A SORAFENIB INDUCED MODEL OF GLOMERULAR KIDNEY DISEASE

Glomerular damage and proteinuria are important pathophysiological signs of chronic kidney disease. This study provides data obtained using a model developed based on the use of the anti-cancer drug sorafenib. Sorafenib is a tyrosine kinase inhibitor that acts through the signaling pathway associated with vascular endothelial growth factor and is widely used to treat various types of cancer. Sorafenib, on the other hand, causes serious side effects in patients, including the development of chronic kidney disease. This study was aimed at using the nephrotoxic properties of sorafenib to model chronic kidney disease in rats. We showed that rats treated with sorafenib for 8 weeks along with a diet high in salt (8% NaCl) develop hypertension with high systolic blood pressure of 80 mmHg, proteinuria with an increase in protein content of 75% higher, and a 4-fold increase in glomerular damage compared to the control group. In case of damage to the renal glomeruli caused by sorafenib, the level of transcripts that are involved in the synthesis of key glomerular proteins such as nephrine, podocin, synaptopodin and subplanin is significantly reduced. Also, when studying this model, activation of the endothelial-mesenchymal transition is observed. In the group of rats treated with sorafenib, the mRNA level for the WT-1 endothelial cell marker was reduced by 20%, while the concentration of the Col III, FSP-1, α-SMA and vimentin mesenchymal cell markers increased by 2–3 times. Thus, we developed a preclinical model of chronic kidney disease, expressed in damage to the renal glomeruli. We also demonstrated that glomerular damage in this model is associated with a reduced expression of key structural glomerular proteins and activation of the endothelial-mesenchymal transition of the kidneys.

Keywords: sorafenib, vascular endothelial growth factor, glomerular injury.

Introduction. Angiogenesis inhibition is a cancer chemotherapeutic approach utilizing either monotherapy or combination chemotherapy and has become a standard treatment for several types of cancers. Anti-angiogenic drugs are particularly effective against solid tumors, such as metastatic renal cell carcinoma (mRCC), non-small cell lung carcinoma, gastrointestinal stromal tumors (GIST), and colorectal carcinoma [20]. One of the main angiogenic growth factors that is targeted to treat cancer is vascular endothelial growth factor (VEGF) and VEGF receptors [6]. Several multi-targeted kinase inhibitors (MTKIs) sorafenib, sunitinib, and pazopanib were approved for the treatment of metastatic renal cell carcinoma (mRCC) and several other cancers [14, 20]. MTKIs are small molecules that target the VEGF receptor VEGFR-2, the platelet-derived growth factor (PDGF) receptor, RAS, and c-KIT [4].

MTKI medications have expanded to many different solid tumors, with ongoing clinical trials with newer formulations [8]. Although very effective in treating mRCC, GIST and non-small cell carcinoma, the MTKIs such as sorafenib have several limiting serious side effects. The most common and serious side-effects of VGEF-targeted therapies are hypertension including salt-sensitive
hypertension, kidney dysfunction with proteinuria, glomerular and renal tubular injuries [4].

In the present study, we have investigated the MTKI sorafenib on the kidney and glomerulus. We demonstrate that sorafenib in combination with a high sodium diet (8% NaCl) causes marked glomerular injury that is associated with a decrease in key glomerular proteins essential for glomerular structure and function. Our data also demonstrate a critical contribution for endothelial-to-mesenchymal transition (EndoMT) in sorafenib-induced kidney and glomerular injury. We propose that sorafenib can be utilized as a glomerular kidney disease model to study pathophysiology during progressive chronic kidney disease.

Material and methods. All experiments in this study were approved and carried out according to the guidelines of the Institutional Animal Care and Use Committee, Medical College of Wisconsin, Milwaukee, USA. Eight to twelve weeks old male Sprague–Dawley rats weighing 200-225 g (Charles River, MA, USA) received an 8% NaCl diet and administered vehicle or sorafenib (20mg/kg/d, p.o.) for 8 weeks. The sorafenib dose was chosen based on a previous rat study [21]. A schematic of the experimental protocol is shown in Figure 1.

