Divergent roles of sphingosine kinases in kidney ischemia–reperfusion injury

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Sphingosine-1-phosphate (S1P), produced by sphingosine kinase 1 (SphK1) or kinase 2 (SphK2), mediates biological effects through intracellular and/or extracellular mechanisms. Here we determined a role for these kinases in kidney injury of wild-type mice following ischemia–reperfusion. SphK1 but not SphK2 mRNA expression and activity increased in the kidney following injury relative to sham-operated animals. Although *SphK1*$^{-/-}$ mice had no alteration in renal function following injury, mice with a disrupted SphK2 gene (*SphK2*$^{tr/tr}$) had histological damage and impaired function. The immune-modulating pro-drug, FTY720, an S1P agonist failed to provide protection in *SphK2*$^{tr/tr}$ mice. Injured kidneys of these mice showed increased neutrophil infiltration and neutrophil chemokine expression along with a 3- to 5-fold increase in expression of the G-protein-coupled receptor $S1P_3$ compared to heterozygous *SphK2*$^{+/tr}$ mice. Kidney function and reduced vascular permeability were preserved in $S1P_3^{-/-}$ compared to $S1P_3^{+/-}$ mice after ischemia–reperfusion injury, suggesting increased $S1P_3$ mRNA may play a role in the injury of *SphK2*$^{tr/tr}$ mice. Our study suggests that constitutive expression of SphK2 may contribute to reduced ischemia–reperfusion injury of the kidney, and its absence may enhance injury due to increased neutrophil infiltration and $S1P_3$ activation. We also confirm that SphK2 is necessary to mediate the protective effects of FTY720.

Kidney International (2009) **75**, 167–175; doi:10.1038/ki.2008.400; published online 29 October 2008

KEYWORDS: SphK; acute kidney injury; S1P; FTY720

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Received 13 September 2007; revised 21 April 2008; accepted 27 May 2008; published online 29 October 2008

Ischemic acute kidney injury is a major clinical problem with high morbidity and mortality and also accounts for delayed graft function of transplanted kidneys.[1,2] Treatment of ischemic acute kidney injury still remains largely supportive and many drugs that reduced injury in animal models have failed in humans.[3] Therefore, a refined understanding of the mechanisms of kidney ischemia–reperfusion injury (IRI) and development and testing of novel compounds is necessary.

Sphingosine-1-phosphate (S1P), a pleiotrophic lipid mediator is produced by phosphorylation of sphingosine by sphingosine kinases (SphKs) in response to a variety of stimuli. S1P is exported out of the cell (inside-out signaling) and serves as a ligand for five different G-protein-coupled receptors ($S1P_{1-5}$).[4] In addition to extracellular function, S1P also has an intracellular role and acts as a second messenger.[5,6] S1P regulates diverse biological processes and functions, including cell growth, survival, and proliferation and angiogenesis; S1P inhibits apoptosis and effects lymphocyte trafficking.[7–12] Furthermore, $S1P_1$ receptor activation has been shown to protect kidneys from IRI.[13,14]

SphK1 and SphK2 are important rate-limiting steps in the formation of S1P. Despite sharing two large conserved regions, these kinases have different kinetics of expression during development as well as different subcellular localization,[15,16] suggesting that these two isoforms may serve different functions. SphK1 promotes cell-survival, proliferation, and regulates cell transformation.[17] In contrast, less is known about SphK2, although several reports suggest that SphK2 serves proapoptotic functions.[18,19] More importantly, SphK2 phosphorylates FTY720,[20] an immune modulatory prodrug currently in clinical trials for multiple sclerosis. Mice deficient in SphK1 or SphK2 do not exhibit conspicuous abnormalities, whereas double knockouts are lethal.[21] The purpose of the current study was to investigate the role of SphK1 and SphK2 in kidney IRI and to determine the role of SphK2 in mediating the kidney-protective effect of FTY720.

RESULTS

SphK gene expression and enzyme activities after kidney IRI

We first examined *SphK1* and *SphK2* gene expression and enzyme activities at various time points after kidney IR in

wild-type (WT) mice. SphK1 mRNA expression showed a robust increase as early as 2 h after reperfusion, peaking at 4–6 h with a 30-fold higher level compared to sham-operated mice and remaining elevated until 96 h. In contrast to the SphK1 mRNA, SphK2 mRNA did not increase significantly after IRI (Figure 1a). As expected from changes in cognate mRNA levels, SphK1 enzyme activity increased by threefold but there was no significant change in SphK2 enzyme activity (Figure 1b). These findings demonstrate that kidney IRI induces an increase in steady-state kidney SphK1 mRNA but not SphK2 mRNA, suggesting that SphK1 may be important in injury following kidney IR.

