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Ursodeoksikolik asidin streptozosin ile diyabet oluşturulmuş farelerde kardiyomyopatideki antiinflamatuar ve anti-fibrotik etkileri

Anti-inflammatory and anti-fibrotic effects of ursodeoxycholic acid in streptozocin-induced diabetic rats

İsmail Polat Canbolat[1], Gürkan Yiğittürk[2], Oytun Erbaş[3]

1Demiroğlu Bilim Üniversitesi Tıp Fakültesi, Kardiyoloji Ana Bilim Dalı, İstanbul, Türkiye
2Muğla Sıtkı Koçma Üniversitesi Tıp Fakültesi, Histoloji ve Embriyoloji Ana Bilim Dalı, Muğla, Türkiye
3Demiroğlu Bilim Üniversitesi Tıp Fakültesi, Fizyoloji Ana Bilim Dalı, İstanbul, Türkiye

ÖZ

GİRİŞ ve AMAÇ: *Diabetik kardiyomyopati, serbest yağ aside oksidasyonu, mitokondrial disfonksiyon, oksidatif stress ve diffuz miyokardiyal fibrozise seconder olarak gelişmektedir. Bu deneysel çalışmada, ursodeoksikolik asidin streptozocin ile tetiklenmiş diabetik fare modelinde anti-inflamatuar ve anti-fibrotik etkilerini araştırmayı hedefledik.*

YÖNTEM ve GEREÇLER: *Sprague Dawley albino 30 erişkin fare 3 gruba ayrıldı: Grup-1: kontrol grubu (n=10); Grup-2 (n=10) diabetik fare grubu; Grup-3 (n=10) ursodeoksikolik asit verilen diabetic fare grubu. Histopatolojik ve biyokimyasal değerlendirmeler 4 hafta sonra kalp dokusundan yapıldı. Fibronektin ve TGF-β immunekspresyonu, TGF-β, malondialdehid, pentraxin-3, pro-BNP ve troponin-T düzeyleri ölçüldü.*

BULGULAR: *Fibronektin immunekspresyonu, TGF-β, pentraxin-3, troponin-t, pro-BNP ve malondialdehid düzeyleri diabetik farelerde control grubuna göre anlamlı olarak artmış saptandı. Ursodeoksikolik asidin inflamasyon belirteçlerini ve fibroz düzeyini anlamlı olarak azalttığı izlendi*

TARTIŞMA ve SONUÇ: *Bu deneysel çalışmada, ursodeoksikolik asidin diabetik farelerde anti-inflammatuar ve anti-fibrotik etkilerini gösterdik. Diabetik hastalarda ursodeoksikolik asidin ilaç olarak kullanımı klinik olarak fayda gösterebilir.*

Anahtar Kelimeler: *diabetik kardiyomyopati, ursodeoksikolik asit, inflamasyon, kardiak fibroz*

ABSTRACT

INTRODUCTION: *Diabetic cardiomyopathy is a consequence of free fatty acid oxidation, dysfunction in mitochondria, oxidative stress and diffuse myocardial fibrosis. We aimed to investigate the anti-inflammatory and anti-fibrotic effect of ursodeoxycholic acid in streptozocin-induced diabetic rat model.*

METHODS: *Male Sprague Dawley albino mature rats were divided into 3 groups: Group 1 (n=10) control group; group 2 (n=10) diabetic rats group; group 3 (n=10): diabetic rats treated with ursodeoxycholic acid group. Diabetes mellitus model was established after injection of intraperitoneal streptozocin. Histopathological and biochemical examinations were done after 4 weeks from heart tissues. Immunoexpression levels of fibronectin and TGF-β were obtained. Malondialdehyde levels were used to determine lipid peroxidation and pentraxin-3 levels were used to determine inflammation. Myocardial damage was also determined with troponin-T and pro-BNP levels.*

RESULTS: *Cardiac muscle cell thickness (hypertrophy), TGF-β levels, fibronectin immunoexpression malondialdehyde, pentraxin-3, troponin-T and pro-BNP levels were increased significantly in groups 2 and 3 when compared to control group. Administration of ursodeoxycholic acid significantly reduced inflammation and fibrosis in group 3 compared to group 2.*

