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Inhibition of tumor necrosis factor-α enhanced the antifibrotic effect of empagliflozin in an animal model with renal insulin resistance

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Abstract

Insulin resistance (IR) has emerged as one of the main risk factors for renal fibrosis (RF) that represents a common stage in almost all chronic kidney disease. The present study aims to investigate the inhibitory effect of empagliflozin (EMPA "a sodium-glucose co-transporter 2 inhibitor") and infliximab [IFX "a tumor necrosis factor-α (TNF-α) antibody"] on RF in rats with induced IR. IR was induced by adding 10% fructose in drinking water for 20 weeks. Thereafter, fructose-induced IR rats were concurrently treated with EMPA (30 mg/kg), IFX (1 dose 5 mg/kg), or EMPA + IFX for 4 weeks, in addition to IR control group (received 10% fructose in water) and normal control (NC) group. Rats with IR displayed hyperglycemia, deterioration in kidney functions, glomerulosclerosis, and collagen fiber deposition in renal tissues as compared to NC. This was associated with downregulation of the renal sirtuin 1 (Sirt 1) expression along with higher renal tissue TNF-α and transforming growth factor-β1 (TGF-β1) levels. Both EMPA and IFX significantly modulated the aforementioned fibrotic cytokines, upregulated the renal Sirt 1 expression, and attenuated RF compared to IR control group. Of note, IFX effect was superior to that of EMPA. However, the combination of EMPA and IFX alleviated RF to a greater extent surpassing the monotherapy. This may be attributed to the further upregulation of renal Sirt 1 in addition to the downregulation of fibrotic cytokines. These findings suggest that the combination of EMPA and IFX offers additional benefits and may represent a promising therapeutic option for RF.

Keywords Empagliflozin · Infliximab · Insulin resistance · Renal fibrosis · Sirt 1

Introduction

It is well documented that insulin resistance (IR) is one of the important metabolic risk factors for chronic kidney diseases, CKD [1, 2]. Several reports demonstrated a strong association between IR/hyperinsulinemia and kidney dysfunction [3, 4]. Additionally, hyperglycemia induces a kidney damage through several mechanisms including the activation of the exacerbated polyol and hexosamine flux, an increase in the advanced glycation end-products (AGEs) formation, and activation of protein kinase C which represents a low grade of chronic inflammation [5]. Furthermore, IR induced in experimental animals from high fructose consumption was associated with a kidney inflammation and a renal fibrosis, RF [6–9] which represents a final common pathway of all progressive kidney diseases regardless the initial cause of injury [10].

A compiling evidence demonstrated the major role of transforming growth factor-β1 (TGF-β1) in mediating CKD associated with progressive RF [11]. TGF-β1 is considered the major driver of matrix synthesis besides inhibiting matrix degradation and stimulating the myofibroblast activation [4, 12, 13]. Additionally, TGF-β1 stimulates mesangial cells, interstitial fibroblasts, and tubular epithelial cells to become matrix-producing fibrogenic cells [14].

A large body of literature indicates that inflammation plays a critical role in the initiation and progression of RF [15]. Tumor necrosis factor-alpha (TNF-α), a potent proinflammatory cytokine that is produced from macrophages, mesangial cells, and renal tubular epithelial cells, is served as an important mediator of inflammatory tissue damage [16,

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17]. In addition to its inflammatory role, it stimulates the release of TGF-β1 and has an important role in glomerular inflammation and fibrosis [18]. Previous studies on different models of fibrosis showed that Infliximab (IFX), a monoclonal antibody for TNF-α [19], attenuates effectively the fibrosis and the induced inflammation via decreasing the TNF-α and inhibiting nuclear factor kappa-B, NF-κB [20, 21]. However its effect on improving RF induced by IR has not been studied yet.

Although RF is considered a major cause of the end-stage renal disease, treatment remains non-specific and clinically ineffective [22]. Sodium-glucose co-transporter 2 (SGLT2) inhibitors are a novel class of glucose-lowering agents with potential renoprotective effects [23]. Recent studies have shown that empagliflozin (EMPA) (a member of SGLT2 inhibitors) possess anti-inflammatory and anti-oxidative stress properties which makes it a prospective renoprotective drug [24–26]. However, few studies about its effect against RF are established. On the other hand, increasing evidence suggests that sirtuin1 (Sirt 1) (a nicotinamide adenine dinucleotide-dependent deacetylase) provides renoprotective effects against the development of different renal disorders due to its anti-fibrosis, anti-oxidative stress [27], and anti-inflammatory effects [28].

Therefore, the present study aims to explore the effect of EMPA and IFX on RF rats with induced IR, to examine if the combination of the two drugs might offer additional benefits and to clarify one of the underlying mechanisms.

Materials and methods

Drugs and chemicals

EMPA (Jardiance® film-coated tablets 25 mg) was supplied from Boehringer Ingelheim, Germany. IFX (Remicade® 1 vial contain 100 mg of IFX) was obtained from Janssen Biotech, USA. Fructose powder was obtained from Unipharma Co., Egypt.

Animals and experimental design

Thirty male Wistar albino rats weighing 150 ± 10 g were obtained from the Faculty of Veterinary Medicine (Zagazig University, Egypt) and acclimated in the Animal Facility of Faculty of Pharmacy, Zagazig University at controlled environmental conditions with free access to standard chow and tap water. After 1 week of acclimatization, rats were fed either normal chow diet and served as normal control (NC, $n = 6$) or normal chow diet + 10% w/v fructose in drinking water for 20 weeks to induce IR [29]. IR was confirmed in animals by high oral glucose tolerance test (data are not shown) and homeostatic model assessment of IR (HOMA-IR) value > 4.0 [30]. Thereafter, fructose-induced IR rats were randomly divided into four groups ($n = 6$). One group received 10% fructose in drinking water and served as IR control group. The other three groups were treated with EMPA (30 mg/kg body weight/day, orally) [31], IFX (1 dose 5 mg/kg body weight, intraperitoneally, IP) [32, 33], and combination of EMPA and IFX (with the same doses as the monotherapy) for four weeks concurrently with control groups. All experimental protocols were performed in accordance with the National Institutes of Health (NIH) guidelines for handling of laboratory animals and approved from the Ethical Committee of Animal Research of Faculty of Pharmacy, Zagazig University, Egypt (No. 10–12-2017).

Blood sampling and tissue harvest

At the end of the experiment, blood samples were obtained via retro-orbital bleeding after overnight fasting and sera were separated and divided into aliquots and stored at − 4 °C for subsequent measuring of the biochemical parameters. Rats were scarified and kidneys were removed immediately, rinsed with normal saline, dried, and weighed. One kidney was snap frozen in liquid nitrogen (− 170 °C, obtained from Veterinary Directorate, Zagazig, Egypt) for 5 min then stored at − 80 °C for further determination of renal SGLT2, TNF-α, and TGF-β1 contents and Sirt 1 gene expression. The other kidney was fixed in 10% neutral buffered formalin at 4 °C for 72 h and processed for histopathological examination.

