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## A DUAL COX-2/sEH INHIBITOR IMPROVES THE METABOLIC PROFILE AND REDUCES KIDNEY INJURY IN ZUCKER DIABETIC FATTY RAT

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### Abstract

Cyclooxygenase (COX) and soluble epoxide hydrolase (sEH) inhibitors have therapeutic potential. The present study investigated efficacy of a novel dual acting COX-2/sEH inhibitor, PTUPB in type 2 diabetic Zucker Diabetic Fatty (ZDF) rats. Male ZDF rats were treated with vehicle or PTUPB (10 mg/kg/d, i.p.) for 8 weeks. At the end of the 8-week experimental period, ZDF rats were diabetic (fasting blood glucose, 287±45 mg/dL) compared to Zucker Diabetic Lean rats (ZDL, 99±6 mg/dL), and PTUPB treatment improved glycemic status in ZDF rats (146±6 mg/dL). Kidney injury was evident in ZDF compared to ZDL rats with elevated albuminuria (44±4 vs 4±2 mg/d) and nephrinuria (496±127 vs 16±4 µg/d). Marked renal fibrosis, tubular cast formation and glomerular injury were also present in ZDF compared to ZDL rats. In ZDF rats, PTUPB treatment reduced kidney injury parameters by 30–80% compared to vehicle. The ZDF rats also demonstrated increased inflammation and oxidative stress with elevated levels of urinary monocyte chemoattractant protein-1 excretion (862±300 vs 319±75 ng/d), renal macrophage infiltration (53±2 vs 37±4 /mm<sup>2</sup>) and kidney malondialdehyde/protein ratio (10±1 vs 5±1 µmol/mg). PTUPB treatment decreased these inflammatory and oxidative stress markers in the kidney of ZDF rats by 25–57%. These data demonstrate protective actions of a novel dual acting COX-2/sEH inhibitor on the metabolic abnormalities and kidney function in ZDF rat model of type 2 diabetes.

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### DISCLOSURE

The dual inhibitor is covered under a University of California Patent with Sung Hee Hwang and Bruce D. Hammock. Other authors declared no conflicts of interest, financial or otherwise.

## Keywords

eicosanoid; diabetes; diabetic nephropathy; inflammation; oxidative stress

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## INTRODUCTION

The incidence of diabetic nephropathy continues to increase worldwide in association with the epidemic rise in diabetes [1]. Approximately 20–40% of all diabetic patients develop diabetic kidney disease in their life time, which often progress to end-stage kidney failure [2,3]. In the United States, diabetic kidney disease is now the major cause of end-stage kidney disease and is associated with co-morbid cardiovascular complications [4].

The mechanisms responsible for the pathophysiology of diabetic nephropathy are complex; however, it is recognized that multiple risk factors interact synergistically during the development of diabetic nephropathy [5,6]. Therefore, there is interest in developing novel therapeutic strategies that target multiple risk factors associated with diabetic kidney diseases in diabetic patients [7]. To improve the patients' quality of life these new therapeutic strategies should slow the progression of diabetic kidney disease, and aim to reduce all causes of morbidity and diabetes-associated mortality [8].

Arachidonic acid metabolism is one pathway that can be targeted to develop a novel therapy for diabetic kidney diseases. In support of this approach, products of arachidonic acid metabolism have been associated with the pathophysiology of metabolic syndrome and diabetes [9,10]. Epoxygenase metabolites of arachidonic acid, epoxyeicosatrienoic acids (EETs) are decreased and cyclooxygenase (COX-2) metabolites are increased in patients with metabolic syndrome and type 2 diabetes [11–13]. COX-2 metabolites have also been implicated in renal injury that occurs in animal models and patients with type 2 diabetes and metabolic syndrome [7,14,15]. COX-2 derived metabolites are increased in these conditions and contribute to glomerular matrix deposition and stimulate glomerular mesangial cell expansion [16,17]. Accordingly, COX-2 inhibition decreases renal injury in animal models of metabolic syndrome and type 2 diabetes [12,18,19]. Unlike COX-2 metabolites, the decrease in epoxygenase metabolites of arachidonic acids that occur in animal models of metabolic syndrome and type 2 diabetes contributes to renal injury [13,16]. EETs have multiple biological actions that are important for kidney protection, and the biological activity of EETs can be enhanced by attenuating their metabolism by soluble epoxide hydrolase (sEH) [20]. Indeed, sEH inhibition decreases inflammation, renal injury, and improve pancreatic function in animal models of hypertension, inflammation, cardiovascular disease, and diabetes [20–22].

Since selective inhibition of either COX or epoxygenase provides beneficial outcomes in models of hypertension, inflammation, cardiovascular disease and diabetes, we developed a novel dual-acting COX-2/sEH inhibitor, PTUPB [4-(5-phenyl-3-{3-[3-(4-trifluoromethylphenyl)-ureido]-propyl}-pyrazol-1-yl)-benzenesulfonamide]. PTUPB has biological activity, as it has recently been shown to have a beneficial action in an animal model of tumor suppression and metastasis [23]. In the present study, we tested the hypothesis that simultaneously inhibiting COX-2 and sEH pathways of eicosanoid

metabolism by PTUPB would be efficacious in attenuating disease using a rat model of type 2 diabetes and metabolic syndrome. We demonstrate that PTUPB markedly prevented the development of metabolic abnormalities and diabetic kidney injury in type 2 diabetic ZDF rat model.