DISCUSSION AND CONCLUSION: *In this experimental study, we demonstrated the anti-inflammatory and anti-fibrotic effects of UDCA on diabetic rats and it can be a good drug candidate for DM patients*

Keywords: *ursodeoxycholic acid, diabetic cardiomyopathy, inflammation, cardiac fibrosis*

İletişim / Correspondence:

Dr. İsmail Polat Canbolat
Demiroğlu Bilim Üniversitesi Tıp Fakültesi, Kardiyoloji Ana Bilim Dalı, İstanbul, Türkiye
E-mail: ismailpolat.canbolat@gmail.com
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INTRODUCTION

Diabetes Mellitus (DM) is a persistent endocrinopathy with a global age-adjusted prevalence of 10% (1). Diabetic patients tend to establish twice-hold risk of heart failure compared with non-diabetic patients (2). Diabetes mellitus affects the heart mostly secondary to coronary atherosclerosis. Diabetic cardiomyopathy (DCM) is a type of heart failure associated with metabolic alterations secondary to diabetes after excluding atherosclerotic, hypertensive and structural heart disease (3). Hyperinsulinemia and insulin resistance exacerbate free fatty acid oxidation, dysfunction in mitochondria, oxidative stress and diffuse myocardial fibrosis (4-6).

Ursodeoxycholic acid (UDCA) is a widely used bile acid mostly for chronic cholestatic liver disease (7). The effect of UDCA is a result of reductions in cell apoptosis and resistance to oxidative stress (8,9). Administration of UDCA had been shown to reduce oxidative stress in aorta in a model of fructose induced metabolic syndrome in rats (10).

We aimed to investigate the beneficial effect of UDCA on the oxidative stress, vascular inflammation, myocardial damage and fibrosis in a diabetic rat model.

MATERIALS AND METHODS

Animals

Totally 30 male Sprague Dawley albino mature rats were used. Animals were able to deliver food and water spontaneously. They were housed in steel cages and kept at room temperature (23□2 □C) with light/dark (12/12 h) cycle. All the animal experiments performed in this study were done under the animal experiment guidelines.

Experimental design

Intraperitoneal (i.p.) injection of streptozocin (STZ) (Sigma-Aldrich, USA) was used to induce diabetes for 20 rats. Rats which were not injected STZ were selected as control group (n=10). Rats with higher than 250 mg/dl blood glucose level after 24 hours were confirmed as diabetic and included in this study. Then, 10 diabetic rats were randomly assigned as diabetes control group and 10 diabetic rats treated with ursodeoxycholic acid (UDCA) 250 mg/kg/day, (Ursofalk) (Diabetes + UDCA) by oral way for 4 weeks as diabetic UDCA treatment group.

The animals were euthanized, and blood samples were collected by cardiac puncture for biochemical analysis and histopathological examination was performed after removal of the heart.

Histopathological examination of heart tissue

Rats were anesthetized with ketamin (80 mg/kg, i.p.) and xylazine (8 mg/kg, i.p.). Heart tissues were fixed with formalin. Hematoxylin and eosin (H&E) stained heart sections with 5 μm thickness were photographed.

Light microscopy was used to determine heart cell hypertrophy degree. The cardiac muscle fiber with the maximum cross section diameter was photographed. Image analysis software (Image- Pro Express 1.4.5, Media Cybernetics, Inc. USA) was used to measure muscle fiber. The analysis was performed after the average of 50 cardiac muscle cells were calculated for each rat.

Fibronectin immunoexpression

Endogenous peroxidase activity was eradicated with 30 min H2O2 (10%) and blocked with 10% normal goat serum (Invitrogen) for 1 hour at room temperature. Subsequently, sections were incubated in primary antibodies (Fibronectin, Bioss, Inc.; 1/100) for 24 h at 4 °C. The antibodies were detected with the Histostain-Plus Bulk kit (Bioss, Inc) and the final product was visualized with 3,3' diaminobenzidine (DAB). Brown cytoplasmic staining was scored as positive for immunoexpression. At least 10 fields of tissue section with 100 magnification was assessed and 100 cardiomyocytes per field was systematically scored.