Analytical methods

Serum biochemical parameters

Glucose level was measured in serum samples by quantitative enzymatic colorimetric determination using diagnostic kits provided from Spectrum kits, Germany, (Catalog No. 250001). Serum insulin level was determined by solid phase enzyme-linked immunosorbent assay (ELISA) using rat insulin kit (RayBiotech, Norcross, GA, Catalog No. ELR-Insulin) according to the manufacturer's instructions. The HOMA-IR was calculated using the formula [[fasting glucose (mmol/L) × fasting insulin (μIU/mL)]/22.5] [34]. HOMA-IR values > 4 are indicator for IR state [30]. Serum BUN and creatinine were measured using Diamond diagnostic assay kits, USA, (Catalog Nos. 215,243 and 242,045, respectively), following the manufacturer's instructions.

Renal tissues SGLT2, TNF-α, and TGF-β1 contents

The kidneys were homogenized with a homogenizer. Then the homogenates were centrifuged at 3000 rpm for 15 min at 4 °C. The supernatants were kept at − 80 °C until being

used for measuring SGLT2, TNF-α, and TGF-β1 concentrations. Renal SGLT2, TNF-α and TGF-β1 contents were determined using ELISA assay kits, (Mybiosource, California, San Diego, USA, Catalog No. MBS763535), (Sigma-Aldrich, St. Louis, MO, USA, Catalog No. RAB0480), and (Biovision, South Milpitas Blvd., California, USA, Catalog No. K4344-100), respectively follows the manufacturer's instructions.

Renal Sirt 1 gene expression

Total RNA was extracted from kidney tissues using Qiagen extraction kit (Qiagen, Valencia, CA, USA). The extracted RNA was reverse transcribed into complementary DNA (cDNA) using reverse transcription kit (Catalog No. K1621, Fermentas, Hanover, MD, USA). cDNA was amplified by reverse transcription polymerase chain reaction (RT-PCR) and then the real time-PCR result was analyzed using an Applied Biosystem (StepOne™, USA). Relative messenger RNA (mRNA) expression relative to the internal control GAPDH gene was calculated by cycle threshold method ($2^{-\Delta\Delta c_t}$) (Table 1) [35].

Histological examination

Paraffin-embedded kidney sections were used for assessment of renal injury. Periodic acid Schiff (PAS) stain was used to assess the basement membranes within the glomerulus and around the renal tubules (which appear red in color) and also to assess the presence of glomerulosclerosis by using 5 µm kidney sections (×400 magnification) [36, 37]. The degree of glomerular damage was scored as follows: 1, < 25%; 2, 25–50%; 3, 50–75%; 4, > 75%; 5, completely sclerotic glomeruli [38]. Masson trichrome was used to detect the presence of collagen fibers and renal interstitial fibrosis (×200 magnification) [39]. Previous stains were examined under the light microscope by an experienced morphologist, who was blinded to the origin of the slides. Morphometric study was done to assess the collagen area percentage/$(\mu m)^2$ surface area in renal sections. This was measured in six randomly selected high-power microscopic fields within the sections for each group using a computerized image system composed of a Leica Qwin 500 image analyser, which is connected to a Leica microscope and were expressed as mean ± standard deviation, SD [40].

Table 1 Sequences of primers used in RT-PCR

Genes	Primer sequences	GeneBank accession number
Sirt 1	F: 5′-TGACTTCAG ATCAAGAGA TGG-3′ R: 5′-TGGCTTGAG GATCTGGGA GAT-3′	XM017601788.1
GAPDH	F: 5′-CACCCTGTT GCTGTAGCCATA TTC-3′ R: 5′-GACATCAAG AAGGTGGTGAAG CAG-3′	XM017592435.1

Sirt 1 Sirtuin 1 or silent mating type information regulation 2 homolog 1, *GAPDH* glyceraldehyde 3-phosphate dehydrogenase

Statistics

Results were statistically analyzed by Prism 7 GraphPad and expressed as mean ± SD. Comparisons between normal and IR control groups were performed by using unpaired Student's *t* test, while the comparisons between groups were done using analysis of variance (ANOVA) followed by Tukey–Kramer post hoc test. *p* values less than 0.05 were considered as statistically significant.

Results

The induction of insulin resistance was confirmed by HOMA-IR values

The induction of IR could be confirmed by HOMA-IR values > 4 [30]. As shown in Table 2, rats received 10% fructose in drinking water for 20 weeks exhibited IR as observed by HOMA-IR > 4.

The combination of empagliflozin with infliximab synergistically improved the glycemic profile and renal tissue SGLT2 levels

From the major manifestations of IR are hyperglycemia and hyperinsulinemia [41], which were apparent here in our model. As shown in Fig. 1 and Table 3, rats maintained on 10% fructose for 20 weeks displayed significant elevation in serum glucose and insulin levels as well as HOMA-IR value in addition to a marked increase in renal tissue SGLT2 contents as compared with NC group ($p < 0.001$). Concurrent treatment of IR rats with EMPA, IFX, or the combined treatments significantly improved the altered glycemic profile

Table 2 Effect of administrating 10% fructose in drinking water for 20 weeks on HOMA-IR values

Parameter	NC	IR
HOMA-IR	1.77 ± 0.11	18.44 ± 2.04*

NC normal control, *IR* insulin-resistant rats ($n = 6$/group)

*Significantly different at $p < 0.05$

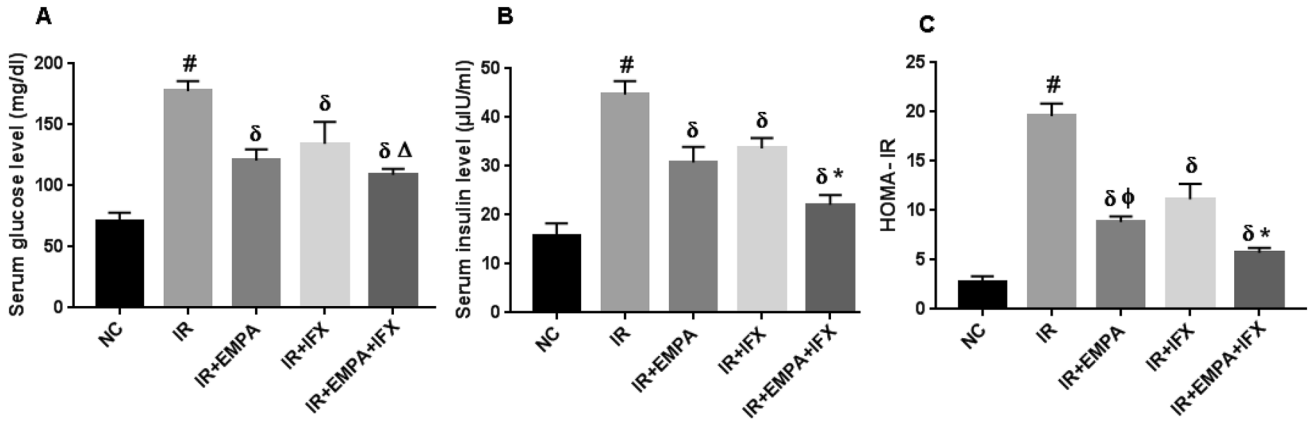

Fig. 1 Effect of empagliflozin (EMPA, 30 mg/kg body weight/day, orally), infliximab (IFX, 1 dose of 5 mg/kg body weight, IP), and the combined treatment for four weeks on **a** serum glucose level, **b** serum insulin level, and **c** HOMA-IR in normal control (NC) and fructose-induced insulin-resistant (IR, 10% fructose in drinking water for 20 weeks) rats ($n=6$ rats/group). Bars represented mean ± SD. $^{\#}p<0.0001$ vs NC, $^{\delta}p<0.0001$ vs IR, $^{\Delta}p<0.01$ vs IR + IFX, $^{*}p<0.0001$ vs IR + EMPA and IR + IFX, $^{\phi}p<0.01$ vs IR + IFX