## MATERIAL AND METHOD

### Chemicals

The chemistry and synthesis process dual COX-2/sEH inhibitor, 4-(5-phenyl-3-{3-[3-(4-trifluoromethylphenyl)-ureido]-propyl}-pyrazol-1-yl)-benzenesulfonamide (PTUPB) described earlier [23]. All chemicals used in this study are purchased from Sigma Aldrich (St Louis, MO, USA) unless and otherwise mentioned.

### Animal Groups

The Medical College of Wisconsin Institutional Animal Care and Use Committee that conforms to the National Institutes of Health Guidelines for Care and Use of Laboratory Animals approved all animal studies. Male obese Zucker Diabetic Fatty (ZDF, strain code 370) and Zucker Lean (ZDL, Lean +/-, strain code 371) rats are obtained from Charles River Laboratories (Wilmington, MA, USA). Animals were housed in the Biomedical Resource Center at the Medical College of Wisconsin with a 12/12h light–dark cycle and free access to water and rodent chow. ZDL rats (n=6) were used as the control group. ZDF rats were divided into two groups. Vehicle treated ZDF group (n=6) received vehicle and treated group received PTUPB (10mg/kg/d, n=6) for 8 weeks. Both vehicle and PTUPB were administered continuously for the 8-week experimental period by intra-peritoneal osmotic pump (ALZET® osmotic pump, DURECT Corporation, Cupertino, CA). All rats were weighed and systolic blood pressure was measured by tail-cuff plethysmography (IITC Life Science Inc., Woodland Hills, CA, USA) after 8 weeks of the treatment protocol.

### Glucose Tolerance Test

Intra-peritoneal glucose tolerance test was carried out at the end of the 8-week treatment protocol in rats that were fasted overnight and injected with glucose (2 g/kg i.p.). Blood samples were collected from the tail vein before and at different time points after glucose injection. The tail vein blood glucose levels were measured using a glucometer LifeScan (Miltipas, CA, USA).

### Urine and Plasma Biochemical Analysis

Urine and plasma samples were collected at the end of the 8-week experimental period. Serum and urinary biochemical analysis were carried out by colorimetric and ELISA assays. Triglyceride, cholesterol, protein and creatinine assay kits were from Cayman (Ann Arbor, MI, USA), albumin and nephrin assay kits were from Exocell (Philadelphia, PA, USA), and monocyte chemoattractant protein-1 (MCP-1) assay kit was from BD Biosciences (San Jose, CA, USA). Serum insulin and C-peptide were measured using ELISA (Mercodia AB, Uppsala, Sweden). Serum glucose was measured using glucose oxidase method (abcam, Cambridge, MA, USA). Homeostatic index of insulin resistance (HOMA-IR) calculated according to the homeostasis of the assessment as described earlier [24]. To determine the

kidney tissue malondialdehyde (MDA) level, the rat kidney was homogenized with buffer containing 1.5% potassium chloride to obtain a 1:10 (w/v) whole kidney homogenate. MDA was measured using colorimetric method after reaction with thiobarbituric acid. Kidney tissue MDA was measured in the kidney using a commercially available kit (Cayman Chemical).

### Histopathological Analysis

The kidney and pancreas were excised and immersion-fixed in 10% neutral buffered formalin and paraffin embedded. The embedded kidney and pancreas sections were cut into 4 $\mu$ m slices for use in histology. Formalin-fixed paraffin-embedded tissue slices were deparaffinized, re-hydrated, and kidney tissue slices were stained with Periodic Acid-Schiff (PAS) and Masson's Trichrome. Glomerulosclerosis and mesangial matrix expansion were blindly scored from kidney sections stained with PAS staining using the following numeric scale: 0= no damage; +1 = very mild; +2 = mild; +3 = moderate and +4 = severe. Two observers in a blinded fashion conducted histological analysis at a magnification of X200 using Nikon NIS Elements Software (Nikon Instruments Inc., Melville, NY, USA). Proteinaceous cast in the kidney was also determined in PAS stained kidney sections at magnification of X200 using Nikon NIS Elements Software. The percentage area positive for proteinaceous cast was calculated from the mean of eight cortical and five medullary fields for each animal. Fibrosis in the kidney was determined in kidney sections stained with Masson's Trichrome at a magnification of  $\times$ 200. The percentage area positive for collagen was calculated as fibrotic area from the mean of eight cortical and five medullary fields for each animal. Renal tubular cast and collagen positive fibrotic areas in the kidney sections were determined by two blinded observers. The pancreas slices were stained with Hematoxylin and Eosin staining and gross histological features of the pancreas were studied in different experimental groups in blinded fashion.

### Immunohistopathological Analysis

Formalin-fixed paraffin-embedded kidney slices were deparaffinized, re-hydrated, and subjected to immunohistochemistry. Kidney sections were immunostained with anti-CD68 (1:100; Serotec, Raleigh, NC, USA) to determine macrophage/monocyte infiltration in the kidney. Biotinylated rat anti-mouse secondary antibody (1:200) was used for development with avidin-biotinylated HRP complex (Vectastain ABC Elite kit, Vector Laboratories, Burlingame, CA, USA) followed by counterstaining with hematoxylin and mounted for image capturing. Stained sections were visualized by light microscopy at 400x magnification and digital images of the stained kidney were taken for analysis using Nikon NIS Elements Software (Nikon Instruments Inc., Melville, NY, USA). Macrophage/monocyte infiltration was determined by point counting CD68-positive cells by two experienced blinded reviewers. The number of positive cells per picture was divided by the metric area of the image to obtain the number of positive cells per mm<sup>2</sup>.