The effects of SphK1 and SphK2 on renal injury

To determine the importance of the *SphK1* and *SphK2* gene products in renal injury, we first evaluated functional and histological changes in $Sphk1^{-/-}$ and $SphK2^{tr/tr}$ mice. We assumed that the marked increase in SphK1 activity following kidney IRI indicated an important role of this enzyme in the kidney. Unexpectedly, the increase in plasma creatinine in $SphK1^{-/-}$ mice following kidney IR was not significantly different from that in $SphK1^{+/+}$ mice (Figure 2a). Surprisingly, although *SphK2* gene expression and enzyme activity were unchanged after kidney IR, kidney injury at 24 h was worse in $SphK2^{tr/tr}$ as indicated by elevated plasma creatinine levels that were significantly greater in $SphK2^{tr/tr}$ than in $SphK2^{+/tr}$ or *WT* mice (Figure 2b). Histological examination with hematoxylin and eosin staining showed extensive tubular injury characterized by an increase in tubular cell necrosis, dilation of tubules, and cast formation in the outer medulla of $SphK2^{tr/tr}$ mice. Kidneys from $SphK2^{+/tr}$ and/or *WT* showed less tubular injury compared to $SphK2^{tr/tr}$ (Figure 3a). A semiquantitative assessment demonstrated histological damage was greater in $SphK2^{tr/tr}$ than in $SphK2^{+/tr}$ mice (Figure 3b).

Compensatory SphK mRNA expression in SphK-deficient mice

To examine the possibility that compensatory changes in expression of SphKs may occur in SphK null mice, we measured SphK1 mRNA expression in $SphK2^{tr/tr}$ and SphK2 mRNA expression in $SphK1^{-/-}$ after 6 h of reperfusion in sham and IR mice. Although the marked increase in expression of SphK1 and SphK2, as shown in Figure 1, was confirmed in this experiment, there were no significant differences in the magnitude of the increase in SphK1 mRNA in $SphK2^{tr/tr}$ compared to $SphK2^{+/tr}$ mice or in SphK2 mRNA in $SphK1^{-/-}$ compared to $SphK1^{+/+}$ mice (Figure 4a and b). These results suggest that following kidney IRI, a compensatory mechanism between SphK1 and SphK2 does not occur when one enzyme is absent.

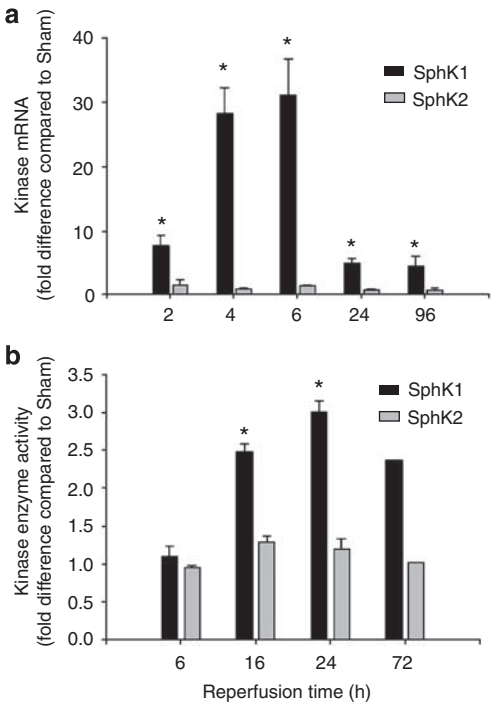

Figure 1 | Time course of SphK mRNA expression and enzyme activities in kidney IRI. Mouse kidneys were subjected to 32 min of ischemia and at various time points after reperfusion, kidneys were harvested for RNA extraction and measurement of SphK enzyme activities. Values are mean ± s.e.m.; $n = 4$–5 for each group; $^*P < 0.05$ compared with sham. (**a**) Time course of SphK mRNA expression following kidney IRI; (**b**) Time course of SphK enzyme activities following kidney IRI.

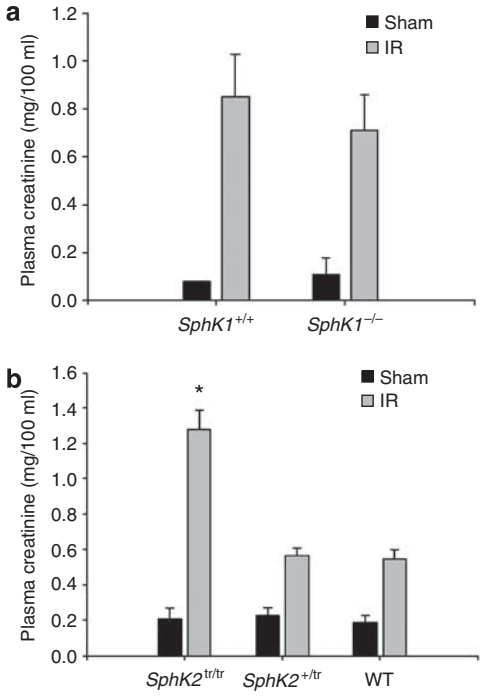

Figure 2 | Effect of the absence of SphK1 and SphK2 on plasma creatinine in kidney IRI. Kidneys from (**a**) SphK1 ($SphK1^{-/-}$ and $SphK1^{+/+}$) or (**b**) SphK2 ($SphK2^{tr/tr}$ $SphK2^{+/tr}$ and *WT*) mice were subjected to 32 min of ischemia and plasma creatinine concentrations were measured after 24 h of reperfusion. Values are mean ± s.e.m.; $n = 4$–5 for each group; $^*P < 0.05$ compared with $SphK2^{+/tr}$ in (**b**).