RESEARCH ARTICLE

Trends in CTGF Expression in Renal and Chorioretinal Tissues Following Metformin and SGLT2 Inhibitor Treatment in Diabetic Rats

Maimun Syukri[1], Lia Meuthia Zaini[1,2,*], Arief Sjamsulaksan Kartasasmita[3,4], Tjahjono Darminto Gondhowiardjo[5], Ronny Lesmana[3], Putri Nabillah Mulya[2]

[1]Department of Medical Education, Faculty of Medicine, Universitas Syiah Kuala, Jl. Teungku Tanoh Abee, Banda Aceh 24415, Indonesia
[2]Department of Ophthalmology, Zainoel Abidin Hospital/Faculty of Medicine, Universitas Syiah Kuala, Jl. Moh. Daud Beureuh No.108, Banda Aceh 24415, Indonesia
[3]Faculty of Medicine, Universitas Padjadjaran, Jl. Raya Bandung, Sumedang Km. 21, Jatinangor 45363, Indonesia
[4]Department of Ophthalmology, Cicendo National Eye Hospital, Jl. Cicendo No. 4, Bandung 40171, Indonesia
[5]Department of Ophthalmology, Cipto Mangunkusumo Hospital/Faculty of Medicine, Universitas Indonesia, Jl. Pangeran Diponegoro No.71, Jakarta 10430, Indonesia

*Corresponding author. Email: liameuthiazaini@gmail.com

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Abstract

BACKGROUND: Connective tissue growth factor (CTGF) plays a central role in fibrotic processes affecting both renal and retinal tissues in diabetes. Although sodium-glucose cotransporter 2 (SGLT2) inhibitors have been shown to exert renoprotective and antifibrotic effects, their impact on CTGF expression in renal and retinal tissues has not been clearly established. This preliminary study was conducted to evaluate whether SGLT2 inhibitors (SGLT2i) could influence CTGF expression in the kidneys and eyes of diabetes-induced rats.

METHODS: After two weeks of adaptation, 24 rats were randomized and distributed equally into four groups (n=6 each): 1) Healthy Control, healthy rats without diabetic induction; 2) Negative Control, diabetic rats induced with streptozotocin (STZ) without treatment; 3) Metformin Group, diabetic rats treated with metformin; and 4) SGLT2i Group, diabetic rats treated with empagliflozin. Following eight weeks of intervention, CTGF expression was analyzed by Western blot in renal tissue (right kidney) and chorioretinal tissue (right eye). Four samples per group yielded analyzable bands and were included in the final quantification.

RESULTS: In renal tissue, CTGF levels (mean±SD) were highest in Negative Control Group (0.81±0.06). Both the Metformin Group (0.58±0.14) and SGLT2i Group (0.57±0.33) demonstrated a trend toward reduced CTGF expression. In chorioretinal tissue, CTGF values were relatively similar across groups (Healthy Control: 0.67±0.05; Negative Control: 0.63±0.12), with Metformin Group (0.61±0.12) and SGLT2i Group (0.64±0.22) showing a modest reduction trend.

CONCLUSION: In diabetic rats, CTGF expression levels are markedly increased. Following treatment with metformin and SGLT2i, CTGF expression demonstrates a noticeable reduction trend.

KEYWORDS: SGLT2 inhibitor, metformin, connective tissue growth factor, cellular communication network factor 2, renal, retina

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Introduction

Diabetes mellitus (DM) is a global metabolic disorder with a rapidly increasing prevalence and substantial health burden. (1) DM leads to microvascular complications, particularly diabetic nephropathy (DN) and diabetic retinopathy (DR), with the kidney and retina being especially vulnerable to hyperglycemia-induced injury.(2) Persistent hyperglycemia triggers oxidative stress, inflammation, and extracellular

matrix (ECM) accumulation, driving structural changes in renal and retinal tissues.(3) These mechanisms contribute to glomerular and tubular fibrosis in the kidney and vascular degeneration in the retina.(4,5) Various upstream inflammatory and profibrotic pathways have been implicated in diabetic fibrosis.(6,7) Within this cascade, connective tissue growth factor (CTGF) acts as a key mediator of fibrosis. Overexpression of CTGF promotes glomerular injury and retinal fibrosis.(8) Several other cascades also mediate fibrotic remodeling, including the AMP-Activated Protein Kinase alpha subunit (AMPK-α), NADPH Oxidase 2 and 4 (NOX2/4), and the Phosphoinositide 3-Kinase/ Protein Kinase B/Mechanistic Target of Rapamycin (PI3K/ Akt/mTOR) pathways.(9) Targeting CTGF is therefore a promising strategy to mitigate diabetes-related fibrosis.