### Statistical analysis

Data are expressed as mean SE and were analyzed using one-way ANOVA followed by Tukey's post-hoc test for multiple group comparisons using Prism version 4.0 software by

GraphPad Software Inc. (La Jolla, CA, USA). Statistical significance was assumed at  $P < 0.05$ .

## RESULTS

### Treatment with a dual COX-2/sEH inhibitor improves the metabolic profile of ZDF Rats

The ZDF rats were diabetic with fasting blood glucose ( $287 \pm 45$  mg/dL) and glucose intolerance, while ZDL rats were normoglycemic ( $99 \pm 6$  mg/dL). The dual COX-2/sEH inhibitor PTUPB lowered fasting blood glucose and improved glucose tolerance in ZDF rats ( $146 \pm 6.2$  mg/dL) (Figure 1A). The ZDF rats also had insulin resistance with a higher glucose area under the curve and higher HOMA-IR compared to ZDL rats, and PTUPB reduced insulin resistance in the ZDF rats (Figure 1B,C). The ZDF rats also had gross morphological changes in islet morphology characterized by irregular islet borders and evidence of endocrine cell death. The morphological changes observed in ZDF islets are largely prevented in ZDF rats treated with PTUPB (Figure 1D). The ZDF rats ( $461 \pm 11$  g) had higher body weight compared to ZDL ( $319 \pm 13$  g) rats, and treatment with PTUPB did not modify the body weight of ZDF rats ( $468 \pm 9$  g). In contrast, PTUPB administration attenuates the elevated systolic blood pressure of ZDF rats ( $177 \pm 16$  mmHg, ZDF;  $125 \pm 17$  mmHg ZDL;  $141 \pm 12$  mmHg treated ZDF). The ZDF rats also had dyslipidemia with elevated levels of cholesterol, triglyceride, free fatty acid, and decreased serum adiponectin compared to ZDL rats, PTUPB reduced dyslipidemia in ZDF rats (Table 1). These findings indicate that PTUPB administration attenuates the development of glucose intolerance and hyperglycemia, reduced insulin resistance and has anti-lipidemic action in ZDF rats.

### Dual COX-2/sEH inhibition reduces renal inflammation and oxidative stress in ZDF Rats

There is increased renal oxidative stress in ZDF rats with 3 times higher kidney malondialdehyde (MDA) level compared to ZDL rats. PTUPB reduced renal oxidative stress in ZDF rats by reducing kidney MDA levels by 57% (Figure 2A). The ZDF rats also demonstrated marked inflammation with 3 times higher urinary excretion of MCP-1 and 2 times higher infiltration of macrophages/monocytes in the kidney when compared to ZDL rats. PTUPB reduced renal inflammation in ZDF rats as evidenced by a decrease urinary excretion of MCP-1 (50%) and lower levels of macrophage infiltration (25%) (Figure 2B–D). These data indicate that oxidative stress and inflammation in the kidney in ZDF rats can be attenuated using the dual COX-2/sEH inhibitor PTUPB.

### Dual COX-2/sEH inhibitor reduced renal damage in ZDF Rats

The ZDF rats demonstrated marked renal damage with 10 times higher albuminuria compared to ZDL rats. Moreover, the elevated albuminuria was associated with histopathological changes including tubular cast formation, renal interstitial fibrosis and glomerular injury (Figure 3). The dual COX-2/sEH inhibitor PTUPB reduced albuminuria (by 30%) (Fig 3A) and tubular proteinaceous cast formation in the kidney of ZDF rats (Figure 3B–C). PTUPB treatment also reduced interstitial fibrosis in the kidney of the ZDF rats (Figure 4). In addition, PTUPB decreased glomerular injury by reducing nephrinuria (Figure 5A) along with reduction in extracellular matrix formation, glomerular sclerosis, and mesangial expansion in the kidney of ZDF rats (Figure 5B,C). These findings support a

kidney protective action of the dual COX-2/sEH inhibitor PTUPB in terms of its ability to reduce tubular and glomerular injury.

## DISCUSSION

Kidney disease is a major complication in type 2 diabetes, and causes almost half of all cases of kidney failure [26]. There is increasing interest in finding novel therapeutic strategies that target multiple risk factors associated with the complex pathophysiology of metabolic syndrome and type 2 diabetes [7]. An important role of eicosanoid metabolites has been identified in the etiopathology of metabolic syndrome and type 2 diabetes [9,10]. Indeed, eicosanoid metabolites are known to impact multiple factors associated with metabolic syndrome and type 2 diabetes including blood pressure, lipid levels, and insulin signaling in these conditions [27]. In the present study we examined the beneficial actions of a novel molecule that simultaneously targets multiple pathways of arachidonic acid metabolism in type 2 diabetes. We demonstrated that the dual acting COX-2/sEH inhibitor PTUPB markedly ameliorated multiple pathophysiological features of metabolic syndrome and reduced diabetic kidney injury in the ZDF rat, a well recognized metabolic syndrome and type 2 diabetes rat model [28,29].