Blood pressure and urine collection were done at baseline and then on days 28 and 56 of the experimental protocol. Urine samples were used for biochemical analysis to measure protein and creatinine. Blood and kidney tissues were collected at the end of the 56 day protocol. Kidney tissues were processed for histology and gene expression analysis. Systolic blood pressure measurements of vehicle and sorafenib administered rat groups were carried out by tail-cuff plethysmography (IITC Life Science Inc., Woodland Hills, CA, USA). Blood pressure measurement was done at baseline, day 28, and day 56 of the experimental protocol. Urine samples collected at baseline, day 28, and day 56 were used for protein measurement using a commercially available kit (Cayman, Ann Arbor, MI, USA). Creatinine levels in urine were measured using a commercially available kit from Cayman (Ann Arbor, MI, USA). Urinary protein and creatinine values were used for comparative threshold cycle (Ct) analysis. To measure proteinuria, we determined by normalizing Ct values to two housekeeping genes. All data are expressed as mean ± S.E.M. GraphPad Prism® Version 4.0 software was utilized statistical analysis.

Results and discussion. Hypertension is the most common cardiovascular event that occurs with VEGF-signaling pathway inhibitors [2]. Sorafenib induces hypertension in around one-quarter of the patients [27]. In agreement with this finding, data in the present study demonstrate that sorafenib treatment along with a high salt diet resulted in a marked hypertension. Systolic blood pressure increased by 80 mmHg in rats administered sorafenib compared to normal control-vehicle rats by the end of the 8-week protocol (Figure 2A).

Wilms Tumor 1 (WT-1) and several mesenchymal markers (fibronectin, collagen III (Col III), α-smooth muscle actin (α-SMA), fibroblast specific protein-1 (FSP-1) and vimentin. Messenger RNA (mRNA) was prepared from each kidney cortical sample using RNeasy Mini Kit (QIAGEN, CA, USA). The mRNA samples were quantified spectrophotometrically and cDNA was synthesized from 1µg of total RNA using iScript™ Select cDNA Synthesis Kit (Bio-Rad, Hercules, CA, USA). Gene expression was quantified by iScript One-Step RT-PCR Kit with SYBR green using the MyiQ™ Single Color RT-PCR Detection System (Bio-Rad Laboratories, Hercules, CA, USA). Dissociation curve analysis was carried out with IQ5 Optical System Software, Version 2.1 (Bio-Rad Laboratories. Hercules, CA, USA), and each amplified sample analyzed for homogeneity. During RT-PCR, the denaturation of the cDNA was done at 95°C for 2 min followed by 40 cycles used at 95°C for 10s and at 60°C for 30s. All mRNA samples were run in triplicate and fold change in gene expression was compared to controls determined by comparative threshold cycle (Ct) method. Expression levels of the gene of interest were determined by normalizing Ct values to two housekeeping genes. All data are expressed as mean ± S.E.M. GraphPad Prism® Version 4.0 software was utilized statistical analysis. (GraphPad Software Inc, La Jolla, CA, USA). Two-tailed unpaired Student's t-test was applied to determine statistical significance between groups. P value of < 0.05 was deemed significant.

