Effect of fenofibrate and gemfibrozil on kynurenic acid production in rat kidney in vitro: old drugs, new possibilities

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Research Article

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Abstract

Kidney dysfunction significantly increases cardiovascular risk, even in the setting of minor function decline. Hypertriglyceridemia is the most common finding among lipid abnormalities in patients with kidney disorders. PPARα (peroxisome proliferator-activated receptor-α) agonists called fibrates are main agents used to lower triglycerides level.

Kynurenic acid (KYNA) is one of tryptophan (Trp) metabolites, directly formed from L-kynurenine (L-KYN) by kynurenine aminotransferases (KATs). KAT I and KAT II are the best studied KAT isoenzymes. KYNA is classified as a uremic toxin, which level correlates with kidney function decline. High fat diet, known as ketogenic diet, was previously shown to increase KYNA concentration.

Purpose: The aim of this study was to analyze the effect of most commonly used fibrates, fenofibrate and gemfibrozil, on KYNA production and KATs activity in rat kidney in vitro.

Methods: The influence of fenofibrate and gemfibrozil on KYNA synthesis, as well as both KATs activity, was tested in rat kidney homogenates in vitro after 2 hours incubation in the presence of KYNA precursor and selected drug. Each drug was examined at increasing concentrations up to 1 mM. KYNA formation was analyzed through high performance liquid chromatography (HPLC).

Results: Fenofibrate and gemfibrozil significantly decreased KYNA synthesis and both KATs activity in rat kidney in vitro.

Conclusion: Fenofibrate and gemfibrozil decrease KYNA production in rat kidney in vitro through inhibition of KAT I and KAT II isoenzymes. Presented results show novel mechanism of fibrates action in the kidney, indicating potential role of examined drugs in kidney function regulation.

Introduction

Kidney diseases remain major health care problem, related with increased morbidity and mortality. Patients with chronic kidney disease (CKD) have 1.4 higher risk for atherosclerotic cardiovascular diseases and almost 3 times higher risk in the co-presence of diabetes mellitus (Mathew et al. 2021). An inverse relationship between kidney function and cardiovascular risk is known to be independent of age, sex and conventional cardiologic risk factors compared to patients without kidney disorders (Ferro et al. 2018). What is important, significantly higher risk of cardiovascular disease was observed even in patients with minor function decline (Vanholder et al. 2005). Impaired lipoprotein profile, chronic inflammation, endothelial dysfunction, high oxidative stress and accumulation of uremic toxins are main contributors of accelerated atherosclerotic plaques formation (Ghiadoni et al. 2004). Abnormalities in lipid profile in patients with CKD are a consequence of postprandial and triglyceride rich lipoproteins altered degradation (Ferro et al. 2018). Increased level of other proatherogenic factors, like small dense LDL molecules, may be of higher importance than standard particles used to evaluate cardiovascular risk (Florens et al. 2016). Apart from increased incidence of cardiac events, deposition of lipids in the kidney
may lead to direct glomerular injury, together with mesangial cell activation and proliferation (Gyebi et al. 2012). Fibric acid derivatives called fibrates are used in the therapy of hypertriglyceridemia (Kostapanos et al. 2013). Beyond lipid lowering effect fenofibrate presented antiproliferative, anti-inflammatory and antioxidative properties as well as lowered blood pressure in animals models of diabetic nephropathy (Zhao and Li 2008). Similarly, gemfibrozil was shown to improve organ function and oxidative stress level, distinct from hypolipidemic effect in the animal model of aging (Hakimizadeh et al. 2023).

Exogenous aminoacid tryptophan (Trp) is metabolized in the body mainly through kynurenine (KYN) pathway. Most KYN metabolites were analyzed in the context of neurological diseases and were reported as neurotoxic agents (Davidson et al. 2022). Among them kynurenic acid (KYNA) presents exceptional properties. KYNA is synthesized by kynurenine aminotransferases (KATs), from which KAT I and KAT II are the best known KAT isoenzymes. KYNA acts as an antagonist of all three glutamatergic ionotropic receptors, also as an antagonist of α7-nicotinic acetylcholine receptors, agonist of G-protein-coupled receptor (GPR35) and ligand of aryl hydrocarbon receptor (AHR) (DiNatale et al. 2010; Hughes et al. 2022). Based on animal and human studies KYNA was shown as neuroprotective agent, especially in neurodegenerative diseases (Ostapiuk and Urbanska 2022). Additionally, KYNA was reported to induce natriuresis (Bądzyńska et al. 2014) and lower heart rate (Bądzyńska et al. 2020) in spontaneously hypertensive rats. However KYNA is also considered as uremic toxin (Vanholder et al. 2022). In recent studies KYNA was suggested to have antidiabetic properties, with potentially beneficial effect on lipid profile (Koziel and Urbanska 2023). Interestingly, high fat diet known as ketogenic diet was reported to elevate KYNA concentration (Zarnowski et al. 2017; Żarnowska et al. 2019). Since fibrates were shown to affect kidney function (Emami et al. 2020) and may influence KYNA formation, the goal of presented study was to analyze the effect of two the most popular fibrates: fenofibrate and gemfibrozil, on KYNA synthesis and KATs activity in rat kidney in vitro.