The PTUPB treatment, which concurrently acts on COX-2 and sEH pathways of arachidonic acid metabolism, ameliorated metabolic abnormalities in ZDF rats. Hyperglycemia, hypertension, and lipid profile in the PTUPB treated ZDF rats were at the levels of control ZDL rats. In regards to the action of PTUPB on the lipid profile, a contribution of sEH pathway in hyperlipidemia as well as the lipid lowering effect of sEH inhibition have been reported in several earlier studies [30,31]. Epidemiological studies have associated sEH polymorphisms with lipid abnormality in human and have shown that the R287Q variant of sEH polymorphisms is associated with increased levels of plasma cholesterol and triglycerides in familial hypercholesterolemia [30]. Moreover, sEH null (*Ephx2*<sup>-/-</sup>) mice have a 25% lower plasma total cholesterol level and 2-fold lower HMG-CoA reductase activity compared to wild type mice [31]. Similar to sEH inhibition, COX-2 inhibition has also been shown to lower lipids [32]. Consistent with our findings, these studies support the view that PTUPB maintained a normal lipid profile in the ZDF rats by acting on COX-2 and sEH pathways of arachidonic acid metabolism.

The current study also demonstrated that PTUPB treatment attenuates hypertension and diabetes in the ZDF rats. Antihypertensive effects of sEH inhibitors are widely reported, and have been attributed to the increase in the ratio of EETs to their less biologically active diols [33–35]. Unlike sEH inhibition, COX-2 inhibition is not antihypertensive [18,36] and did not affect blood pressure in humans and animals [37]. However, several clinical trials have shown that chronic use of COX-2 inhibitors for periods of 6–9 months can induce hypertension in patients [38,39]. Thus, it is likely that the antihypertensive effect of PTUPB in this study are due to its inhibitory action on sEH.

In addition to the antihypertensive action, PTUPB improves glucose tolerance in ZDF rat. This anti-diabetic action of PTUPB can be linked to the effects of sEH inhibition. Indeed, several *in vivo* studies showed that sEH inhibition ameliorated type 2 diabetic phenotypes in

mice [11,23]. Studies also demonstrated that sEH inhibitors improved  $\beta$ -cell function and reduce  $\beta$ -cell death in streptozotocin-induced type 1 diabetic mice [40,41]. It is further reported that epoxygenase enzymes present in human and rat pancreas generate EETs, and EETs stimulate insulin secretion from isolated rat pancreatic islets [42,43]. These studies clearly indicate an important role of sEH inhibition and EETs in the pathophysiology of diabetes, and support the view that sEH inhibition and EETs possess anti-diabetic effect. Unlike sEH inhibition, there is limited evidence regarding COX-2 inhibition and pancreatic function in humans with obesity, type 2 diabetes, and metabolic syndrome. Nonetheless, it is reported that high-dose aspirin ameliorates insulin resistance and improves glucose tolerance in patients with type 2 diabetes [44]. Overall, we demonstrated that PTUPB prevented the development of metabolic abnormalities in type 2 diabetic ZDF rats, and these actions of PTUPB is attributed to its ability to inhibit both sEH and COX-2 pathways of arachidonic acid metabolism. Importantly, these findings indicate that dual acting COX-2/sEH inhibitors have great therapeutic potential in treating/preventing metabolic abnormalities in metabolic syndrome and type 2 diabetes.

One of the leading causes of mortality in diabetic patients is diabetic nephropathy that occurs in 20–40% diabetic patients, and its incidence is increasing dramatically worldwide [45,46]. In the current study we demonstrated that the dual COX-2/sEH inhibitor, PTUPB prevented kidney injury in type 2 diabetic ZDF rat. The kidney protective actions of PTUPB can be attributed to its ability to concurrently inhibit COX-2 and sEH pathways. Indeed, an important pathophysiological role of COX-2 pathway has been reported in diabetic kidney diseases. Up-regulated renal COX-2 expression and elevated urinary excretion of pro-sclerotic COX-2 arachidonic acid metabolites are present in rat models of type 1 and type 2 diabetes [19,47]. COX-2 inhibition reduced renal injury in pre-clinical diabetic models including type 2 diabetic ZDF rat by decreasing glomerular and tubulointerstitial injury mediators in the kidney [18,19]. Apart from the COX-2 pathway, sEH pathway of arachidonic acid metabolism also contributes to the pathophysiology of diabetic kidney injury, and sEH inhibition ameliorated experimental diabetic nephropathy [40,48,49]. These earlier reports along with our data in the present study provide convincing evidence that in type 2 diabetic fatty ZDF rats the novel dual acting COX-2/sEH inhibitor PTUPB prevented the diabetic kidney injury by concurrently acting on COX-2 and sEH pathways.

The kidney injury in ZDF rats was associated with marked renal oxidative stress and inflammation. In several earlier studies, others and we reported such renal oxidative stress and inflammation along with marked kidney injury in ZDF rats [19,50]. Consistent with these earlier findings, there are marked elevation in urinary excretion of MCP-1, renal infiltration of macrophages, and the kidney levels of MDA is elevated in the ZDF rats [19,50]. This is an important finding as elevated oxidative stress and inflammation play important roles in the pathophysiology of diabetic kidney diseases [6,51]. Interestingly, in the present study we demonstrated that the dual acting COX-2/sEH inhibitor PTUPB attenuates renal inflammation and oxidative stress in ZDF rats. These anti-oxidative and anti-inflammatory actions of PTUPB are likely due to the inhibition of both COX-2 and sEH pathways, as inhibitors of each pathway can reduce oxidative stress and inflammation in the kidney [28,37]. Inhibition of sEH has been shown to reduce inflammation and oxidative stress in mouse models of hypertension and diabetes. The sEH null (*Ephx2*<sup>-/-</sup>) mice treated