Results and discussion. Hypertension is the most common cardiovascular event that occurs with VEGF-signaling pathway inhibitors [2]. Sorafenib induces hypertension in around one-quarter of the patients [27]. In agreement with this finding, data in the present study demonstrate that sorafenib treatment along with a high salt diet resulted in a marked hypertension. Systolic blood pressure increased by 80 mmHg in rats administered sorafenib compared to normal control-vehicle rats by the end of the 8-week protocol (Figure 2A).
Evidence indicates that VEGF inhibition causes hypertension as a consequence of decreased nitric oxide production [11]. Another proposed mechanism by which VEGF-signaling pathway inhibition leads to hypertension is that inhibition of the VEGF-signaling pathway increases the production of endothelin-1, a potent vasoconstrictor [2]. It is important to note that sorafenib is known to cause salt-sensitivity [26], hence, it is very likely that the placing the sorafenib treated rats on high salt diet is also played an important pathophysiological role in the development of hypertension and kidney injury assessed from proteinuria. In relation a role of salt sensitivity in VEGF inhibition associated hypertension and kidney injury, in normotensive Sprague-Dawley (SD) rats, the tyrosine kinase inhibitor SU5416 induced salt-sensitive hypertension and kidney injury. In a study performed in normotensive Wistar-Kyoto rats exposed to a low dose of sunitinib, a similar VEGF inhibitor like sorafenib, it is found that a high salt diet augmented the rise in blood pressure and proteinuria [16]. Different mechanisms may account for the salt sensitive hypertension during VEGF inhibition.

As suggested by Gu et al, the VEGF inhibition–induced decrease in NO production by renal proximal tubular cells may impair the pressure-natriuresis response because of impaired vasodilatation in the vasa recta [10]. Additionally, it is suggested that the VEGF inhibition can activate endothelin system and contribute to salt-sensitive hypertension [19].

In the present study, our data demonstrate that the sorafenib treated rats develop marked proteinuria with a 40-fold higher urinary protein excretion compared to normal control-vehicle rats (Figure 2B). Like hypertension, development of proteinuria is another common side effect associated with VEGF inhibitors including sorafenib [5, 12]. Other findings substantiate that VEGF inhibition by drugs like sorafenib causes damage to the glomerular filtration system and leads to proteinuria [12]. In the present study, we demonstrate that rats administered sorafenib in combination to a high sodium diet develop marked 4-fold higher glomerular injury characterized by glomerular sclerosis, mesangiolysis, and glomerular capillary injury (Figure 3A, 3B).
These findings corroborate those of several earlier studies that described glomerular injury during VEGF inhibition [3, 22]. Not only others, in an earlier study, we demonstrated that sorafenib treatment along with high salt diet caused marked glomerular injury in rats [21]. The glomerulus is a highly specialized filtration apparatus with selective permeability that allows free passage of water and solutes, but not protein. The permselectivity of the glomerular filtration barrier restricts protein passage into Bowman’s space. Glomerular barrier breakdown and loss of permselectivity leads to proteinuria, which is common in renal diseases. The current study found increased urinary protein and glomerular barrier breakdown in rats administered sorafenib. Although the details of glomerular filtration permselectivity and barrier remain unknown, it is clear that the glomerular epithelial cell and podocyte are important glomerular filtration barrier components. Podocyte injury is frequently involved in the pathogenesis of glomerular diseases [24]. Podocyte damage can be the result of changes in individual podocyte-associated proteins including those that assemble and stabilize the slit diaphragm and those that anchor the foot process to the glomerular basement membrane. In the present study, we evaluated kidney cortical mRNA expression of these proteins including nephrin, podocin, synaptopodin, and podoplanin. Our findings demonstrate that in rats administered sorafenib there is 20-80% lower mRNA expression of glomerular proteins compared to normal control-vehicle rats (Figure 3C–F). It has been proposed that decreased expression of glomerular proteins could be linked to decreased VEGF signaling that occurs with sorafenib administration. Indeed, VEGF is crucial to the maintenance of normal renal function, and both VEGF over- and under-expression can disrupt normal glomerular function. The interaction between VEGF generated by podocytes and VEGFR-2 on glomerular endothelial cells is necessary to maintain glomerular slit diaphragm barrier integrity [8, 9, 15]. Several studies underscored the importance of VEGF signaling in kidney health. Selective depletion of one VEGF allele in podocytes in mice leads to down regulation of the slit-diaphragm protein nephrin, resulting in proteinuria and structural and functional glomerular damages [7, 25].

