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See related article, "Suppression of microRNA-29 Expression by TGF- $\beta$ 1 Promotes Collagen Expression and Renal Fibrosis," on pages 252–265.

## Sphingosine Lipids in the Resolution of Renal Ischemia and Reperfusion Injury

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Despite all research endeavors in the field of AKI, its severity and incidence are still increasing without substantial improvements in its prevention or therapy.<sup>1</sup> Thus, AKI is still associated with a dramatic increase in morbidity and mortality in patients; a recent study in hospitalized patients indicated that a rise of serum creatinine of only 0.3 mg/dl is associated with a 70% increase in the risk of death.<sup>2</sup> The increase in morbidity and mortality in conjunction with the lack of available therapies and the incredible costs associated with renal failure make it an area of intense investigations.

One of the leading causes of AKI is renal ischemia with attenuated blood supply to the kidneys associated with sepsis.<sup>1,3–7</sup> Ischemic tissue damage has multifaceted effects on renal tissues, including renal inflammation, direct tubular damage, and alterations of vascular responses. AKI caused by ischemia can occur in different clinical settings such as surgical procedures, where cross-clamping of the aorta and renal vessels is associated with a renal failure rate of up to 30%.<sup>8</sup> Similarly, acute renal failure after cardiac surgery occurs in up to 10% of patients under normal circumstances and is associated

with dramatic increases in mortality.<sup>9</sup> Therefore, new potential therapeutic targets are of urgent need to prevent renal injury caused by ischemia.<sup>10–15</sup> Luckily, mouse models of AKI are very well established, and studies in gene-targeted or conditional mice offer great potential for investigating interesting targets protective of AKI.

Lee and colleagues<sup>16–21</sup> have intensively investigated the role of adenosine receptors, which are G-protein-coupled receptors, in the pathophysiology of AKI and have opened novel and promising potential therapeutic pathways in treating AKI caused by ischemia. In this issue of *JASN*, Park *et al.*<sup>22</sup> investigated the impact of sphingosine 1-phosphate (S1P) and its G-protein-coupled receptor, so called S1P receptor 2 (S1P<sub>2</sub>R), in renal ischemia reperfusion (IR) injury.

Sphingosine and its receptors belong to the sphingolipid family. One characteristic of lipids compared with other messenger molecules is they can freely diffuse across membranes. Thus, they cannot be stored in vesicles but are biosynthesized on demand. Sphingolipids are a class of lipids containing a backbone of sphingosine bases and aliphatic amino alcohols that includes sphingosine. Sphingosine is generated from *N*-deacylation of ceramide by ceramidase. It can be phosphorylated by sphingosine kinases (SK1 and SK2) to S1P. Once phosphorylated to S1P, it can activate all of the five known S1P receptors.

Five S1P receptors, discovered in the early 1990s, have been cloned thus far (S1P<sub>1</sub>R, S1P<sub>2</sub>R, S1P<sub>3</sub>R, S1P<sub>4</sub>R, and S1P<sub>5</sub>R).<sup>23</sup> S1P receptors and their mediators have been recently shown to play an important role as potent bioactive messengers in cell differentiation, proliferation, apoptosis, migration, and angiogenesis.<sup>24</sup> Moreover, modulators of S1P receptor attenuated vascular leak during acute lung injury, attenuated ischemia reperfusion injury in the heart and the kidneys,<sup>25,26</sup> and improved graft survival<sup>27</sup> in animal models. Furthermore, synthetic S1P receptors show therapeutic efficacy in clinical trials in multiple sclerosis.<sup>28</sup> The S1P<sub>1</sub> receptor is the most extensively studied receptor in immune-modulatory processes, whereas the role of the S1P<sub>2</sub> receptor in IR injury is largely unknown. Genetic deletion of the S1P<sub>1</sub> receptor in mice causes embryonic lethality due to incomplete vascular maturation,<sup>29</sup> whereas genetic deletion of S1P<sub>2</sub> leads to deafness.<sup>30</sup> In the kidney, S1P receptors are expressed on proximal tubules, endothelial cells, and immune cells.<sup>31,32</sup>

Thus, the present study of Park *et al.*<sup>22</sup> is of interest, based on recent findings, and develops comprehensive insights into the complex role of the sphingosine receptor, S1P<sub>2</sub>R, and its mediator, S1P, and sphingosine kinases in renal IR injury. Park *et al.* observed that pharmacological inhibition of S1P<sub>2</sub>R provides dose-dependent protection against IR injury. On a genetic level, they could show that gene-targeted mice for the S1P<sub>2</sub>R or mice treated with small interfering RNA targeting S1P<sub>2</sub>R were protective of IR injury. To confirm that S1P<sub>2</sub>R is the most important of the five known S1PRs, the authors determined transcript and protein levels and showed that receptor 2 was the one most upregulated after renal ischemia compared with the other four known receptors.

