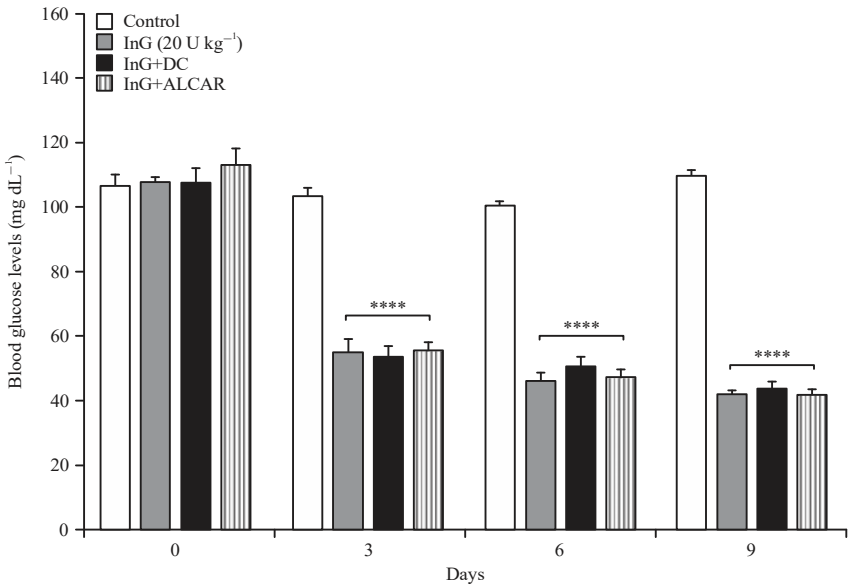

Fig. 2: Blood glucose levels in control, InG, InG+DC and InG+ALCAR groups

Measurement of blood glucose levels in saline (control), InG (20 U kg^{-1}), InG+DC and InG+ALCAR groups at day 0, 3, 6 and 9, values are expressed as Mean±SEM (n = 5), ****p<0.0001 for InG (20 U kg^{-1}), InG+DC and InG+ALCAR vs control, analysis were performed using two-way ANOVA followed by Tukey's multiple comparisons test

Table 3: Hematological parameters in InG-treated animals

	Hematological parameters			
Groups	WBCs×10^3 (cell μL^{-1})	RBCs×10^6 (cell μL^{-1})	Hb (g dL^{-1})	HCT (%)
Control	10.90±0.90	10.93±0.14	15.26±0.12	63.53±0.78
10 U	7.15±1.25	10.65±0.01	14.90±0.30	61.85±2.35
15 U	6.65±0.35	11.28±0.11	16.10±0.30	64.90±3.00
20 U	3.55±0.55*	11.22±0.28	15.13±0.66	61.20±1.70
25 U	6.05±2.15	9.71±1.39	14.15±2.05	56.40±10.10

Measurement of WBC, RBC, Hb and HCT levels in all groups treated with saline (control), 10, 15, 20 and 25 U/kg/day of InG, values are expressed as Mean±SEM (n = 3), WBC levels decreased in the InG (20 U kg^{-1})-treated group, compared to the control group (*p<0.05), analysis were performed using one-way ANOVA followed by Tukey's multiple comparisons test

Table 4: Hematological parameters in control, InG, InG+DC and InG+ALCAR groups

	Hematological parameters				
Groups	WBCs×10^3 (cell μL^{-1})	RBCs×10^6 (cell μL^{-1})	PLT X 103 (cell μL^{-1})	Hb (g dL^{-1})	HCT (%)
Control	12.62±1.45	11.02±0.09	894.75±63.67	15.70±0.41	58.37±1.33
InG (20U/kg)	6.93±1.58*	9.90±0.05	706.00±62.58	14.42±0.36	57.00±2.03
InG+DC	5.47±0.77**	10.00±0.31	872.50±69.08	14.20±0.23	55.65±0.43
InG+ALCAR	9.82±1.18	11.47±0.54*	1018.25±89.67*	17.27±0.79**	60.82±1.41