Table 3 Effect of empagliflozin (EMPA, 30 mg/kg body weight/day, orally), infliximab (IFX, 1 dose of 5 mg/kg body weight, IP), and the combined treatment for four weeks on renal tissue tumor necrosis factor α (TNF-α), transforming growth factor β1 (TGF-β1), and sodium-glucose co-transporter 2 (SGLT2) levels and renal silent mating type information regulation 2 homolog 1(Sirt1) gene expression in normal control (NC) and fructose-induced insulin-resistant (IR, 10% fructose in drinking water for 20 weeks) rats ($n=6$ rats/group)

Parameter	Group				
	NC	IR	IR + EMPA	IR + IFX	IR + EMPA + IFX
TNF-α (pg/mg tissue)	151.4 ± 21.45	$806.7 \pm 50.63^{\#}$	$316.7 \pm 8.84^{\delta}$	$230 \pm 35.31^{\delta,\Delta}$	$196.8 \pm 12.21^{\delta,\phi}$
TGF-β1 (pg/mg tissue)	266.3 ± 31.91	$911.8 \pm 45.63^{\#}$	$517.3 \pm 23.09^{\delta}$	$368.3 \pm 19.20^{\delta,\phi}$	$240.7 \pm 11.41^{\delta,\theta}$
SGLT2 (ng/mg tissue)	6.65 ± 0.06	$30.6 \pm 1.78^{\#}$	$15.6 \pm 0.71^{\delta}$	$16.25 \pm 0.93^{\delta}$	$11.05 \pm 0.49^{\delta,\theta}$
Sirt 1 expression	2.75 ± 0.22	$0.84 \pm 0.06^{\#}$	$1.83 \pm 0.26^{\delta}$	$2.45 \pm 0.13^{\delta,\Delta}$	$2.62 \pm 0.34^{\delta,\phi}$

Values are expressed as mean ± SD

$^{\#}p<0.001$ vs NC, $^{\delta}p<0.0001$ vs IR, $^{\Delta}p<0.001$ vs IR + EMPA, $^{\phi}p<0.0001$ vs IR + EMPA, $^{\theta}p<0.0001$ vs IR + EMPA and IR + IFX

and decreased renal tissue SGLT2 levels when compared to IR group ($p<0.0001$). The effect of the combined treatments was superior to the individual treatments regarding insulin level, HOMA-IR value, and renal SGLT2 levels, while the effect on glucose level was nearly similar to EMPA treatment.

Empagliflozin, infliximab, and the combined therapy improved kidney hypertrophy without significant improvement in the kidney function parameters

Previous studies demonstrated that hyperinsulinemia plays an important role in promoting kidney dysfunction by inducing glomerular hyperfiltration, endothelial dysfunction, mesangial hyperplasia, and renal hypertrophy [42, 43]. Figure 2 showed that induction of IR significantly increased kidney weight and serum BUN and creatinine levels in IR group compared to NC group ($p<0.05$). Treatment with EMPA, IFX, or the combination showed significant decrease in kidney weight as compared with IR group ($p<0.05$), while there was no improvements in renal function parameters in all treated groups.

Treatment with empagliflozin plus infliximab significantly improved renal fibrosis

It was reported that IR and the released inflammatory cytokines are responsible for glomerular mesangial expansion, basement membrane thickening, podocytopathy, and the loss of slit pore diaphragm integrity, which leads to glomerulosclerosis and tubulointerstitial injury [44]. As shown in Fig. 3, both PAS and Masson trichrome representative images of IR group showed thickening in the glomerular (glomerulosclerosis) and tubular basement membrane (score 4) indicated by the increase in PAS-positive staining area with loss of renal tubule brush border as well as a marked increase in collagen fibers

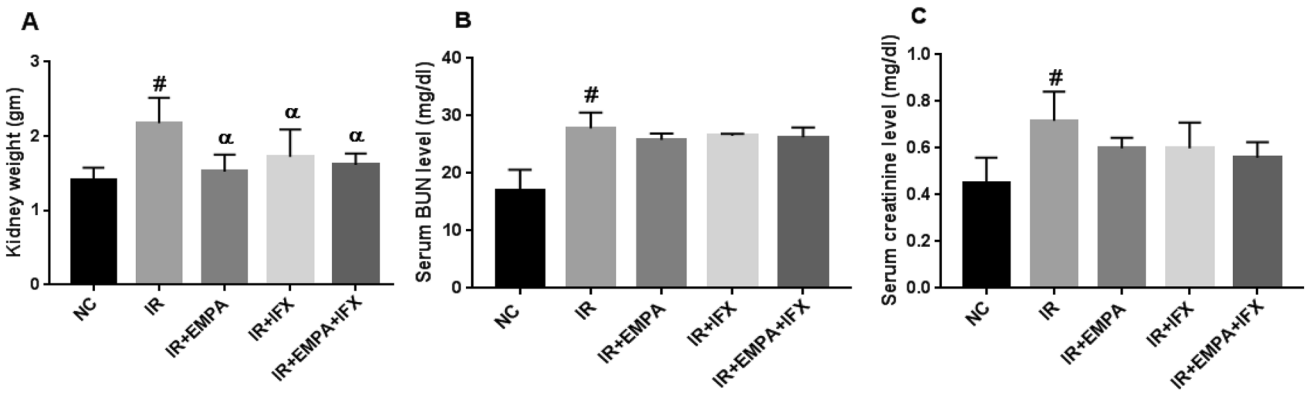

Fig. 2 Effect of empagliflozin (EMPA, 30 mg/kg body weight/day, orally), infliximab (IFX, 1 dose of 5 mg/kg body weight, IP), and the combined treatment for four weeks on **A** kidney weight, **B** serum blood urea nitrogen (BUN) level, **C** serum creatinine level in normal control (NC) and fructose-induced insulin-resistant (IR, 10% fructose in drinking water for 20 weeks) rats ($n=6$ rats/group). Bars represented mean ± SD. $^{\#}p<0.001$ vs NC, $^{\alpha}p<0.01$ vs IR

deposition around renal glomeruli and in between the renal tubules as compared with NC group ($p<0.0001$). In contrast, the PAS stain in both treatments showed preserved brush borders of the tubules with thin basement membrane (decreases in PAS-positive area) in addition to a significant decrease in the amount of collagen fibers in comparison with IR group ($p<0.001$). Moreover, IFX (score 2) achieved better effect than EMPA (score 3) on glomerulosclerosis. Co-administration of EMPA with IFX induced a better improvement in decreasing glomerulosclerosis (score 1) and collagen fibers deposition than the individual treatments.