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In a next step, the authors observed that pharmacologic inhibition or genetic deletion of S1P<sub>2</sub>R reduces renal tubular necrosis after IR injury. Along this line, proinflammatory cytokines (TNF $\alpha$  and intercellular adhesion molecule 1) were reduced in mice with pharmacological inhibition of S1P<sub>2</sub>R. Because S1P<sub>2</sub>R is known to activate the Rho GTPase and the Rho-associated, coiled-coil containing protein kinase 1, also known as ROCK1 pathways, the authors suggest a possible role by blocking S1P<sub>2</sub>R. They performed an elegant experiment by pretreating the mice with selective Rho and ROCK inhibitors before activating these pathways with a S1P<sub>2</sub>R agonist. Both inhibitors attenuated the renal dysfunction induced by an S1P<sub>2</sub>R agonist and protected against renal IR injury.

Since it has been shown that a downregulation or deletion of this receptor in mice goes along with an increase of SK1 and SK2, and since an upregulation of SK1 and SK2 is protective against cardiac and renal IR injury,<sup>33,34</sup> the authors question whether the protective effect they see by inhibition or deletion of the S1P<sub>2</sub>R receptor might be caused by an upregulation of SK1 or SK2. Furthermore, the phosphorylation of SKs resulted in the formation of S1P, which could be the mediator of renal injury. Interestingly, S1P<sub>2</sub>R deletion is accompanied by a marked upregulation in SK1, and a selective inhibition of SK1 in S1P<sub>2</sub>R-deleted mice abolished its protection against IR injury. Furthermore, the protective effect of S1P<sub>2</sub>R inhibition was abolished in mice with additional pharmacological blockade of the S1P<sub>2</sub>R, suggesting that S1P synthesis is necessary for the protective effect. Thus far, S1P<sub>2</sub>R deletion seems to mediate protection by an upregulation of SK1 and an increase of S1P mediated by S1P<sub>1</sub>R.

Hypoxia-inducible factors (HIFs) are an important mediator under hypoxic conditions and during inflammatory processes. HIF-1 $\alpha$  is stabilized and induces the expression of genes that are known to be protective in inflammation and organ injury due to ischemia/hypoxia.<sup>35</sup> Therefore, the authors hypothesize that the S1P pathways might be stimulated by HIF-1 $\alpha$ . Furthermore, it is known that SK is a hypoxia-regulated gene.<sup>36</sup> Interestingly, pretreatment of cells with an HIF-1 $\alpha$  inhibitor before exposure to hypoxia significantly attenuates levels of mRNA encoding SK1. Furthermore, inhibition of S1P<sub>2</sub>R increases nuclear HIF-1 $\alpha$ . Moreover, inhibition of HIF-1 $\alpha$  abolishes the protective effect of S1P<sub>2</sub>R blockade in IR injury in mice. These data suggest that HIF-1 $\alpha$  plays a critical role in renal protection by S1P<sub>2</sub>R inhibition.

In a last experiment, the authors wanted to clarify which renal cell type is involved in that protective mechanism. They could show that SK1 was only induced in isolated tubules and not in isolated endothelial cells from mouse kidneys after IR injury. Thus, their data suggest S1P<sub>2</sub>R directly modulates the synthesis of HIF-1 $\alpha$ , which increases SK1 synthesis and thus mediates renal protection in tubular cells.

Lipids play a pivotal role in the resolution process during organ inflammation.<sup>37–39</sup> The resolution phase is a pretty recently defined period during organ inflammation that has to be separated from active processes of inflammation, particularly the reduction of neutrophil infiltration. The resolution process is a more passive process where various molecules promote the

clearance of inflammatory cells. Members of the lipid family, acting as pro-resolution molecules, can be determinant in attenuating inflammation. The role of the sphingosine lipids, as described by Park *et al.*<sup>22</sup> during renal IR injury, fits this resolution scheme of protection from renal IR injury. However, more studies are warranted to clarify the precise mechanism of these sphingosine lipids during renal IR injury.

## DISCLOSURES

None.

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See related article, "Inhibition of Sphingosine 1-Phosphate Receptor 2 Protects against Renal Ischemia-Reperfusion Injury," on pages 266–280.

## The Crossroad of RAAS Modulation, Inflammation, and Oxidative Stress in Dialysis Patients: Light at the End of the Tunnel?

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After four decades of fully reimbursed chronic dialysis therapy in the United States under the justification of lifesaving treatment, the survival rate of dialysis patients remains worse than many fatal cancers.<sup>1</sup> Most recorded causes of mortality on death certificates are cardiovascular or infectious; however, the true etiology of poor survival is unknown. Some slight improvement in the survival of dialysis patients reported over the past few years may be attributed to increased life expectancy in the background healthy population, although in the interest of fairness, the increased use of cardio-protective and other pharmacologic agents should also be noted as potential contributors.<sup>1</sup> Nevertheless, we have not succeeded in saving lives more significantly than before.

In line with ongoing attempts to uncover the underlying etiology of death in dialysis patients, sporadic attention has been paid to the evil axis of malnutrition, inflammation, and oxidative stress. There are compelling reasons why we cannot afford to ignore this axis: up to two-thirds of long-term dialysis

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