Measurement of WBC, RBC, PT, Hb and HCT levels in all groups treated with saline (control), InG (20 U kg^{-1}), InG+DC and InG+ALCAR, values are expressed as Mean±SEM (n = 5), WBC number decreased in InG (20 U kg^{-1}) and InG+DC treated groups, compared to the control group (*p<0.05 for control vs., InG (20 U kg^{-1}) and control vs., InG+DC, respectively), RBC, PT and Hb levels increased in the InG+ALCAR treated group compared to the InG (20 U kg^{-1}) and InG+DC treated groups (*p<0.05), analysis were performed using one-way ANOVA followed by Tukey's multiple comparisons test

compared to the control group (Table 4). The ALCAR increased levels of RBCs, PTs and Hb in the InG+ALCAR treated group, compared to the InG (20 U kg^{-1}) and InG+DC treated groups (Table 4). However, Carnitine have shown an essential role in the prevention of hypoglycemic complications on the hematological parameters.

NT-proBNP and CK-MB measurements: To assess the role of carnitine in the avoidance of hypoglycemia-induced cardiac hypertrophy, NT-proBNP and CK-MB levels were measured in serum samples as biomarkers of cardiac hypertrophy. The NT-proBNP and CK-MB levels increased in the InG (20 U kg^{-1}) and InG+DC treated groups, compared to the

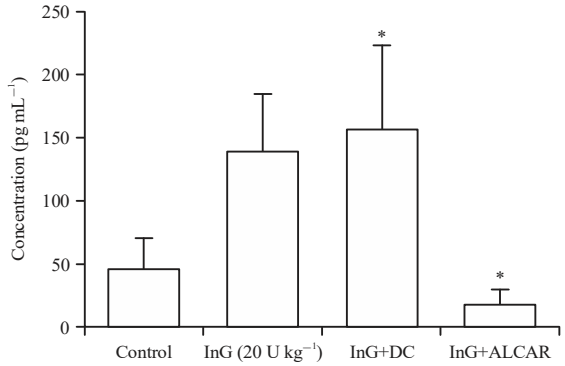

Fig. 3: Serum levels of NT-proBNP

Serum levels of NT-proBNP in all groups treated with saline (control), InG (20 U kg^{-1}), InG+DC and InG+ALCAR, values are expressed as Mean±SEM, NT-proBNP level was induced in the InG+DC treated group compared to the control group (*p<0.05), ALCAR decreased the NT-proBNP levels in the InG+ALCAR treated group compared to the InG+DC group (*p<0.05), Analysis were performed using one-way ANOVA followed by Tukey's multiple comparisons test

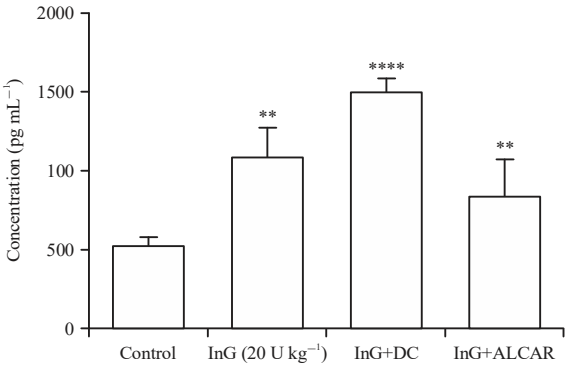

Fig. 4: Serum levels of CK-MB

Serum levels of CK-MB in all groups treated with saline (control), InG (20 U kg^{-1}), InG+DC and InG+ALCAR, values are expressed as Mean±SEM, CK-MB level increased in the InG (20 U kg^{-1}) and InG+DC treated groups compared to the control group (**p<0.01 and ****p<0.0001), ALCAR decreased the CK-MB level in the InG+ALCAR treated group compared to the InG+DC treated group (**p<0.01), Analysis were performed using one-way ANOVA followed by Tukey's multiple comparisons test

control group (Fig. 3, 4). Carnitine deficiency in the InG+DC treated group exaggerated induced NT-proBNP and CK-MB levels, compared to the InG-treated group (Fig. 3, 4). In the InG+ALCAR treated group, ALCAR treatment maintained NT-proBNP and CK-MB levels to within the normal levels during InG-induced hypoglycemia (Fig. 3, 4). Therefore, carnitine plays a pivotal role in the prevention of hypoglycemia-induced cardiac hypertrophy.