Hyperglycemia-driven sodium glucose cotransporter-2 (SGLT2) overactivity increases intracellular glucose load, generating oxidative stress and activating the advanced glycation end-products (AGE)–receptor for AGEs (RAGE), and transforming growth factor (TGF)-β1 pathways, which are upstream regulators of CTGF. Because CTGF is a major downstream effector that drives extracellular matrix accumulation and fibrotic progression, reductions in glucose flux and oxidative signaling through SGLT2 inhibition may attenuate CTGF production. Evaluating this mechanism is essential to determine whether SGLT2 inhibitors exert antifibrotic effects beyond glycemic control, particularly in tissues highly vulnerable to diabetic microvascular injury such as the kidney and retina.(10, 11)

Among pharmacological options, SGLT2 inhibitors (SGLT2i) exhibit reno- and cardioprotective effects beyond glycemic control through anti-oxidative and anti-fibrotic mechanisms.(10,12,13) SGLT2 inhibition suppresses CTGF-mediated epithelial-mesenchymal transition and collagen deposition in diabetic kidneys, and alleviates retinal microvascular injury.(11,14) Empagliflozin and metformin downregulate SGLT2 and glucose transporter (GLUT)-1 in the chorioretina, suggesting ocular benefit.(15) Recent Indonesian studies reinforce these findings: empagliflozin reduced fibrosis-related markers (miR-21, TGF-β1) in hyperglycemic rats (16), while combining pharmacological and lifestyle interventions to prevent fibrotic complications (17). Despite such progress, there is limited evidence examining how SGLT2 inhibition modulates CTGF expression in both renal and retinal tissues within a diabetic model. Therefore, the effects of empagliflozin on CTGF expression in renal and chorioretinal tissues of streptozotocin (STZ)-induced diabetic rats to clarify the antifibrotic potential of SGLT2i in diabetes is evaluated.

Methods

Study Design

This was an *in vivo* experimental study with a post-test only control group design. There were 24 healthy male Wistar rats aged 8–10 weeks weighing 250–350 g. Rats of the same strain were housed under uniform environmental conditions and provided the same standard laboratory diet. Animals were obtained from the BioFarma Laboratory, Bandung. Inclusion criteria were healthy male Wistar rats aged 8–10 weeks, weighing 250–350 g, and showing normal activity. Exclusion criteria were rats that became ill or died during the study, or failed to develop hyperglycemia after STZ induction.

All procedures were approved by the Ethics Committee of the Faculty of Medicine, Universitas Padjadjaran (No. 710/UN6.KEP/EC/2020) and were conducted in accordance with the Association for Research in Vision and Ophthalmology (ARVO) Statement for the Use of Animals in Ophthalmic and Vision Research. The sample size was calculated using Federer's formula, ensuring adequacy for statistical analysis while adhering to the 3Rs principle, particularly the reduction principle. A total of 24 rats were used and were divided into four groups of six animals each.

Diabetic Induction

Male Wistar rats were induced with diabetes using a single intraperitoneal injection of STZ at 60 mg/kg BW. STZ was commonly used to create type 1 diabetes models through DNA damage and pancreatic β-cell destruction. This process resulted in reduced insulin production.

Animal Treatment

Animals were acclimatized for two weeks under controlled room temperature (20–28°C) and humidity (50–97%), with sawdust bedding and free access to food and water. After adaptation, rats were induced with STZ and randomized into the following groups: 1) Healthy Control, healthy rats without STZ induction and no treatment; 2) Negative Control, STZ-induced diabetic rats without treatment; 3) Metformin Group, diabetic rats treated with 100 mg/kg BW metformin; and 4) SGLT2i Group, diabetic rats treated with 30 mg/kg BW empagliflozin. Treatments were administered orally via probe once daily for eight weeks. Dosages were based on previous studies using these drugs in diabetic rat models.(14,18) Empagliflozin was chosen because it was widely available in Indonesia, with well-documented experimental dosing protocols. Only rats with blood glucose

>250 mg/dL on three consecutive measurements after STZ induction were included in the intervention phase. At the end of treatment, animals were euthanized, and retinal and renal tissues were collected for protein analysis. The renal samples were obtained from the right kidney, and chorioretinal samples were obtained from the right eye.