Endothelial-to-mesenchymal transition (EndoMT) is a subtype of epithelial–mesenchymal transition. EndoMT is a novel source for myofibroblasts and contributes importantly to fibrosis and chronic kidney disease progression. During EndoMT, endothelial cell progressively changes their endothelial phenotype into a mesenchymal phenotype resulting in the loss of specific endothelial markers like WT-1 and gain in mesenchymal markers, such as α-SMA or FSP-1 [1, 23]. Previous findings indicate that EndoMT plays a critical role in glomerular injury and results in albuminuria during diabetic nephropathy. The findings described in this study and several other studies demonstrate a role EndoMT in causing podocyte damage leading to glomerular injury [17, 18, 28]. In the present study, we demonstrate that sorafenib administration caused proteinuria and glomerular injury in rats. Considering a role of EndoMT in glomerular injury we investigated if EndoMT contributes to sorafenib-induced glomerular injury. The current findings reveal that sorafenib administration to rats decreased renal cortical endothelial cell marker WT-1 mRNA expression by 20 % and increased mesenchymal marker Col III, FSP-1, α-SMA and vimentin mRNA expression by 2–3 fold when compared to normal control-vehicle rats (Figure 4 A–F).
These results indicate a potential contribution for EndoMT on glomerular endothelial cells to glomerular injury caused by sorafenib.

Conclusion. VEGF signaling inhibition by sorafenib administration resulted in hypertension and kidney injury. Sorafenib administration induced glomerular injury was associated with increased EndoMT and decreased glomerular barrier proteins. Accordingly, our findings establish sorafenib administration as a glomerular disease model in which glomerular injury is associated with decreased key glomerular structural proteins and activated kidney EndoMT.

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Figure 4

Figure 4. Kidney cortical expression of an endothelial cell marker, WT-1 (A), and several mesenchymal markers (Col III, α-SMA, FSP-1 and vimentin) in different experimental groups (B–E)

*P vs. Normal Control-Vehicle. All data are expressed as mean +/- SEM. N = 6.
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Особливості формування еубактеріального комплексу ризосфери пшениці озимої (TRITICUM DURUM) за різних систем удобріння

Досліджено кількісний, якісний склад і таксономічну структуру еубактеріального комплексу у ризосфері пшениці озимої при застосуванні різних систем удобріння. Мікробіологічними методами визначали вміст у ризосфері пшениці озимої мікроорганізмів, а молекулярними – таксономічну структуру та метагеному-бактеріальні комплекс мікроорганізмів. Встановлено, що на варіантах біологічної системи удобріння пшениці озимої зростала частка мікроорганізмів родин Proteobacteria до 80,3 %, а чисельність представників Actinobacteria зменшувалась до 12,4 %, аналітичні значення зростали при варіанті удобріння домінуючого виду мікроорганізмів родини Alcaligeneae в 3,5 раза, за застосування біологічної системи зменшилася в 6,4 раза, а в наявності мінеральних, сприяла формуванню різноманіття бактерій. Наискорість значення індексу Шеннона було за біологічної системи удобріння – 4,82.

Встановлено, що застосування біологічної системи удобріння супроводжувалося збільшенням видового різноманіття грунтової мікрофлори за рахунок філ Acidobacteria, Actinobacteria, Bacteroidetes, Firmicutes, Proteobacteria, Verrucomicrobia, використання екологічної системи удобріння – за рахунок представників Actinobacteria, Bacteroidetes, Firmicutes, Proteobacteria. Абсолютним домінантам в дослідженні грунтової мікрофлори, незалежно від застосованої системи удобріння, були представники бактеріальних філ Proteobacteria – 79,1 % та Actinobacteria – 14,9 %.

Ключові слова: грунтовая мікрофлора, системи удобріння, метагеном, піросеквенування, ризофаера.