Expression of (pro)renin receptor (PRR) in cardiac tissues: On mRNA and protein levels, PRR expression was assessed in the cardiac tissues using RT-PCR and WB techniques, respectively (Fig. 5). The PRR mRNA levels were induced

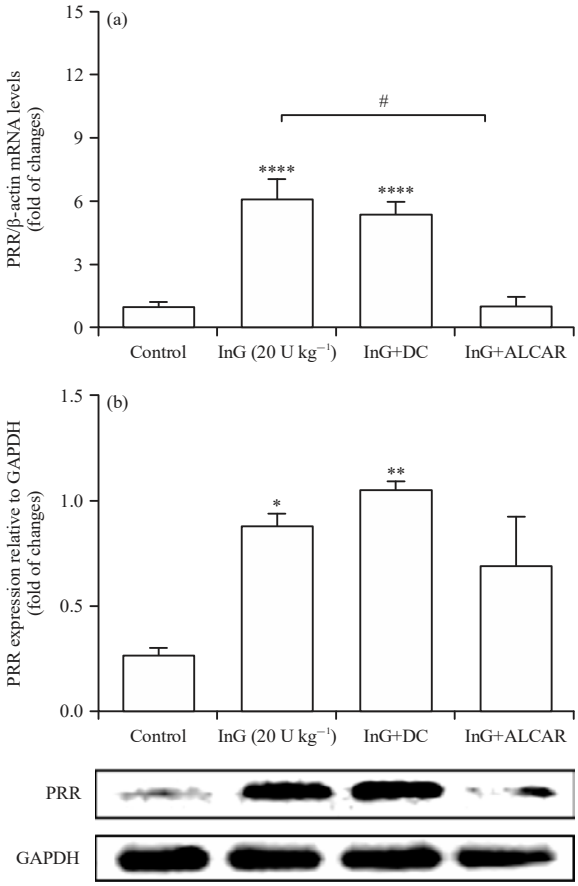

Fig. 5(a-b): mRNA and protein levels of PRR in cardiac tissues, (a) mRNA levels of cardiac PRR increased in the InG (20 U kg^{-1}) and InG+DC treated groups compared to the control group (****p<0.0001), ALCAR inhibited induced mRNA PRR in the InG+ALCAR treated group compared to the InG (20 U kg^{-1}) and InG+DC treated groups ($^{\#}$p<0.0001) (one-way ANOVA followed by Tukey's multiple comparisons test) and (b) Protein levels of cardiac PRR increased in the InG (20 U kg^{-1}) and InG+DC treated groups compared to the control group (*p<0.05 and **p<0.01)

Measurement of mRNA and protein levels of PRR expression in the cardiac tissues in all groups treated with saline (control), InG (20 U kg^{-1}), InG+DC and InG+ALCAR, values are expressed as Mean±SEM, one-way ANOVA followed by Dunnett's multiple comparisons test

significantly in the InG-treated group, compared to the control group (Fig. 5a). The DC treatment increased the mRNA expression of PRR in the InG+DC treated group, compared the control group (Fig. 5a). In the InG+ALCAR group, ALCAR treatment attenuated the induction of PRR mRNA expression during hypoglycemic conditions, compared to InG (20 U kg^{-1})