Western Blot Analysis

The tissue preparation process began with the collection of approximately 20 mg of retinal and renal tissue, respectively, which was crushed and homogenized in radioimmunoprecipitation (RIPA) buffer containing protease inhibitors to prevent protein degradation. The homogenate was centrifuged at 12000 rpm and the supernatant containing total protein (lysate) was collected. Bromophenol blue dye was added as a tracking agent, and the samples were denatured at 95°C for 5 minutes, then cooled to room temperature. Equal amounts of protein were loaded into Sodium Dodecyl Sulfate–Polyacrylamide (SDS–PAGE) wells and electrophoresed to separate proteins based on molecular weight.

Following electrophoresis, proteins were transferred onto nitrocellulose membranes using electroblotting with a Mini Blot Module (Thermo Fisher Scientific, Waltham, MA, USA). Membranes were blocked with 5% bovine serum albumin (BSA) or casein to prevent nonspecific binding, then incubated overnight at 4 °C with a primary antibody against CTGF 1:1000 dilution (Cat. No. ab6992; Abcam, Cambridge, UK). After washing with Tris-Buffered Saline (TBST) buffer, membranes were incubated with horseradish peroxidase (HRP)–conjugated secondary antibodies for 1 hour at room temperature. Detection was performed using enhanced chemiluminescence (ECL) reagents, and the intensity of protein bands was quantified using ImageJ software (NIH, Bethesda, MD, USA).

Protein Carbonylation Assay

Protein carbonylation was assessed using the Protein Carbonyl Assay Kit (Western Blot) (Cat. No. ab178020; Abcam). Tissue lysates were derivatized with 2,4-dinitrophenylhydrazine (DNPH) to form DNP-hydrazones, separated by Sodium dodecyl-sulfate polyacrylamide gel electrophoresis (SDS–PAGE), and transferred to polyvinylidene difluoride (PVDF) membranes. Membranes were probed with an anti-DNP antibody provided in the kit, followed by HRP-conjugated secondary antibody. The carbonylated protein bands were visualized using chemiluminescence and quantified densitometrically.

Statistical Analysis

Data were analyzed using one-way ANOVA followed by multiple comparison tests with GraphPad Prism version 10 (GraphPad Software, San Diego, CA, USA). Results were presented as mean±standard deviation (SD), and $p<0.05$ were considered statistically significant.

Results

Blood Glucose Levels Before and After STZ Induction

Fasting blood glucose levels were measured before and after STZ induction to confirm the establishment of the diabetic model (Table 1). Prior to induction, all groups showed normal glucose levels, with mean values ranging from approximately 107 to 114 mg/dL. After STZ administration, a marked increase in blood glucose was observed in all STZ-induced groups compared to the Healthy Control. The mean post-induction glucose levels were 354.67±24.11 mg/dL in the Negative Control, 333.76±31.52 mg/dL in the Metformin Group, and 336.83±52.52 mg/dL in the SGLT2i Group, while the Healthy Control rats remained normoglycemic (116.00±8.04 mg/dL). These findings confirm the successful induction of hyperglycemia in the diabetic rat model before treatment interventions were initiated.

CTGF Expression was Highest in Diabetic Rats

Of the six animals assigned to each group, only four samples per group produced clearly readable CTGF bands and were used for quantitative analysis. Samples with faint or unreadable bands were excluded due to technical issues and mortality. Western blot analysis revealed that CTGF expression was higher in the chorioretinal and proximal tubular renal tissues of diabetic rats (Negative Control) compared with those of normoglycemic rats (Healthy Control). Band intensities were quantified using ImageJ (Table 2). The highest CTGF expression was observed in Negative Control group, whereas treatment with metformin

Table 1. Baseline data of blood glucose levels before and after the induction of STZ.

Group	Mean±SD	
	Pre-STZ	Post-STZ
Healthy Control	107.50±17.08	116.00±8.04
Negative Control	110.00±18.08	354.67±24.11
Metformin Group	109.42±17.97	333.76±31.52
SGLT2i Group	113.83±22.55	336.83±52.52

Table 2. CTGF expression in renal and chorioretinal tissues.

Group	Mean±SD	
	Renal	Chorioretinal
Healthy Control	0.45±0.20	0.67±0.05
Negative Control	0.81±0.06	0.63±0.12
Metformin Group	0.58±0.14	0.61±0.12
SGLT2i Group	0.57±0.33	0.64±0.22

or empagliflozin reduced CTGF expression in both renal and chorioretinal tissues (Figure 1).