Empagliflozin, infliximab, and the combined treatments ameliorated renal tissue TNF-α and TGF-β1 levels

Several studies confirmed the relationship between high fructose diet consumption and production of proinflammatory cytokines such as TNF-α [7, 45–47]. Additionally, hyperglycemia as previously described increased the formation of AGEs, which in turn stimulates the release of numerous cytokines including TNF-α and TGF-β1 [48, 49]. As illustrated in Table 3, using 10% fructose in drinking water for 20 weeks produced a dramatic increase in profibrotic cytokines levels in renal tissues, which were significantly decreased by both the individual and combined treatments. Noteworthy, the combined treatments reduced the renal tissue TNF-α and TGF-β1 levels to greater extent than the individual treatment, so the combination achieved the best result.

Treatment with empagliflozin plus infliximab synergistically upregulated renal Sirt 1 gene expression

Several studies reported the downregulation of Sirt 1 expression during IR state [50–53]. Here, in this study, and as shown in Table 3, the renal expression of Sirt 1 gene was significantly downregulated in the induced IR rats as compared to NC group**.** On the other hand, the administration of EMPA, IFX, and the combined treatment for four weeks resulted in a significant upregulation of renal Sirt 1 expression. The combined treatment exerted greater effect than treatment with EMPA alone regarding Sirt 1 expression ($p<0.0001$).

Discussion

The present study demonstrated that the treatment either with EMPA or IFX ameliorated RF, which resulted from induced IR mainly via improving hyperglycemia, decreasing renal tissues TNF-α and TGF-β1, and the upregulation of Sirt 1 gene expression. It is worth mentioning that the renal antifibrotic effect of IFX on RF was more remarkable than that of EMPA. Moreover, the inhibition of TNF-α by IFX enhanced the antifibrotic effect of EMPA against RF besides the upregulation of Sirt 1 gene expression in renal tissues by combined drugs. This adds extra benefits to the combined treatment in ameliorating RF.

IR is considered an important clinical and biochemical determinant, not only of diabetes but also of many other clinical states because it represents an underlying mechanism for several diseases including type 2 diabetes mellitus, obesity, cardiovascular and CKD [41, 54]. Furthermore, several

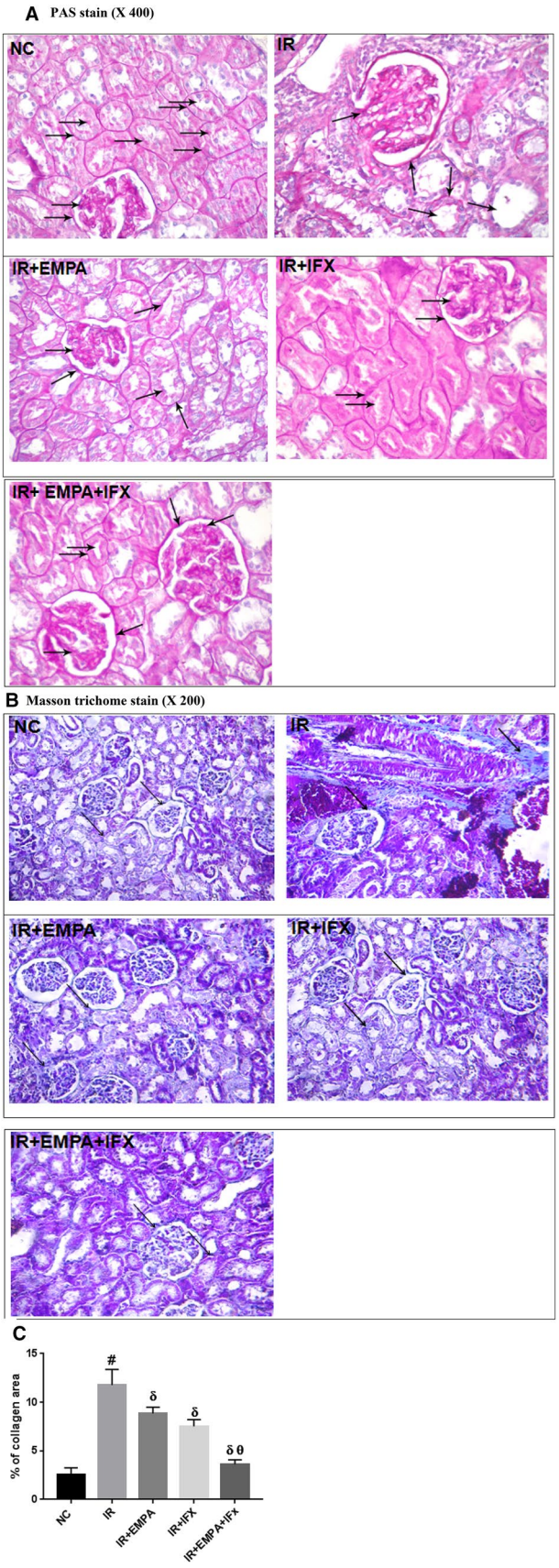

Fig. 3 photomicrographs of renal tissues in rats **A** representative PAS-stained sections × 400 magnification, **B** representative Masson's trichrome-stained sections × 200 magnification, and **C** percentage of collagen area in different groups. *NC* normal control, *IR* insulin resistance rats + 10% fructose in water, *IR* + *EMPA* insulin resistance rats + 30 mg/kg/day body weight of empagliflozin, orally for 4 weeks, *IR* + *IFX* insulin resistance rats + 1 dose 5 mg/kg body weight of infliximab IP, *IR* + *EMPA* + *IFX* insulin resistance rats + empagliflozin + infliximab (with the same doses of the monotherapy). Bars represented mean ± SD. $^{\#}p < 0.0001$ vs NC, $^{\delta}p < 0.0001$ vs IR, $^{\theta}p < 0.0001$ vs IR + EMPA and IR + IFX

studies have reported the association between IR and kidney dysfunction [3, 44] in addition to its role in promoting RF [2]. Spoto et al. reported that high insulin level induces the growth of mesangial cells and inhibits its apoptosis during IR states, in addition to reduce the activity of matrix metalloproteinases, leading to RF [54].