with deoxycorticosterone acetate plus high salt (DOCA-salt) had markedly lower renal inflammation in terms lower expression of inflammatory genes and less infiltrated macrophages in their kidney compared to similarly treated wild type mice [52]. Moreover, in a renal fibrosis model, sEH inhibition markedly reduced renal oxidative stress and protected the kidney from fibrotic injury by reducing renal lipid peroxidation [53]. In ZDF rats, the diabetic kidney injury associated with elevated renal oxidative stress and inflammation is also ameliorated by COX-2 inhibition [19]. These earlier findings on the contributions of anti-oxidative and anti-inflammatory actions of COX-2 and sEH inhibition support the concept that the marked kidney protective effect of PTUPB in the ZDF rat is linked to its anti-oxidative and anti-inflammatory actions.

## CONCLUSION

The present study investigated the efficacy of a novel dual acting therapeutic that can concurrently inhibit two arachidonic acid metabolic pathways on the development of type 2 diabetes and associated kidney injury. We demonstrate that this novel dual acting COX-2/sEH inhibitor PTUPB attenuates the development of metabolic abnormalities and kidney injury in a rat model of type 2 diabetes. We also provide evidence that correlates the beneficial actions of PTUPB in the ZDF rats with reduced oxidative stress and inflammation in the kidney. The findings support the use of this dual and concurrent inhibitor of COX-2 and sEH as a potential new therapeutic for metabolic syndrome and type 2 diabetes.

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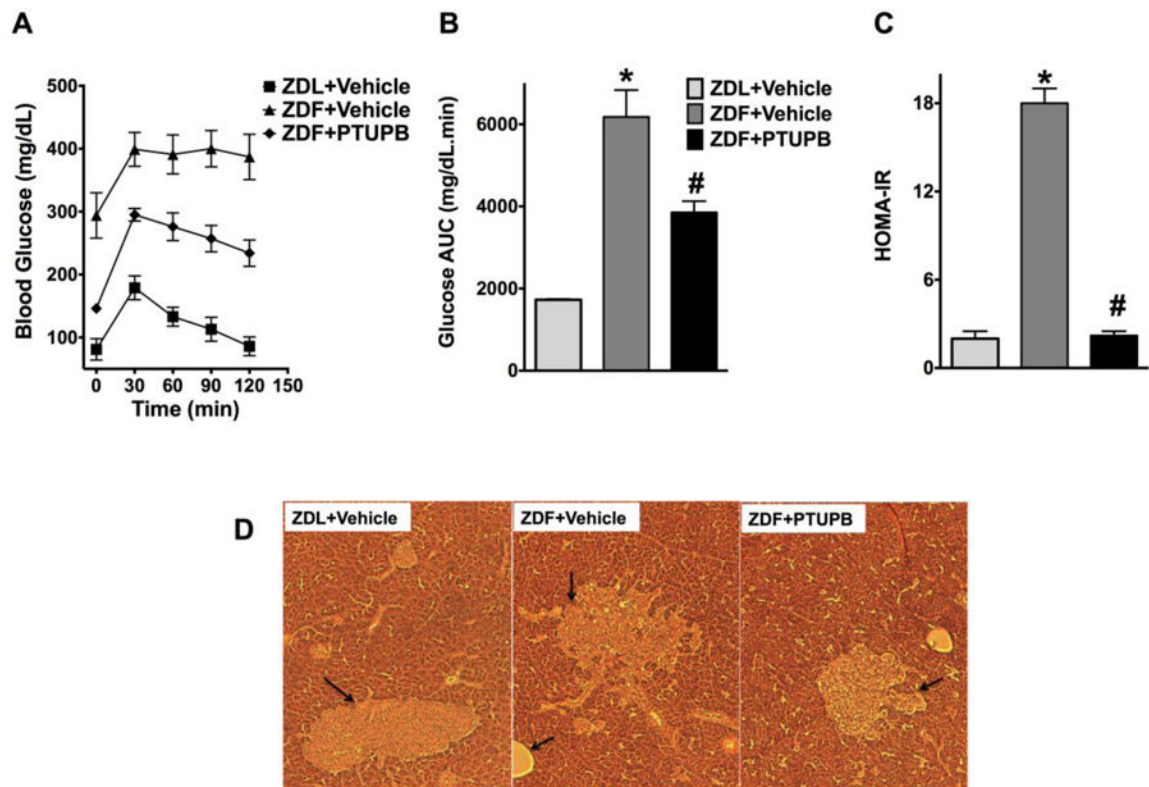
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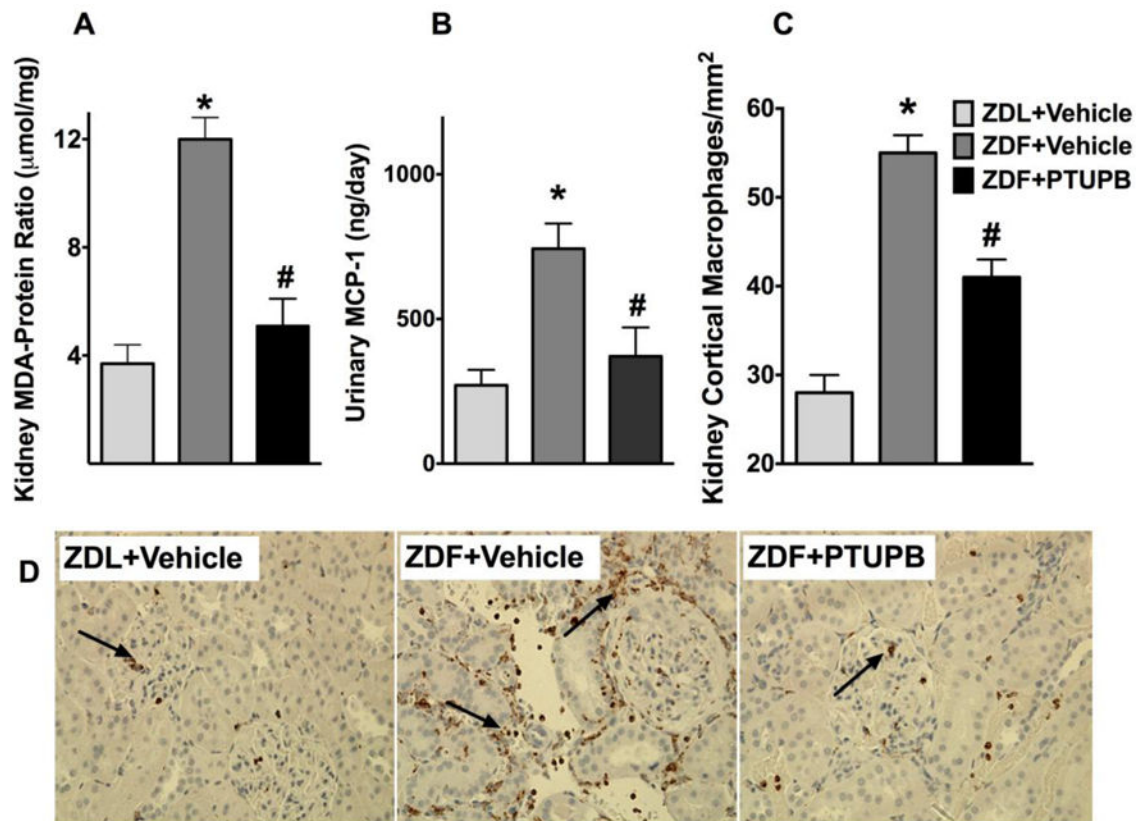
**HIGHLIGHTS**