**Materials And Methods**

**Animals**

Male Wistar rats (Experimental Medicine Center, Lublin, Poland) weighing 150–200 g, were housed under standard laboratory conditions (12 h light-dark cycle, standard humidity and temperature) with food and water available without limits. Every experimental procedure was performed between 7 a.m. and 1 p.m. All procedures presented in this study were in accordance with the international, national and institutional guidelines for the care and use of animals.

**Chemical substances**

L-kynurenine (sulfate salt), fenofibrate, gemfibrozil, dimethyl sulfoxide (DMSO), components of Krebs-Ringer buffer: sodium chloride, potassium chloride, magnesium sulfate heptahydrate, calcium chloride anhydrous, sodium phosphate dibasic dodecahydrate, sodium phosphate monobasic dihydrate, glucose,
distilled water; substances for KATs analysis: Trizma base, acetic acid, pyridoxal 5'-phosphate hydrate, 2-mercaptoethanol, pyruvate and glutamine were purchased from Sigma-Aldrich. Substances used for high-performance liquid chromatography (HPLC) were from J.T. Baker Chemicals and from Sigma-Aldrich.

The analysis of KYNA production in rat kidney in vitro

Animals kidneys were collected immediately after decapitation and placed on ice. Every kidney was weighed and homogenized in preformed oxygenated Krebs-Ringer buffer at pH 7.4 (1:4; w/v). After that, 100 µL of kidney homogenate was put into test tubes, pre-filled with oxygenated Krebs-Ringer buffer (800 µL in every tube). Then, the homogenate was incubated for 2 h at 37°C in the presence of 10 µM Lkynurenine (50 µL) and one from the tested compound: fenofibrate or gemfibrozil (50 µL). Six concentrations of each drug were tested: 1 µM, 10 µM, 50 µM, 100 µM, 500 µM and 1 mM. Due to limited solubility each drug was dissolved in DMSO. For each set of experiments at least six independent tissue samples were analyzed. Homogenate incubation was stopped on ice by adding 1 N HCl (100 µL per each sample). All samples were centrifuged (15 133 × g, 15 min), then obtained supernatants were examined by the HPLC (Thermo Fisher Scientific HPLC system, ESA catecholamine HR-80, 3 µm, C18 reverse-phase column) and the KYNA level was assessed fluorometrically.

KAT I and KAT II activity analysis in rat kidney in vitro

KATs activity in rat kidney in vitro were quantified as described previously (Gramsbergen et al. 1992) with minor modifications. Shortly, to evaluate KAT I and KAT II activity, rat kidneys were immediately homogenized in dialysate buffer (1:9; w/v) containing 5 mM Tris-acetate buffer (pH 8.0) with 50 µM pyridoxal 5'phosphate and 10 mM 2-mercaptoethanol. Afterwards, kidney homogenate was centrifuged (15 133 × g, 15 min) and the obtained supernatant was dialyzed against 4 L of the dialysate buffer for 12 hours at 8°C in cellulose membrane dialysis tubing. Later, the obtained enzyme sample was incubated for 2 h at 37°C with L-kynurenine (2 µM), glutamine (2 mM) and selected fbrate (at 1 µM, 10 µM, 50 µM, 100 µM, 500 µM and 1 mM concentration) at pH 9.5 or 7.0, optimal for KAT I or KAT II activity, respectively. KAT I inhibitor, glutamine (2 mM) was given to samples to assess KAT II activity. The reaction was ended by putting all samples into ice cold bath. At the end samples were centrifuged and tested by the means of HPLC as samples from KYNA synthesis analysis. All assays were carried out in triplicates.

Statistical analysis

Presented data were expressed as mean ± standard deviation (SD), until stated otherwise. Differences between the means of the treatments were examined using one-way analysis of variance (one-way ANOVA) followed by Tukey’s multiple comparison test. Data were statistically analyzed with the use of GraphPad Prism 6. p < 0.05 was set as statistically significant.
Results

**Evaluation of KYNA formation in rat kidney *in vitro* in the presence of fibrates**

*De novo* production of KYNA in analyzed rat kidney homogenates under standard conditions in the presence of 10 µM KYN was 9.95 ± 2.69 pmol/mg tissue. Fenofibrate at 100 µM, 500 µM and 1 mM concentration suppressed KYNA synthesis in rat kidney homogenates *in vitro* to 72% (p < 0.05), 60% (p < 0.001) and 51% (p < 0.001) of control value, respectively (Fig. 1).