Previous studies showed that IR was often accompanied by NF-κB activation [55, 56]. The activation of NF-κB pathway in turn regulates the expression of target genes encoding inflammatory mediators, such as TNF-α [57]. On the other hand, a negative crosstalk between NF-κB pathway and Sirt 1 expression was reported [28, 56, 58]. Yeung et al. stated that Sirt 1 inhibits NF-κB activity by deacetylating the p65 subunit, blocking NF-κB ability to bind DNA, thereby inhibiting the transcription of TNF-α [59]. In 2014, Du et al. demonstrated that Sirt 1 expression was decreased by TNF-α in a time- and dose-dependent manner [60]. Additionally, a previous study showed that a significant upregulation in Sirt 1 expression in patients with inflammatory bowel disease successfully treated with IFX [61].

More importantly, evidence of a potential link between Sirt 1 and TGF-β1 has emerged from previous studies [62, 63]. It was reported that Sirt 1 inhibits TGF-β1 signaling by deacetylating Smad3 and represses the effect of TGF-β1 on RF progression [13]. Noteworthy, phosphorylation of Smad3 is considered as a key signaling mechanism underlying the fibrogenesis in response to TGF-β1 [64]. This effect provides an additional benefit for the Sirt 1 upregulation against RF.

Although RF represents a common stage of almost all CKD [65], currently there are no effective treatments for preventing the progression of RF [66]. Therefore, new medical therapies or combined therapies that hinder RF are highly required**.** In this study, the researchers propose that inhibiting TNF-α by using IFX will increase renal Sirt 1 expression and in turn inhibit TGF-β1 and attenuate the progression of RF. Therefore, a combination of IFX and EMPA would enhance the antifibrotic effect of EMPA against RF.

In the current study, rats received 10% fructose in drinking water for 20 weeks demonstrated marked hyperglycemia, significant elevation of profibrotic cytokines, thickness in the basement membrane of the glomeruli (glomerulosclerosis), and the renal tubules with a loss of tubular brush borders as

well as collagen fibers deposition. These changes concurred with the biochemical features typically seen in RF and in agreement with previous reports [29, 67, 68]. Therefore, our results indicated that fructose-fed rats could be a perfect model for studying the pathological mechanisms of RF and the therapeutic interventions.

EMPA is a new oral antidiabetic drug, exerts its effect by inhibiting SGLT2 in kidney [69]. This fact was shown in the present results and evidenced by a significant decrease in the glycemic index. EMPA reduces the plasma glucose concentration by preventing the reabsorption of glucose from the S1 segment of proximal convoluted tubule, thereby increasing the urinary glucose excretion [70]. IFX, which is a chimeric (mouse–human) monoclonal Immunoglobulin G1 antibody, binds specifically to TNF-α and prevents its interaction with the TNF-α receptors. It also induces the lysis of activated immune cells and apoptosis in activated macrophages and T cells [71, 72]. IFX has been used to treat several autoimmune and chronic inflammatory diseases such as rheumatoid arthritis, Crohn's disease, and ulcerative colitis [73].

So, the observed marked decrease in serum glucose and insulin levels by IFX mainly through improving the peripheral actions of insulin agreed with previous studies regarding the ability of IFX to restore blood glucose homeostasis [74, 75].

It is known that TNF-α production increases under chronic hyperglycemia conditions. TNF-α affects insulin sensitivity through its ability to decrease the tyrosine kinase activity of insulin receptors in addition to induce delays in insulin-mediated glucose uptake in skeletal muscle [76]. Moreover, IFX decreased renal SGLT2 through autocrine manner. It was reported that blocking renal TNF-α in cultured kidney epithelial cells decreases the renal SGLT2 [77].

The treatment either with EMPA or IFX significantly attenuated RF in consistent with previous studies [78, 79], which is confirmed in our model by a significant decrease in kidney weight, renal TNF-α, TGF-β1 levels, glomerulosclerosis, preserved renal tubule brush border with thin basement membrane, and decrease in percentage of collagen area. These findings render them as potential therapeutic approaches that could prevent or delay the progression of RF. However, the effect of the combined treatments was superior to individual ones.

Several studies demonstrated that TNF-α is critically involved in the pathogenesis of RF [15]. It was reported that TNF-α has a prominent role in glomerular inflammation and fibrosis [18, 80]. Therefore, the inhibitory effect of EMPA on renal TNF-α level appeared to be mediated mainly through the reduction of NF-κB pathway which is involved in TNF-α synthesis [26].

Apart from the anti-inflammatory properties of EMPA, several studies have shown that EMPA could reduce TGF-β1, which is a key fibrogenic cytokine, promoting RF by increasing the ECM accumulation [12]. It is known that hyperglycaemia increases the formation of AGEs, which provokes the production of proinflammatory and profibrotic cytokines such as TGF-β1 [81]. So, EMPA decreased hyperglycemia and in turn the induced TGF-β1. Supportively, it was reported that EMPA was able to reduce the high glucose-induced tubular expression of inflammatory and fibrotic markers in a previous in vitro study [82].

It was reported that TNF-α signals are required for the endothelial cells to release TGF-β1 [80]. Additionally, macrophages and fibroblasts are stimulated by TNF-α for producing TGF-β1 [83]. As for IFX, the significant decrease in renal TNF-α and TGF-β1 contents was likely due to the specific binding of IFX to TNF-α, preventing its binding with receptors [84] and consequently decreased TGF-β1 synthesis [20].

A key finding of the current study was the significant decrease in renal Sirt 1 in IR rats, which increased significantly after treatment with EMPA, IFX, or the combined therapy. Interestingly, the effect of the combination of EMPA and IFX on the upregulation of renal Sirt 1 was more remarkable than that of EMPA alone. Accumulating evidences suggested the inhibitory role of IR on Sirt 1 [85]. The present results showed that EMPA effectively inhibited SGLT2 and in turn upregulated Sirt 1 expression in IR. This is in agreement with previous study [86] suggesting that EMPA could serve as Sirt 1 upregulator beside its hypoglycemic effect. Interestingly, more elevation in renal Sirt 1 expression was observed in IFX-treated group over EMPA-treated group, which might be attributed to the remarkable inhibition of TNF-α by IFX more than EMPA.

Conclusion

Collectively, our findings confirmed the protective role of EMPA and IFX against RF, which might broaden their therapeutic effect. This work highlighted EMPA/IFX combination as a promising therapeutic strategy to ameliorate RF associated with IR. The TNF-α inhibiting effect of IFX and the upregulation of renal Sirt 1 expression by IFX and also by EMPA through blocking SGLT2 offers additional benefits against RF. Surely, further experimental and clinical studied are required to certify these results.

Study limitations

In this study, non-significant improvement in kidney function was observed after both treatments, which might be due to the short duration of our treatment model or small dose used regarding IFX. Therefore, further investigations with longer treatment duration and higher doses of IFX should be carried out to verify our results.

Author contributions HM: conception and design of the research, revision of the manuscript, and gave final approval. MA: conception and design of the research, revision of the manuscript, and gave final approval. MK: data analysis, interpretation of results, and manuscript editing. RH: carried out the histological examination. YM: carried out the experiments, data analysis, interpretation of results, and writing the drafted manuscript. All authors revised and approved the final manuscript for submission.