Renal CTGF Expression Showed a Reduction Trend following Treatment

Mean CTGF expression in renal tissue was 0.81±0.06 in the Negative Control, compared with 0.45±0.20 in the Healthy Control, 0.58±0.14 in the Metformin Group, and 0.57±0.33 in the SGLT2i Group. One-way ANOVA yielded $F(3,12)=2.10$ ($p=0.154$). Although statistical thresholds were not reached, the SGLT2i Group demonstrated a comparatively stronger reduction trend in renal CTGF levels (Table 2).

Chorioretinal CTGF Expression Showed a Minimal Reduction Trend across Treatment Groups

The Negative Control exhibited a mean CTGF value of 0.63±0.12, compared with 0.67±0.05 in the Healthy Control, 0.61±0.12 in the Metformin Group, and 0.64±0.22 in the SGLT2i Group. One-way ANOVA yielded $F(3,19)=0.56$ ($p=0.647$). A slight trend toward lower CTGF expression was observed in the Metformin Group (Table 2).

Protein Carbonylation Levels Showed a Reduction Trend in Treated Groups

As a preliminary assessment of oxidative stress, protein carbonylation was examined in renal and chorioretinal tissues. Representative western blot images showed increased protein carbonyl levels in Negative Control compared with the Healthy Control. A trend toward reduced protein carbonylation was observed following treatment, with empagliflozin appearing more effective than metformin in renal tissue (Figure 2). Because only one representative sample was available per group, densitometric quantification and statistical analysis were not performed, and the data was interpreted qualitatively.

Discussion

The result of this study demonstrated that CTGF expression was elevated in both renal and chorioretinal tissues of STZ-induced diabetic rats. This reinforces the concept that persistent hyperglycemia activates profibrotic pathways, with CTGF serving as a central mediator of fibrosis in diabetic complications.(13) Although the current analysis did not reach statistical significance, the overall trend indicated increased CTGF expression in diabetic animals compared with controls, while both metformin and empagliflozin attenuated this response. These findings suggest that CTGF downregulation may contribute to the protective actions of antidiabetic therapies.

The observed upregulation of CTGF is consistent with earlier studies reporting enhanced CTGF expression in renal and retinal tissues under hyperglycemia.(13) CTGF has been implicated in ECM accumulation, capillary basal lamina thickening, and vascular remodeling, which collectively drive the development of DN and DR. Our results therefore provide additional evidence that CTGF is a pivotal factor in the pathogenesis of diabetic microvascular complications.

Metformin treatment was associated with reduced CTGF expression in both renal and retinal tissues, with a more pronounced effect in the renal. This aligns with

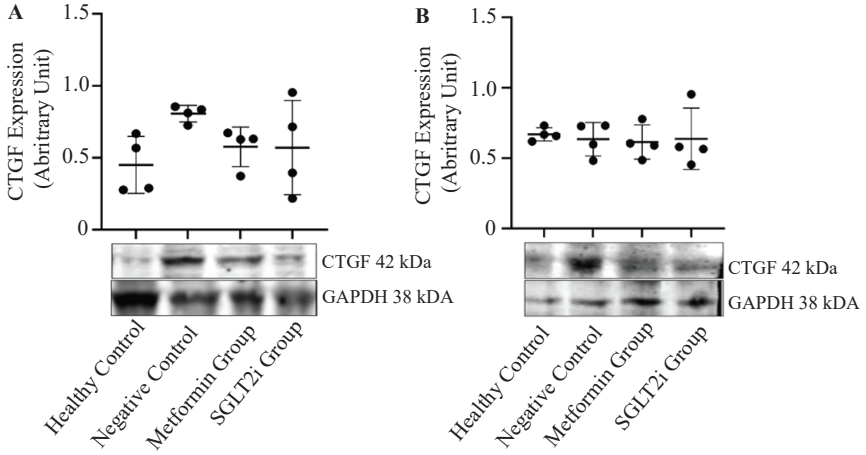

Figure 1. CTGF expression in renal and chorioretinal tissues. A: Renal CTGF expression (mean±SD, n=4). B: Chorioretinal CTGF expression (mean±SD, n=4).