- A novel dual acting molecule that concurrently inhibits two arachidonic acid metabolic pathways (cyclooxygenase-2 and soluble epoxide hydrolase; PTUPB) was tested for therapeutic actions on type 2 diabetes and the associated kidney injury.
- PTUPB decreased blood glucose and reduced renal oxidative stress and inflammation in the kidney of type 2 diabetic Zucker diabetic fatty rat.
- PTUPB has potential to be developed as new therapeutic for type 2 diabetes and associated diabetic nephropathy.



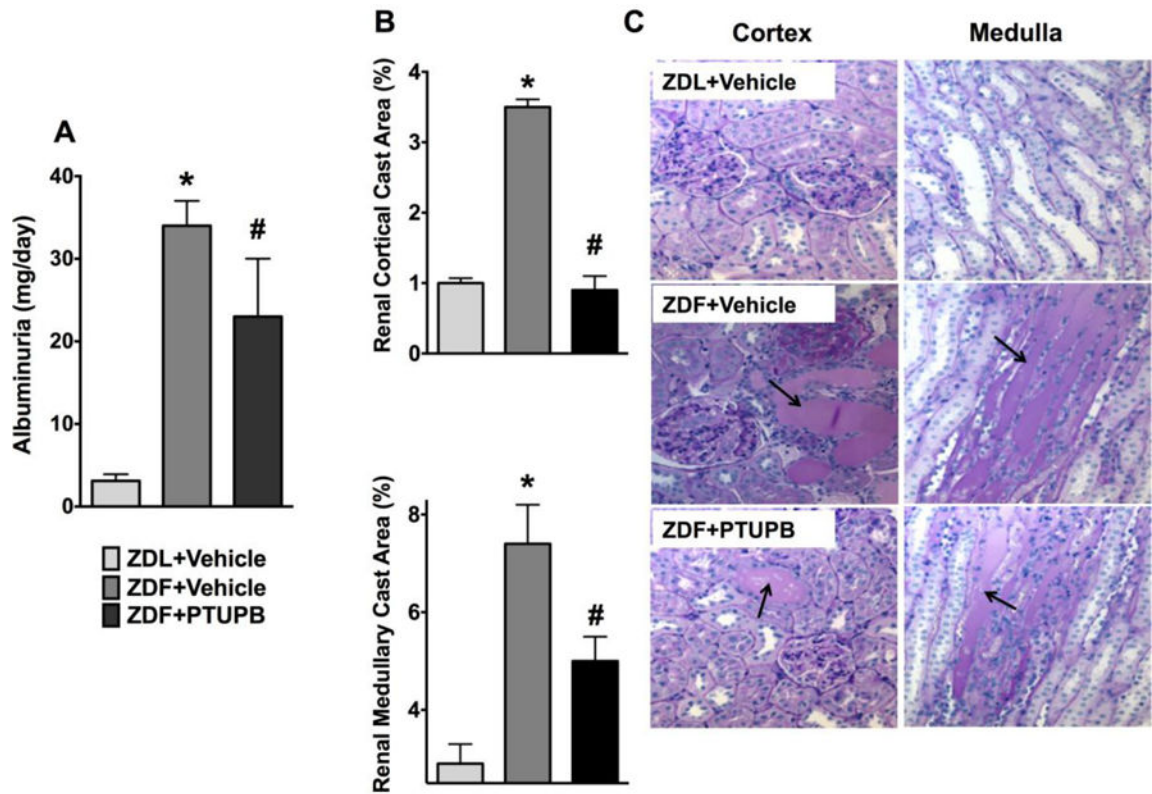
**Figure 1.**

(A) Blood glucose levels during intra-peritoneal glucose tolerance test, (B) glucose area under the curve in Zucker Lean (ZDL) and Zucker Diabetic Fatty rats (ZDF) after 8 weeks of vehicle or the dual COX-2/sEH inhibitor PTUPB treatment. (C) HOMA-IR index as an indicator of the level of insulin resistance in different experimental groups. (D) The