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Compliance with ethical standards

Conflicts of interest The authors declare that they have no conflict of interest.

References

1. Nistala R, Whaley-Connell A (2013) Resistance to insulin and kidney disease in the cardiorenal metabolic syndrome; role for angiotensin II. Mol Cell Endocrinol 378:53–58
2. Artunc F, Schleicher E, Weigert C, Fritsche A, Stefan N, Haering H-U (2016) The impact of insulin resistance on the kidney and vasculature. Nat Rev Nephrol 12:721
3. De Cosmo S, Menzaghi C, Prudente S, Trischitta V (2012) Role of insulin resistance in kidney dysfunction: insights into the mechanism and epidemiological evidence. Nephrol Dial Transplant 28:29–36
4. Declèves A-E, Sharma K (2015) Obesity and kidney disease: differential effects of obesity on adipose tissue and kidney inflammation and fibrosis. Curr Opin Nephrol Hypertens 24:28
5. Dronavalli S, Duka I, Bakris GL (2008) The pathogenesis of diabetic nephropathy. Nat Rev Endocrinol 4:444
6. Gersch MS, Mu W, Cirillo P, Reungjui S, Zhang L, Roncal C, Sautin YY, Johnson RJ, Nakagawa T (2007) Fructose, but not dextrose, accelerates the progression of chronic kidney disease. Am J Physiol Ren Physiol 293:F1256–F1261
7. Palanisamy N, Kannappan S, Anuradha CV (2011) Genistein modulates NF-κB-associated renal inflammation, fibrosis and podocyte abnormalities in fructose-fed rats. Eur J Pharmacol 667:355–364
8. Oudot C, Lajoix AD, Jover B, Rugale C (2013) Dietary sodium restriction prevents kidney damage in high fructose-fed rats. Kidney Int 83:674–683
9. Qiao Y, Xu L, Tao X, Yin L, Qi Y, Xu Y, Han X, Tang Z, Ma X, Liu K (2018) Protective effects of dioscin against fructose-induced renal damage via adjusting Sirt3-mediated oxidative stress, fibrosis, lipid metabolism and inflammation. Toxicol Lett 284:37–45
10. Yu J, Mao S, Zhang Y, Gong W, Jia Z, Huang S, Zhang A (2016) MnTBAP therapy attenuates renal fibrosis in mice with 5/6 nephrectomy. Oxidative Med Cell Longev. https://doi.org/10.1155/2016/7496930
11. Meng X-M, Tang PM-K, Li J, Lan HY (2015) TGF-β/Smad signaling in renal fibrosis. Front Physiol 6:82
12. Lan H (2011) Diverse roles of TGF-β/Smads in renal fibrosis and inflammation. Int J Biol Sci 7:1056
13. Huang XZ, Wen D, Zhang M, Xie Q, Ma L, Guan Y, Ren Y, Chen J, Hao CM (2014) Sirt1 activation ameliorates renal fibrosis by inhibiting the TGF-β/Smad3 pathway. J Cell Biochem 115:996–1005
14. Liu Y (2006) Renal fibrosis: new insights into the pathogenesis and therapeutics. Kidney Int 69:213–217
15. Lv W, Booz GW, Wang Y, Fan F, Roman RJ (2018) Inflammation and renal fibrosis: recent developments on key signaling molecules as potential therapeutic targets. Eur J Pharmacol 820:65–76
16. Vielhauer V, Mayadas TN (2007) Functions of TNF and its receptors in renal disease: distinct roles in inflammatory tissue injury and immune regulation. Semin Nephrol 27:286–308
17. Lee S-Y, Kim SI, Choi ME (2015) Therapeutic targets for treating fibrotic kidney diseases. Transl Res 165:512–530
18. Idasiak-Piechocka I, Oko A, Pawliczak E, Kaczmarek E, Czekalski S (2010) Urinary excretion of soluble tumour necrosis factor receptor 1 as a marker of increased risk of progressive kidney function deterioration in patients with primary chronic glomerulonephritis. Nephrol Dial Transplant 25:3948–3956
19. Braun J, Deodhar A, Dijkmans B, Geusens P, Sieper J, Williamson P, Xu W, Visvanathan S, Baker D, Goldstein N (2008) Efficacy and safety of infliximab in patients with ankylosing spondylitis over a two-year period. Arthritis Care Res Off J Am Coll Rheumatol 59:1270–1278
20. Altintas N, Erboga M, Aktas C, Bilir B, Aydin M, Sengul A, Ates Z, Topcu B, Gurel A (2016) Protective effect of infliximab, a tumor necrosis factor-alfa inhibitor, on bleomycin-induced lung fibrosis in rats. Inflammation 39:65–78
21. Zhang H, Sui J-N, Gao L, Guo J (2018) Subcutaneous administration of infliximab-attenuated silica-induced lung fibrosis. Int J Occup Med Environ Health 31:503–515
22. Meng X-M, Zhang Y, Huang X-R, Ren G-L, Li J, Lan HY (2015) Treatment of renal fibrosis by rebalancing TGF-β/Smad signaling with the combination of asiatic acid and naringenin. Oncotarget 6:36984
23. Satirapoj B (2017) Sodium-glucose cotransporter 2 inhibitors with renoprotective effects. Kidney Dis 3:24–32
24. Kawanami D, Matoba K, Takeda Y, Nagai Y, Akamine T, Yokota T, Sango K, Utsunomiya K (2017) SGLT2 inhibitors as a therapeutic option for diabetic nephropathy. Int J Mol Sci 18:1083
25. Abbas NA, Salem AE, Awad MM (2018) Empagliflozin, SGLT 2 inhibitor, attenuates renal fibrosis in rats exposed to unilateral ureteric obstruction: potential role of Klotho expression. Naunyn-Schmiedeberg's Arch Pharmacol 391:1347–1360
26. Jigheh ZA, Haghjo AG, Argani H, Roshangar L, Rashtchizadeh N, Sanajou D, Ahmad SNS, Rashedi J, Dastmalchi S, Abbasi MM (2019) Empagliflozin alleviates renal inflammation and oxidative stress in streptozotocin-induced diabetic rats partly by repressing HMGB1-TLR4 receptor axis. Iran J Basic Med Sci 22:384
27. Wakino S, Itoh H (2018) High basolateral glucose increases sodium-glucose cotransporter 2 and reduces sirtuin-1 in renal tubules through glucose transporter-2 detection. Sci Rep 8:6791
28. Xie J, Zhang X, Zhang L (2013) Negative regulation of inflammation by SIRT1. Pharmacol Res 67:60–67
29. Sanghavi M, Vajir M, Kumar S, Tikoo K (2015) NFAT inhibitor tributylhexadecylphosphoniumbromide, ameliorates high fructose induced insulin resistance and nephropathy. Chemicobiol Interact 240:268–277
30. Emoto M, Nishizawa Y, Maekawa K, Hiura Y, Kanda H, Kawagishi T, Shoji T, Okuno Y, Morii H (1999) Homeostasis model assessment as a clinical index of insulin resistance in type 2 diabetic patients treated with sulfonylureas. Diabetes Care 22:818–822
31. Vickers SP, Cheetham SC, Headland KR, Dickinson K, Grempler R, Mayoux E, Mark M, Klein T (2014) Combination of the sodium-glucose cotransporter-2 inhibitor empagliflozin with orlistat or sibutramine further improves the body-weight reduction and glucose homeostasis of obese rats fed a cafeteria diet. Diabetes Metab Syndr Obesity Targets Ther 7:265