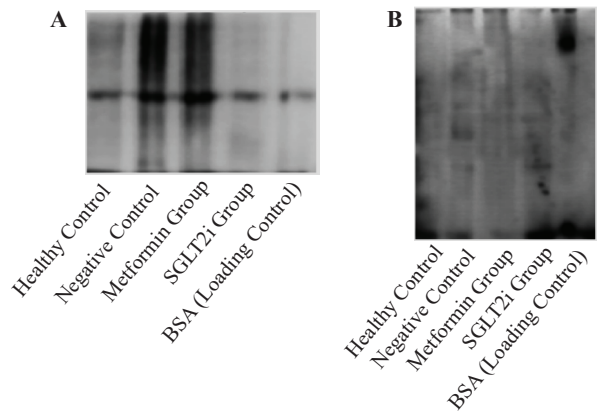

Figure 2. Protein carbonylation in renal and chorioretinal tissues. Representative immunoblots showing protein carbonyl accumulation in (A) renal tissue and (B) chorioretinal tissue.

studies showing that metformin activates AMPK, which subsequently suppresses Smad3-driven transcription and fibrotic signaling.(19) Beyond glycemic control, metformin has been shown to inhibit collagen synthesis, reduce CTGF expression, and attenuate profibrotic responses in renal fibroblasts. Further evidence from animal models indicates that metformin ameliorates renal injury, preserves microvascular integrity, and can even halt the progression of chronic kidney disease.(18,20) These multifaceted effects likely contribute to the observed CTGF reduction in our study.

Empagliflozin, a SGLT2i, also decreased CTGF expression, with stronger effects observed in renal tissue compared to the retina. The current findings in the kidney are consistent with previous study, who demonstrated that empagliflozin attenuated renal fibrosis in diabetic rats by inhibiting CTGF-mediated epithelial-to-mesenchymal transition.(11) However, other studies in db/db mice reported that while empagliflozin reduced several profibrotic markers such as TGF-β1 and Cd14, CTGF expression remained elevated compared with non-diabetic controls. Interestingly, when empagliflozin was co-administered with metformin, the combination abolished the diabetes-induced upregulation of most profibrotic genes, and CTGF expression was significantly lower than in either drug alone. (14) These findings suggest that dual therapy may provide synergistic antifibrotic benefits.

Previous studies have shown that empagliflozin reduces CTGF expression and ameliorates renal injury, partly by inhibiting the advanced glycation end product–receptor axis. (10) Other work has demonstrated its antifibrotic properties through increased klotho expression and attenuation of renal fibrosis.(12) Additional mechanisms have been proposed, including suppression of epithelial-to-mesenchymal transition, modulation of Vascular endothelial growth factor C (VEGF-C)/VEGF receptor 3 (VEGFR3) signaling, and reduction of inflammation and oxidative stress.(21) Taken together, these findings support the potential antifibrotic role of empagliflozin in diabetic complications.

When comparing the two agents, mmetformin showed a modest reduction trend in chorioretinal CTGF, whereas both agents demonstrated similar reduction trends in renal tissue. These tissue-specific differences may reflect distinct pathogenic drivers: retinal CTGF is closely associated with angiogenesis and fibrosis in DR, while renal CTGF expression is strongly influenced by SGLT2-mediated hyperfiltration and metabolic overload.(22,23) Complementary actions observed suggest that combining these therapies may provide broader protection against microvascular complications.

This study has several limitations, including the absence of post-intervention glucose measurements and the lack of comparison with additional antidiabetic agents. In this study, the post-intervention blood glucose levels were not assessed within this experiment because the glycemic data had been analyzed and reported separately in a prior publication, and duplicating the dataset was avoided for ethical and editorial reasons. Consequently, we could not fully distinguish whether the reduction in CTGF expression was mediated through improved glycemic control or represented a direct antifibrotic effect. In addition, the study did not include comparison with other glucose-lowering agents, which could strengthen the evidence regarding drug-specific versus glycemia-independent effects. Future research should incorporate serial glucose monitoring, evaluate multiple antidiabetic classes, and explore earlier disease stages such as prediabetes to better delineate the mechanisms by which SGLT2i and metformin modulate fibrotic pathways. Larger studies with longer treatment durations and complementary methods such as immunohistochemistry and transcriptomic profiling are warranted to validate these findings.

Conclusion

CTGF expression is highest in diabetic rats, and treatment with metformin or SGLT2i shows a trend toward reduced CTGF levels in both renal and chorioretinal tissues. These findings indicate that CTGF may serve as a relevant fibrosis-related marker in diabetic microvascular complications and highlight the potential of CTGF modulation as a therapeutic target.

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Authors Contribution

MS, LMZ, ASK, TDG, and RL were involved in concepting and planning the research. LMZ and PNM performed the data acquisition/collection, calculated the experimental data, performed the analysis, and interpreted the results, drafted the manuscript, and designed the figures. All authors took parts in giving critical revision of the manuscript.

Conflict of Interest

The authors declare no conflict of interest.

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