Similarly, gemfibrozil at 100 µM, 500 µM and 1 mM concentration decreased basal KYNA production in rat kidney *in vitro* to 66% (p < 0.001), 58% (p < 0.001) and 41% (p < 0.001), respectively.

**Evaluation of KAT I activity in rat kidney *in vitro* in the presence of fibrates**

The mean KYNA production by KAT I in examined kidney homogenates under standard conditions in the presence of 2 µM KYN was 59.12 ± 6.98 pmol/mg tissue. Fenofibrate only at 500 µM and 1 mM concentration inhibited KAT I activity in rat kidney *in vitro* to 68% (p < 0.05) and 59% (p < 0.05) of control value, respectively (Fig. 2).

Gemfibrozil was more potent KAT I inhibitor, suppressing enzyme's activity in rat kidney *in vitro* at 100 µM, 500 µM and 1 mM concentration to 68% (p < 0.05), 56% (p < 0.01) and 52% (p < 0.01) of standard value, respectively.

**Evaluation of KAT II activity in rat kidney *in vitro* in the presence of fibrates**

The mean KYNA production by KAT II in tested kidney homogenates under standard conditions in the presence of 2 µM KYN was 93.71 ± 16.98 pmol/mg tissue. Fenofibrate at 100 µM, 500 µM and 1 mM concentration lowered standard KAT II activity in rat kidney *in vitro* to 78% (p < 0.05), 63% (p < 0.01) and 64% (p < 0.05), respectively (Fig. 3).

Compared to that, gemfibrozil at 500 µM and 1 mM concentration stronger suppressed KAT II activity in rat kidney *in vitro* to 47% (p < 0.001) and 26% (p < 0.001) of control value, respectively.

**Discussion**

Results of the present study indicate for the first time that drugs lowering triglyceride level, fenofibrate and gemfibrozil, decrease KYNA production and KATs activity in rat kidney *in vitro*. Presented findings
propose potentially protective effect of this class of drugs on kidney tissue.

The association between the rate of Trp degradation and kidney damage was the aim of recently published studies. Although CKD pathogenesis is very complex, the activation of immune system and KYN pathway in the course of disease are gaining a lot of interest. Debnath et al. showed that the circulating level of KYN, KYNA and quinolinic acid (QA) was significantly related with the severity of CKD in patients with diabetes mellitus (Debnath et al. 2017). Similar observations were made by Klawitter et al., who reported correlation between plasma KYNA level, immune activation and CKD severity in patients with autosomal dominant polycystic kidney disease (Klawitter et al. 2022). Interestingly, a recent paper by Pires et al. on the Lewis polycystic kidney (LPK) rat model not only demonstrated age dependent KYN accumulation in the plasma and the kidney but also showed stimulation of enzymatic Trp degradation, namely through increased activity of KATs (Pires et al. 2022). Since KYN and its metabolites are classified as uremic toxins, inhibitory effect of fibrates on KYNA synthesis should be considered as novel mechanism of kidney protection. It was already shown that fenofibrate by mitochondrial dysfunction improvement delays kidney damage (Liu et al. 2022). Other mechanisms, like reduction of oxidative stress level and increased endothelial nitric oxide synthase expression were presented after fenofibrate treatment in nicotine induced model of acute kidney injury (AKI) (Chakkarwar and Kawtikwar 2021).

Similar findings were observed in ischemia reperfusion AKI model (Kaur et al. 2021) and renal fibrosis model (Xu et al. 2020). Adding to that, gemfibrozil showed the same beneficial effect on kidney function in D-galactose aging model (Hakimizadeh et al. 2023) and in obese Dahl salt sensitive rats (Shields et al. 2020). Gemfibrozil benefits exceeded lipid lowering effects, leading to lower glomerular damage, decrease in proteinuria level, lower blood pressure and improved kidney function. Additionally, gemfibrozil was able to attenuate doxorubicin kidney damage in rats (Hosseinzadeh et al. 2020). Direct cytoprotective effect on podocytes by gemfibrozil was reported as well (Miglio et al. 2012). Data from human studies indicate similar benefits of fibrate therapy on kidney function. In an analysis on 169 198 CKD patients from Taiwan fenofibrate use lead to significantly lower cardiovascular death risk (hazard ratio [HR]: 0.84; 95% CI, 0.75–0.94) and permanent need for dialysis (HR 0.78; 95% CI, 0.77–0.80) compared to statin users (Yen et al. 2021). Available data raise the question about usefulness of triglyceride lowering agents in kidney function preservation (Khurana et al. 2022) and cardiovascular prevention in patients with kidney function decline (Ananthakrishnan and Kaysen 2016). Since increased Trp degradation predisposes to cardiovascular events in CKD patients (Benitez et al. 2022), our study may support the usefulness of fibrates in cardiovascular prevention by decreasing KYNA synthesis.