Measurement of plasma TGF-β, Troponin T, pro-BNP, pentraxin-3

A commercially available ELISA kit was used for measuring plasma TGF-β, troponinT, pro-BNP, pentraxin-3 levels.

Evaluation of lipid peroxidation

Plasma malondialdehyde (MDA) levels were measured to evaluate lipid peroxidation.

Statistical analysis

Descriptive analyses were presented as mean values ± standard derivation. Non-parametric parameters were analyzed with Mann-Whitney U test. The differences between groups was analyzed with Student's-t test. Statistical significance was regarded as a p value equal or lower than 0.05. All analyses were performed using SPSS v.21.0 for Windows (SPSS, Inc., Chicago, Illinois, USA).

RESULTS

Histopathological changes

The histopathological changes in rats are demonstrated in Figure-1. Cardiac muscle cell thickness and immunoexpression of fibronectin were increased in diabetes group compared to the control group ($p<0.01$ for each). UDCA treatment in diabetic rats significantly decreased cardiac muscle cell thickness ($p<0.05$) and fibronectin immunoexpresion ($p<0.001$). The histopathological scores of the groups are shown in Table-1.

Oxidative stress, inflammation markers

Serum levels of MDA, TGF-β, pentraxin-3 were significantly elevated in diabetic rats compared to the control group ($p<0.001$ for each). Administration of UDCA significantly decreased the level of MDA ($p<0.001$), TGF-β ($p<0.001$) and pentraxin-3 ($p<0.05$) as shown in Table-1.

Myocardial damage markers

Serum levels of troponin-T and pro-BNP were significantly elevated in diabetic rats compared to the control group ($p<0.001$ for each). This elevation was significantly reduced by UDCA treatment ($p<0.05$ for pro-BNP and $p<0.001$ for troponin-T) as showed in Table-1.

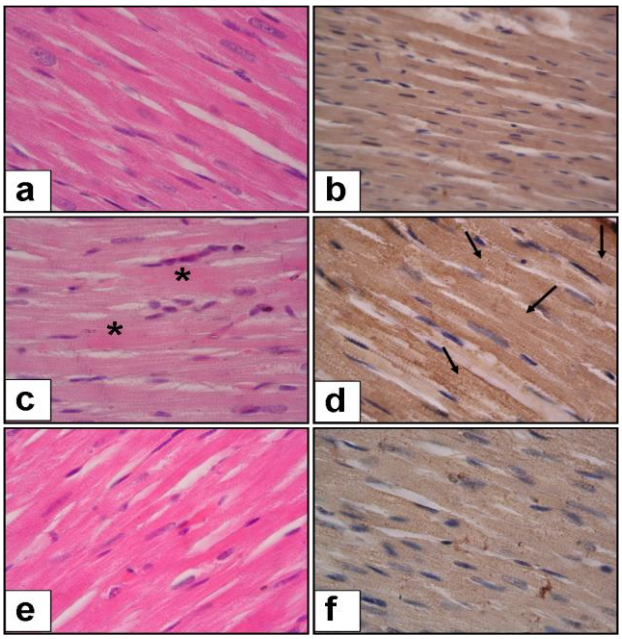

Figure 1: Histopathological changes in streptozocin-induced diabetic rats. a-Control Group, H & E stain x100 magnification. b- Control Group, Fibronectin expression x100 magnification. c- Diabetic Rats (Control group), H & E stain x100 magnification (* shown increased cardiac muscle cell thickness and arrow shown increased fibronectin expression). d- Diabetic Rats (Control group), Fibronectin expression x100 magnification. e- Diabetic Rats (UDCA treatment group), H & E stain x100 magnification. f- Diabetic Rats (UDCA treatment group), Fibronectin expression x100 magnification

Table-1: Cardiac muscle hypertropy, immunoexpression, biomarker levels according to groups