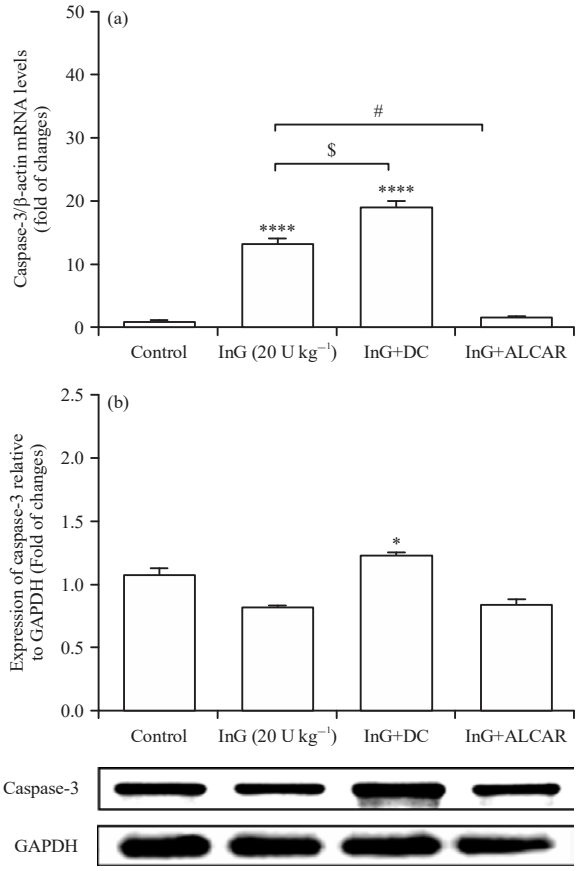

Fig. 6(a-b): mRNA and protein levels of caspase-3 in cardiac tissues, (a) mRNA levels of cardiac caspase-3 increased in the InG (20 U kg^{-1}) and InG+DC treated groups, compared to the control group (****p<0.0001), ALCAR inhibited induced mRNA caspase-3 in the InG+ALCAR treated group, compared to the InG (20 U kg^{-1}) and InG+DC treated groups (#p<0.0001) and (b) Protein levels of cardiac caspase-3 were induced in the InG+DC treated group compared to the control group (*p<0.05)

Measurement of mRNA and protein levels of Caspase-3 expression in the cardiac tissues in all groups treated with saline (control), InG (20 U kg^{-1}), InG+DC and InG+ALCAR, values are expressed as Mean±SEM, analysis were performed using one-way ANOVA followed by Tukey's multiple comparisons test

and InG+DC treated groups (Fig. 5a). For the protein levels, PRR expression increased in InG (20 U kg^{-1}) and InG+DC treated groups, compared to the control group (Fig. 5b). In the InG+ALCAR group, ALCAR treatment attenuated the protein expression of PRR during hypoglycemic conditions, compared to the InG (20 U kg^{-1}) and InG+DC treated groups (Fig. 5b).

Caspase-3 mRNA and protein levels: The cardioprotective role of carnitine against hypoglycemia-induced cardiac hypertrophy was studied for its ability in the prevention of cardiomyocyte apoptosis. However, caspase-3 as a biomarker of apoptosis was assessed in the cardiac tissues for the mRNA and protein levels (Fig. 6). Caspase-3 mRNA levels were induced significantly in the InG-treated group, compared to the control group (Fig. 6a). The DC treatment increased the mRNA expression of caspase-3 in the InG+DC treated group compared to the InG-treated group (Fig. 6a). In the InG+ALCAR group, ALCAR treatment attenuated the induction of caspase-3 mRNA expression during hypoglycemic conditions, compared to the InG (20 U kg^{-1}) and InG+DC treated groups (Fig. 6a). For the protein levels, caspase-3 expression increased in the InG+DC treated group, compared to the control group (Fig. 6b). In the InG+ALCAR group, ALCAR treatment attenuated the protein expression of caspase-3 during hypoglycemia, compared to the InG+DC treated groups (Fig. 6b).

DISCUSSION

The present study was performed to identify the effect of sustained hypoglycemia on the hematological profile and the role of carnitine in the prevention of hypoglycemia-induced hematological abnormalities and cardiac hypertrophy.

At the hematological levels, more than 50% reduction was found in the number of WBCs leading to leukopenia in the InG (20 U kg^{-1})-treated group and carnitine deficiency exaggerated hypoglycemia-induced leukopenia. A recent study showed that oral hypoglycemic drugs, such as sulfonylureas, can induce leukopenia in patients with diabetes, but no prospective study has identified the link between InG-induced hypoglycemia and leukopenia[29,30]. However, future studies will be conducted to investigate whether InG-induced leukopenia is related to hypoglycemia or hyperinsulinemia. Current results showed that carnitine supplementation (ALCAR) blocks InG-induced leukopenia and maintains WBC levels to within the normal values and increases other parameters, such as RBC, PT and Hb levels. As shown in a recent study, carnitine improves the hematological parameters in patients with hepatitis C receiving interferon-α 2b and ribavirin[31].