In the present study, among tested drugs gemfibrozil seems to show stronger inhibitory effect on KYNA production observed from 100 µM concentration, compared to fenofibrate, which decreased less KYNA formation and KATs activity in kidney homogenates. As the inhibition of KYNA formation in the kidney can be considered as nephroprotective, gemfibrozil should be more effective in the kidney function preservation. Indeed, in a study of Song et al. a therapeutically equivalent dose of gemfibrozil led to significantly higher urine output than fenofibrate in mice, indicating novel pharmacodynamic effect of this PPARα agonist (Song et al. 2018).
Contrary to potential benefits of PPARα agonists on kidney function preservation, decrease in KYNA synthesis after fibrates administration may have negative renal effects. According to study of Hsieh et al. KYNA prevented kidney damage and hypotension in rats after heatstroke (Hsieh et al. 2011). Additionally, KYNA counteracted kidney injury in ischemia reperfusion induced AKI animal model (Arora et al. 2014). Of note, gemfibrozil and fenofibrate were already shown to impair kidney function under specific conditions. Whereas gemfibrozil was reported to cause AKI secondary to rhabdomyolysis (Dalugama et al. 2018), more controversies are related with fenofibrate. In clinical studies, despite well-known antiproteinuric and antioxidant effect, fenofibrate was reported to elevate serum creatinine level and decrease glomerular filtration rate (Jun et al. 2012). Although this phenomenon is claimed to be reversible and its mechanism is not well elucidated, special precautions were introduced regarding this drug (Emami et al. 2020). Among possible mechanisms involved in creatinine concentration elevation an impaired kidney blood flow secondary to decrease in prostaglandin synthesis and reduced vasodilation of the afferent arteriole was postulated (Kostapanos et al. 2013). Interestingly, it was suggested that serum creatinine elevation itself does not reflect kidney function impairment after fenofibrate use (Ncube et al. 2012). Adding to these findings, KYNA was recently shown to promote vessel relaxation through endothelial nitric oxide synthase pathway (Wang et al. 2022). Since in our study fenofibrate reduces KYNA synthesis and significantly inhibits KATs activity, it can be suggested that decreased vasodilation related with fibrate administration is also connected with lower KYNA content in the kidney.

In conclusion, presented study indicates novel mechanism of action of fibrates. Inhibition of KYNA synthesis and KATs activity in rat kidney *in vitro* by PPARα agonists suggests their involvement role in kidney function regulation.

**Declarations**

**Ethical approval**

All applicable international, national and institutional guidelines for the care and use of animals were followed. All procedures performed in the presented study were in accordance with the European Directive 2010/63/EU on the protection of animals used for scientific purposes and ethical standards of the Local Ethics Committee for Animal Experiments in Lublin.

**Competing interests**

The authors have no relevant financial or non-financial interests to declare.

**Authors’ Contributions**

IZ and WZ contributed to the study conception and design, IZ conducted experiments, analyzed data and wrote first draft of the manuscript. WZ critically reviewed the manuscript. All authors read and approved the manuscript.

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Availability of data and materials

The datasets generated during the current study are available from the corresponding author upon reasonable request.

References


**Figures**

**Figure 1**

Effect of fibrates on KYNA production in rat kidney *in vitro*. Data are shown as percentage of KYNA synthesis, mean ± SD, n = 6, are plotted against drug concentration on a logarithmic scale. ANOVA followed by Tukey’s multiple comparison test. Figure was prepared in the GraphPad Prism 6. * p < 0.05, *** p < 0.001
Figure 2

Effect of fibrates on KAT I activity in rat kidney in vitro. Data presenting enzymatic activity as percentage of KYNA synthesis, mean ± SD, n = 3, are plotted against drug concentration on a logarithmic scale. ANOVA followed by Tukey’s multiple comparison test. Figure was prepared in the GraphPad Prism 6. * p < 0.05, ** p < 0.01

Figure 3

Effect of fibrates on KAT II activity in rat kidney in vitro. Data presenting enzymatic activity as percentage of KYNA synthesis, mean ± SD, n = 3, are plotted against drug concentration on a logarithmic scale. ANOVA followed by Tukey’s multiple comparison test. Figure was prepared in the GraphPad Prism 6. * p < 0.05, ** p < 0.01, *** p < 0.001