histological photomicrograph of pancreas showing general structure of pancreas in different experimental groups. Values are mean  $\pm$  SEM. n=6 rats per group; \*P<0.05 vs. ZDL + Vehicle; #P<0.05 vs. ZDF + Vehicle.



**Figure 2.**

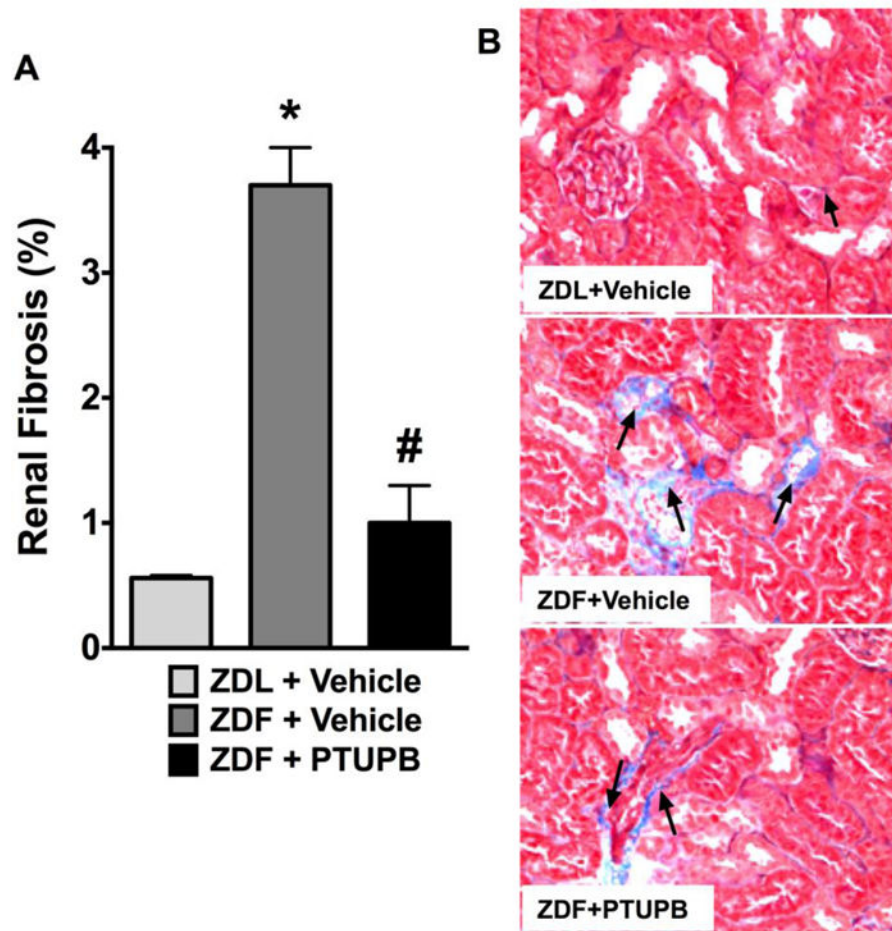
(A) Kidney level of malondialdehyde (MDA) expressed as MDA-Protein ratio, (B) urinary excretion of monocyte chemotactic protein-1 (MCP-1), and (C) macrophage/monocyte levels in the kidney of Zucker Lean (ZDL) and Zucker Diabetic Fatty rats (ZDF) after 8 weeks of vehicle or the dual COX-2/sEH inhibitor PTUPB treatment. (D) A representative photomicrographs ( $\times 200$ ) showing macrophage/monocyte infiltration in the kidney (black arrows) of different experimental groups after 8 weeks of vehicle or PTUPB treatment. Values are mean  $\pm$  SEM.  $n=6$  rats per group; \* $P<0.05$  vs. ZDL + Vehicle; # $P<0.05$  vs. ZDF + Vehicle.