32. Tasdemir C, Tasdemir S, Vardi N, Ates B, Parlakpinar H, Kati B, Karaaslan MG, Acet A (2012) Protective effect of infliximab on ischemia/reperfusion-induced damage in rat kidney. Ren Fail 34:1144–1149
33. Barbuio R, Milanski M, Bertolo MB, Saad MJ, Velloso LA (2007) Infliximab reverses steatosis and improves insulin signal transduction in liver of rats fed a high-fat diet. J Endocrinol 194:539–550
34. Yin Q, Ma Y, Hong Y, Hou X, Chen J, Shen C, Sun M, Shang Y, Dong S, Zeng Z (2014) Lycopene attenuates insulin signaling deficits, oxidative stress, neuroinflammation, and cognitive impairment in fructose-drinking insulin resistant rats. Neuropharmacology 86:389–396
35. Schmittgen TD, Livak KJ (2008) Analyzing real-time PCR data by the comparative C T method. Nat Protoc 3:1101
36. Bancroft JD, Stevens A (1990) Theory and practice of histological techniques. Churchill Livingstone, Edinburgh
37. Wang S, Yang S, Zhao X, Chen F, Shi J (2017) Expression of the Wnt/β-catenin signal pathway in patients with acute renal injury. Eur Rev Med Pharmacol Sci 21:4661–4667
38. Souza AC, Tsuji T, Baranova IN, Bocharov AV, Wilkins KJ, Street JM, Alvarez-Prats A, Hu X, Eggerman T, Yuen PS (2015) TLR 4 mutant mice are protected from renal fibrosis and chronic kidney disease progression. Physiol Rep 3:e12558
39. Drury RA, Wallington EA (1980) Histological techniques, 5th edn. Oxford University Press, Oxford, pp 27–32
40. Mohamad HE, Askar ME, Hafez MM (2011) Management of cardiac fibrosis in diabetic rats; the role of peroxisome proliferator activated receptor gamma (PPAR-gamma) and calcium channel blockers (CCBs). Diabetol Metab Syndr 3(4):1–12
41. Singh B, Saxena A (2010) Surrogate markers of insulin resistance: a review. World J Diabetes 1:36
42. Groop P-H, Forsblom C, Thomas MC (2005) Mechanisms of disease: pathway-selective insulin resistance and microvascular complications of diabetes. Nat Rev Endocrinol 1:100
43. Ferrannini E, Nannipieri M (2000) Effects of insulin on the kidney and the cardiovascular system. The kidney and hypertension in diabetes mellitus. Springer, New York, pp 141–153
44. Liao M-T, Sung C-C, Hung K-C, Wu C-C, Lo L, Lu K-C (2012) Insulin resistance in patients with chronic kidney disease. Biomed Res Int. https://doi.org/10.1155/2012/691369
45. Lazar MA (2006) The humoral side of insulin resistance. Nat Medi 12:43
46. Padiya R, Chowdhury D, Borkar R, Srinivas R, Bhadra MP, Banerjee SK (2014) Garlic attenuates cardiac oxidative stress via activation of PI3K/AKT/Nrf2-Keap1 pathway in fructose-fed diabetic rat. PLoS ONE 9:e94228
47. Kelany ME, Hakami TM, Omar AH (2016) Curcumin improves the metabolic syndrome in high-fructose-diet-fed rats: role of TNF-α, NF-κB, and oxidative stress. Can J Physiol Pharmacol 95:140–150
48. Tan AL, Forbes JM, Cooper ME (2007) AGE, RAGE, and ROS in diabetic nephropathy. Semin Nephrol 27:130–143
49. Sudamrao Garud M, Anant Kulkarni Y (2014) Hyperglycemia to nephropathy via transforming growth factor beta. Curr Diabetes Rev 10:182–189
50. Zabolotny JM, Kim Y-B (2007) Silencing insulin resistance through SIRT1. Cell Metab 6:247–249
51. de Kreutzenberg SV, Ceolotto G, Papparella I, Bortoluzzi A, Semplicini A, Dalla Man C, Cobelli C, Fadini GP, Avogaro A (2010) Downregulation of the longevity-associated protein sirtuin 1 in insulin resistance and metabolic syndrome: potential biochemical mechanisms. Diabetes 59:1006–1015
52. Gillum MP, Kotas ME, Erion DM, Kursawe R, Chatterjee P, Nead KT, Muise ES, Hsiao JJ, Frederick DW, Yonemitsu S (2011) SirT1 regulates adipose tissue inflammation. Diabetes 60:3235–3245
53. Fröjdö S, Durand C, Molin L, Carey AL, El-Osta A, Kingwell BA, Febbraio MA, Solari F, Vidal H, Pirola L (2011) Phosphoinositide 3-kinase as a novel functional target for the regulation of the insulin signaling pathway by SIRT1. Mol Cell Endocrinol 335:166–176
54. Spoto B, Pisano A, Zoccali C (2016) Insulin resistance in chronic kidney disease: a systematic review. Am J Physiol Ren Physiol 311:F1087–F1108
55. Hirabara SM, Gorjao R, Vinolo MA, Rodrigues AC, Nachbar RT, Curi R (2012) Molecular targets related to inflammation and insulin resistance and potential interventions. Biomed Res Int. https://doi.org/10.1155/2012/379024
56. Song Z, Wang H, Zhu L, Han M, Gao Y, Du Y, Wen Y (2015) Curcumin improves high glucose-induced INS-1 cell insulin resistance via activation of insulin signaling. Food Funct 6:461–469
57. Rahman MM, McFadden G (2011) Modulation of NF-κB signalling by microbial pathogens. Nat Rev Microbiol 9:291
58. Kauppinen A, Suuronen T, Ojala J, Kaarniranta K, Salminen A (2013) Antagonistic crosstalk between NF-κB and SIRT1 in the regulation of inflammation and metabolic disorders. Cell Signal 25:1939–1948
59. Yeung F, Hoberg JE, Ramsey CS, Keller MD, Jones DR, Frye RA, Mayo MW (2004) Modulation of NF-κB-dependent transcription and cell survival by the SIRT1 deacetylase. EMBO J 23:2369–2380
60. Du G, Song Y, Zhang T, Ma L, Bian N, Chen X, Feng J, Chang Q, Li Z (2014) Simvastatin attenuates TNF-α-induced apoptosis in endothelial progenitor cells via the upregulation of SIRT1. Int J Mol Med 34:177–182
61. Caruso R, Marafini I, Franzè E, Stolfi C, Zorzi F, Monteleone I, Caprioli F, Colantoni A, Sarra M, Sedda S (2014) Defective expression of SIRT1 contributes to sustain inflammatory pathways in the gut. Mucosal Immunol 7:1467
62. Simic P, Williams EO, Bell EL, Gong JJ, Bonkowski M, Guarente L (2013) SIRT1 suppresses the epithelial-to-mesenchymal transition in cancer metastasis and organ fibrosis. Cell Rep 3:1175–1186
63. García-Vizcaíno EM, Liarte S, Alonso-Romero JL, Nicolás FJ (2017) Sirt1 interaction with active Smad2 modulates transforming growth factor-β regulated transcription. Cell Commun Signal 15:50
64. Roberts AB, Tian F, Byfield SD, Stuelten C, Ooshima A, Saika S, Flanders KC (2006) Smad3 is key to TGF-β-mediated epithelial-to-mesenchymal transition, fibrosis, tumor suppression and metastasis. Cytokine Growth Factor Rev 17:19–27
65. Loboda A, Sobczak M, Jozkowicz A, Dulak J (2016) TGF-β1/Smads and miR-21 in renal fibrosis and inflammation. Mediat Inflamm. https://doi.org/10.1155/2016/8319283
66. Nogueira A, Pires MJ, Oliveira PA (2017) Pathophysiological mechanisms of renal fibrosis: a review of animal models and therapeutic strategies. Vivo 31:1–22
67. Yang M, Liu C, Jiang J, Zuo G, Lin X, Yamahara J, Wang J, Li Y (2014) Ginger extract diminishes chronic fructose consumption-induced kidney injury through suppression of renal overexpression of proinflammatory cytokines in rats. BMC Complement Altern Med 14:174
68. Sutariya B, Saraf M (2017) Betanin, isolated from fruits of *Opuntia elatior* Mill attenuates renal fibrosis in diabetic rats through regulating oxidative stress and TGF-β pathway. J Ethnopharmacol 198:432–443
69. Byrne NJ, Parajuli N, Levasseur JL, Boisvenue J, Beker DL, Masson G, Fedak PW, Verma S, Dyck JR (2017) Empagliflozin prevents worsening of cardiac function in an experimental model of pressure overload-induced heart failure. JACC Basic Transl Sci 2:347–354