In the present study, it was found that chronic InG-induced hypoglycemia induces cardiac hypertrophy via the measurement of NT-proBNP and CK-MB levels in the circulation. Carnitine deficiency exaggerated the circulatory levels of NT-proBNP and CK-MB in the hypoglycemic rats.

In contrast, ALCAR attenuated the induction of cardiac hypertrophy during hypoglycemia. However, the essential function of carnitine in the prevention of hypoglycemia-induced cardiac hypertrophy was proven. Carnitine plays a critical role in energy supply and antioxidative stress through fatty acid oxidation and free radical scavenging, respectively[32,33]. However, several studies have shown that carnitine supplementation deactivates various pathological mechanisms, including RAS and MAPK signaling pathways in diabetes and multiple CVDs[25,34,35]. In various diabetic and hypertensive rodent models, ALCAR alleviated diabetic complications, including insulin resistance and hypertension and managed RAS components leading to the control of blood pressure and cardioprotection[25,35].

At the molecular level, the present study identified the role of carnitine in the prevention of RAS activation and cardiomyocyte apoptosis during hypoglycemia through the attenuation of cardiac PRR and caspase-3 expression, respectively. However, endogenous carnitine can alleviate hypoglycemia-induced RAS activation through the downregulation of PRR expression in the cardiac tissues. The high expression of cardiac PRR has been reported on mRNA and protein levels in various animal models with cardiac remodeling and heart failure[36]. Overexpressed PRR was detected in diabetes-induced cardiac hypertrophy, whereas the blockage or downregulation of PRR attenuated cardiac hypertrophy in the diabetic model[37]. A recent study showed that the imbalance in free radical production in an obese rat model caused the overproduction of RAS components, inducing hypertension[38]. However, hypoglycemia with carnitine deficiency might augment oxidative stress-induced PRR expression leading to hypoglycemic complications.

Moreover, the results of this study proved that hypoglycemia increases cardiomyocyte apoptosis through the upregulation of caspase-3. Carnitine depletion promoted hypoglycemia-induced caspase-3 expression, which might increase apoptosis in the cardiac tissues. The ALCAR alleviated hypoglycemia-induced caspase-3 expression and prevented induced cardiomyocyte apoptosis during hypoglycemia. Recently, the role of carnitine has been identified in the prevention of apoptosis in various tissues with different etiologies, such as muscle myopathy and renal-CVDs[39-41]. In a previous study, carnitine supplements reduced the circulatory levels of tumor necrosis factor-α (TNF-α) and Ang-II leading to the downregulation of caspase-3 and caspase-9 and blocking the apoptosis of muscular cells in heart failure-induced muscle myopathy[39]. The anti-apoptotic role of carnitine in myocardial ischemia has been revealed through the regulation of the PI3K/Akt/Bcl-2/Bax signaling pathway[40]. In diabetic cardiomyopathy, overexpressed PRR has been implicated in the induction of inflammation and apoptosis through the activation of the ERK signaling pathway[20]. In an RAS-independent manner, PRR mediated caspase-3 expression via the p38/MAPK signaling pathway in hypoxia-induced cardiomyocyte apoptosis[42]. However, carnitine might manage caspase-3 expression in cardiac tissues through the downregulation of PRR expression.

Taken together, carnitine provided cardiovascular protection against hypoglycemic complications through the improvement of hematological parameters and the downregulation of PRR-induced RAS signaling pathways, leading to cardiomyocyte apoptosis and cardiac hypertrophy.

CONCLUSION

In summary, HU is a common complication developed from frequent hypoglycemic episodes that causes permanent endothelial damage and CVDs. The current study found that hypoglycemia led to leukopenia and upregulation of PRR and caspase-3 in the cardiac tissues. Carnitine supplementation attenuated the hematological abnormalities and cardiac hypertrophy through the prevention of PRR and caspase-3 expression in cardiac tissues.

SIGNIFICANCE STATEMENT

This study discovered the beneficial roles of carnitine in the prevention of hypoglycemia-induced hematological abnormalities and cardiac hypertrophy through attenuation of cardiac PRR and caspase-3 expression. This study will help the researcher to uncover the critical areas of carnitine function in regulation of hypoglycemia-induced cardiovascular disturbances that many researchers were not able to explore. Thus a new theory on management of cardiovascular complications of chronic hypoglycemia may be arrived at.

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