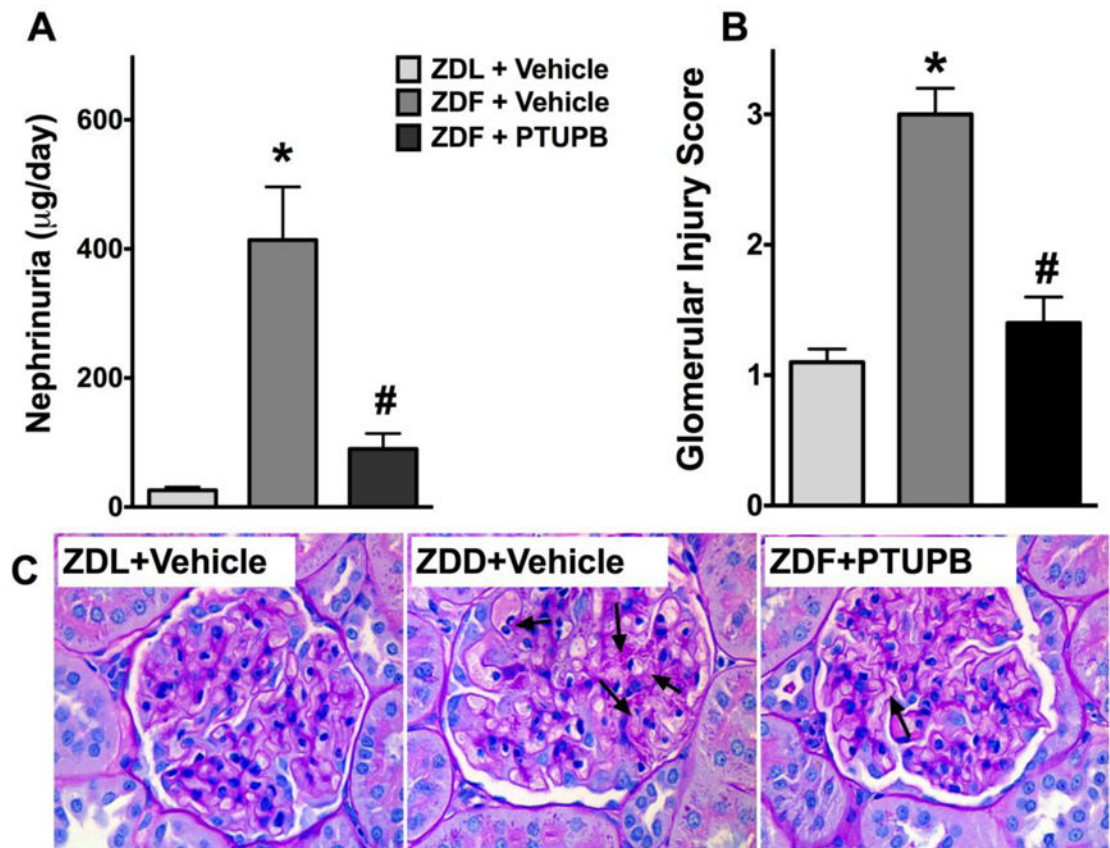
**Figure 3.**

(A) Urinary albumin excretion, (B) semi-quantitative scoring of kidney cast formation in Zucker Lean (ZDL) and Zucker Diabetic Fatty rats (ZDF) after 8 weeks of vehicle or the dual COX-2/sEH inhibitor PTUPB treatment. (C) Representative photomicrographs of Periodic-Acid Schiff staining ( $\times 200$ ) depicting tubular cast formation (black arrows) in the kidney of different experimental groups after 8 weeks of vehicle or PTUPB treatment. Values are mean  $\pm$  SEM.  $n=6$  rats per group; \* $P<0.05$  vs. ZDL + Vehicle; # $P<0.05$  vs. ZDF + Vehicle.





**Figure 4.** (A) Semi-quantitative kidney fibrosis scoring in Zucker Lean (ZDL) and Zucker Diabetic Fatty rats (ZDF) after 8 weeks of vehicle or the dual COX-2/sEH inhibitor PTUPB treatment. (B) Representative photomicrographs of Masson's trichrome staining ( $\times 200$ ) depicting kidney fibrosis (black arrows) in the kidney of different experimental groups after 8 weeks of vehicle or PTUPB treatment. Values are mean  $\pm$  SEM.  $n=6$  rats per group; \* $P<0.05$  vs. ZDL + Vehicle; # $P<0.05$  vs. ZDF + Vehicle.



**Figure 5.**

(A) Urinary nephrin excretion, and (B) semi-quantitative glomerular injury scoring in Zucker Lean (ZDL) and Zucker Diabetic Fatty rats (ZDF) after 8 weeks treatment with vehicle or the dual COX-2/sEH inhibitor PTUPB. (C) Representative photomicrographs of Periodic-Acid Schiff staining ( $\times 200$ ) depicting glomerular injury with mesangial expansion (black arrows) and other changes related to glomerular sclerosis in the kidney of different experimental groups after 8 weeks of vehicle or PTUPB treatment. Values are mean  $\pm$  SEM.  $n=6$  rats per group; \* $P<0.05$  vs. ZDL + Vehicle; # $P<0.05$  vs. ZDF + Vehicle.

**Table 1**

Effects of dual acting COX-2/sEH inhibitor PTUPB on lipid profile of ZDF rat.

Measurement	ZDL+Vehicle	ZDF+Vehicle	ZDF+PTUPB
Serum Triglyceride (mg/dL)	14±2	111±19 <sup>¶</sup>	69±17 <sup>¶, #</sup>
Serum Cholesterol (mM)	2.6±0.6	3.11±0.06 <sup>¶</sup>	2.7±0.03 <sup>¶, #</sup>
Serum Free Fatty Acid (µM)	129±12	675±19 <sup>¶</sup>	253±14 <sup>#</sup>
Serum Adiponectin (µg/mL)	17±2	8.01 <sup>¶</sup>	131 <sup>¶</sup>

Values are mean ± SEM. n=4–6 rats per group;

<sup>¶</sup>P<0.05 vs. ZDL + Vehicle;<sup>#</sup>P<0.05 vs. ZDF + Vehicle.