	Normal Control (Group-1)	Diabetic rat (control group) (Group-2)	Diabetic rat (UDCA treatment) (Group-3)
Cardiac muscle cell thickness (% of control)	100	125.3 ± 4.6 *	**113.4 ± 4.1 #**
Immunoexpression Fibronectin percent (%)	13.2 ± 3.15	45.6 ± 8.3 *	**15.1 ± 5.06 ##**
Blood glucose (mg/dl)	91.3 ± 8.8	367.1 ± 15.9 **	**408.1 ± 16.4**
TGF-Beta (pg/ml)	10.2 ± 2.03	34.5 ± 4.4 **	**16.8 ± 4.08 ##**
MDA (nM)	80.1 ± 13.6	365.6 ± 15.3 **	**113.9 1± 6.4 ##**
Pro-BNP (pg/ml)	3.08 ± 0.68	16.7 ± 4.2 **	**8.6 ± 1.57 #**
Troponin T (pg/ml)	0.73 ± 0.1	3.4 ± 1.1 **	**1.6 ± 0.72 ##**
Pentraxin-3 (ng/ml)	1.38 ± 0.16	3.01 ± 0.25 **	**1.75 ± 0.33 #**

* p<0.01, Group-2 compared to Group-1
** p<0.001, Group-2 compared to Group-1
# p<0.05, Group-3 compared to Group-2
# # p<0.001, Group-3 compared to Group-2

DISCUSSION

In this experimental study, we demonstrated the beneficial effects of UDCA on the end products of lipid peroxidation, vascular inflammation, myocardial damage and fibrosis.

Diabetes mellitus can affect heart from different mechanisms (11). Diabetic cardiomyopathy is a complication of DM related with impairment of microcirculation, abnormalities in subcellular components, alterations in lipid metabolism, maladaptive immune responses and can be diagnosed after ruling out coronary atherosclerosis, hypertension and structural heart disease (12).

Inflammation is a contributor of DCM and different mechanisms of action had been shown in previous animal studies (13,14). Anti-inflammatory effects of UDCA had been shown in experimental models of acute liver injury, non-alcoholic liver disease (15,16). Pentraxin-3, a marker for vascular inflammation was significantly reduced in diabetic rats after UDCA administration in our study.

TGF-β stimulates collagen production by coupling with angiotensin-1 receptor and promotes fibrosis in tissues (17). Pathil et al. demonstrated that fibrosis of liver was suppressed in hepatic stellate cells by blocking TGF-β1/Smad2/3 signaling pathway after the administration of UDCA (18). The role of TGF-β on pulmonary fibrosis was studied by Ko et al and demonstrated that TGF-β alters mRNA to promote lung fibrosis (19). In our study, UDCA administration significantly reduced serum TGF-β levels and immunoexpression of fibronectin leading to decreased cardiac muscle cell thickness and fibrosis. We demonstrated the reduced myocardial damage in diabetic rats treated with UDCA compared to diabetic control group via reduced troponin and pro-BNP levels which are cardiac biomarkers used for clinical assessment (20,21).

Oxidative stress had been described as a major contributor to DCM (22,23). Increased free fatty acid peroxidation is associated with overproduction of reactive oxygen species (24,25). Malondialdehyde (MDA) is a product of lipid peroxidation and reflects cellular damage (26). In an experimental model of cholestatic liver disease, UDCA administration was associated with decreased lipid peroxidation (27). In our study, the use of UDCA lowered serum MDA levels reflecting lowered lipid peroxidation.

Ursodeoxycholic acid has been shown to protect heart muscles in different types of action. Gorelik et al. showed that UDCA protects cardiomyocytes from taurocholic acid's arrhythmia risk by improving abnormal calcium dynamics (28). Hanafi et al. demonstrated the cardioprotective effect of UDCA from hypoxia by regulating ERK and Akt pathway (29). In our study, we demonstrated the cardioprotective role of UDCA in anti-inflammatory and anti-fibrotic ways.

Treatment with UDCA in heart transplant patients was retrospectively analyzed and UDCA was found to lower acute rejections, but the mechanism was not understood (30). von Haehling et al studied the beneficial effect of UDCA on inflammation in heart failure patients (31). In this study, UDCA had no beneficial effect on inflammatory cytokines, functional class or 6-min walk test, but UDCA was well tolerated in heart failure patients and improved endothelial functions. Treatment of UDCA may be more beneficial before overt heart failure occurs.

In conclusion, in this experimental study, we demonstrated the anti-inflammatory and anti-fibrotic effects of UDCA on diabetic rats and it can be a good drug candidate in diabetic patients for protecting from DCM.

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