70. Singh HP, Kaur I, Sharma G (2015) Sodium glucose co-transporter-2 (SGLT2) inhibitors as a new class of anti-diabetic drugs: pharmacokinetics, efficacy and clinical significance. Int J Pharm Sci Rev Res 33(1):40–47
71. Di Paola R, Genovese T, Impellizzeri D, Ahmad A, Cuzzocrea S, Esposito E (2013) The renal injury and inflammation caused by ischemia–reperfusion are reduced by genetic inhibition of TNF-αR1: a comparison with infliximab treatment. Eur J Pharmacol 700:134–146
72. Ma X, Xu S (2013) TNF inhibitor therapy for rheumatoid arthritis. Biomed Rep 1:177–184
73. Méndez-García LA, Trejo-Millán F, Martínez-Reyes CP, Manjarrez-Reyna AN, Esquivel-Velázquez M, Melendez-Mier G, Islas-Andrade S, Rojas-Bernabé A, Kzhyshkowska J, Escobedo G (2018) Infliximab ameliorates tumor necrosis factor-alpha-induced insulin resistance by attenuating PTP 1B activation in 3T3L1 adipocytes in vitro. Scand J Immunol 88:e12716
74. Araujo EP, De Souza CT, Ueno M, Cintra DE, Bertolo MB, Carvalheira JB, Saad MJ, Velloso LA (2007) Infliximab restores glucose homeostasis in an animal model of diet-induced obesity and diabetes. Endocrinology 148:5991–5997
75. Burska AN, Sakthiswary R, Sattar N (2015) Effects of tumour necrosis factor antagonists on insulin sensitivity/resistance in rheumatoid arthritis: a systematic review and meta-analysis. PLoS ONE 10:e0128889
76. Miranda-Filloy J, Llorca J, Carnero-López B, González-Juanatey C, Blanco R, González-Gay M (2012) TNF-alpha antagonist therapy improves insulin sensitivity in non-diabetic ankylosing spondylitis patients. Clin Exp Rheumatol 30:850–855
77. Maldonado-Cervantes M, Galicia O, Moreno-Jaime B, Zapata-Morales J, Montoya-Contreras A, Bautista-Perez R, Martinez-Morales F (2012) Autocrine modulation of glucose transporter SGLT2 by IL-6 and TNF-α in LLC-PK 1 cells. J Physiol Biochem 68:411–420
78. Ojima A, Matsui T, Nishino Y, Nakamura N, Yamagishi S (2015) Empagliflozin, an inhibitor of sodium-glucose cotransporter 2 exerts anti-inflammatory and antifibrotic effects on experimental diabetic nephropathy partly by suppressing AGEs-receptor axis. Horm Metab Res 47:686–692
79. Gallo LA, Ward MS, Fotheringham AK, Zhuang A, Borg DJ, Flemming NB, Harvie BM, Kinneally TL, Yeh S-M, McCarthy DA (2016) Once daily administration of the SGLT2 inhibitor, empagliflozin, attenuates markers of renal fibrosis without improving albuminuria in diabetic db/db mice. Sci Rep 6:26428
80. Khasnis AA, Calabrese LH (2010) Tumor necrosis factor inhibitors and lung disease: a paradox of efficacy and risk. Semin Arthritis Rheum 40:147–163
81. Komala MG, Gross S, Mudaliar H, Huang C, Pegg K, Mather A, Shen S, Pollock CA, Panchapakesan U (2014) Inhibition of kidney proximal tubular glucose reabsorption does not prevent against diabetic nephropathy in type 1 diabetic eNOS knockout mice. PLoS ONE 9:e108994
82. Panchapakesan U, Pegg K, Gross S, Komala MG, Mudaliar H, Forbes J, Pollock C, Mather A (2013) Effects of SGLT2 inhibition in human kidney proximal tubular cells—renoprotection in diabetic nephropathy? PLoS ONE 8:e54442
83. Oikonomou N, Harokopos V, Zalevsky J, Valavanis C, Kotanidou A, Szymkowski DE, Kollias G, Aidinis V (2006) Soluble TNF mediates the transition from pulmonary inflammation to fibrosis. PLoS ONE 1:e108
84. Lis K, Kuzawińska O, Bałkowiec-Iskra E (2014) Tumor necrosis factor inhibitors—state of knowledge. Arch Med Sci 10:1175
85. Liang F, Kume S, Koya D (2009) SIRT1 and insulin resistance. Nat Rev Endocrinol 5:367
86. Umino H, Hasegawa K, Minakuchi H, Muraoka H, Kawaguchi T, Kanda T, Tokuyama H, Wakino S, Itoh H (2018) High basolateral glucose increases sodium-glucose cotransporter 2 and reduces sirtuin-1 in renal tubules through glucose transporter-2 detection. Sci Rep 8:6791