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Targeting sphingosine 1 phosphate receptor type 1 receptors in acute kidney injury

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Abstract

Sphingosine 1-phosphate analogs have a multitude of effects with the best characterized one being mediated through sphingosine 1-phosphate type 1 receptors (S1P1 receptor). Currently, S1P1 receptor agonists are being developed and tested for human disease. Because of the potent effect of S1P1 agonists to modulate the immune system, these compounds are ideal for blocking immune mechanisms that mediate acute kidney injury (AKI). This disorder continues to remain an important disease that is characterized by high morbidity and mortality. Currently there are no FDA approved drugs for the treatment of AKI. This review summarizes current knowledge on the mechanism of AKI due to ischemia-reperfusion and early studies that target S1P1 receptors for the treatment and prevention of AKI.

Acute kidney injury (AKI) is a burgeoning medical disease. Based upon the National Health Statistics and National Hospital Discharge Survey, between 1979 and 2002 there has been an increase in hospitalization for AKI (ICD9 = 584.0 through 584.9) from 35,000 to 650,000 cases per year (P. Eggers, Am Soc. Nephrol., 2004). Overall mortality has been reported to be 40–60% in critically ill patients [1–3]. The estimated annual health care expenditures attributed to hospital-acquired AKI exceed $10 billion [4]. Furthermore there is recognition that there is an increase in the end stage renal disease (ESRD) population due to AKI. In patients suffering from AKI, 13.4% of patients (or 30% of patients with AKI superimposed on chronic kidney disease) will progress to ESRD in 3 years (P. Eggers, Am Soc. Nephrol, 2004). Thus to overcome barriers to successful treatment of AKI, well designed clinical trials will need to be based on a precise understanding of the molecular, cellular and immunological basis of AKI [5]. Novel pharmacological agents need to be tested in preclinical and clinical trials. This review focuses on the pathogenesis of ischemia-reperfusion and one class of agents, agonists of sphingosine 1-phosphate receptors, that are potential agents in the treatment of AKI.

Pathogenesis of acute kidney ischemia reperfusion injury

AKI from ischemia is initiated by unfavorable changes in renal blood flow as a consequence of vasospasm, alterations in ultrafiltration coefficient, tubular obstruction and/or back-leak. The renal medulla is particularly susceptible to renal ischemia because of a low oxygen tension (PO_2 of 10–20 mmHg) [6]. With loss of blood flow, oxygen content is reduced even farther due to red blood cell and leukocyte trapping and a decrease in medullary blood flow. Hypoxic injury or reperfusion injury results in endothelial cell dysfunction that alters the balance of vascular tone of vasoactive agents such as endothelin and nitric oxide [7,8,9–11]. In addition

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to alterations in medullary vascular tone, medullary vascular congestion due to leukostasis contributes to a decrease in blood flow [12,13]. The cascade of events following IR leading to endothelial cell dysfunction and activation of tissue-resident and infiltrating leukocytes [13] consists of the coordinated action of cytokines/chemokines, reactive oxygen intermediates and adhesion molecules [14,15]. Bone marrow-derived cells are important in mediating injury associated with IRI [16]. Most studies have demonstrated that neutrophil infiltration of kidneys subjected to IR is associated with injury [13–15,17–19]. Additional studies provide strong support for the role of macrophages [20,21] and T cells in the early antigen-independent inflammatory response following reperfusion [18,22]. Although most studies have focused on infiltrating leukocytes very little is known about activation of kidney resident immune cells as an early event leading to the release of chemokines and later infiltration of additional leukocytes.

Dendritic cell activation of NKT cells and IFN-γ in kidney IRI

Tissue resident macrophages and dendritic cells originate from a heterogeneous population of bone marrow-derived monocytes [23–26] that are characterized by low surface expression of chemokine receptor 2 (CCR2), Gr-1, and Ly-6C and high surface expression of the fractalkine receptor (CX3CR1) [26,27]. These heterogeneous circulating monocytes infiltrate normal tissue and differentiate into resident Dendritic cells and macrophages [25]. In contrast to the focus on infiltrating leukocytes, there are few studies examining the role of kidney resident dendritic cells in kidney IRI despite their abundant expression in kidney [28]. Following severe IRI, antigen presentation by kidney resident dendritic cells is thought to be involved in the classical pathway of T cell activation through adaptive immunity [29]. On stimulation, dendritic cells can convert from an immature cell type characterized by high phagocytic capacity and low levels of class II major histocompatibility complex (MCH class II) expression to a mature cell type characterized by high levels of MHC class II and low levels of expression of co-stimulatory molecules. Mature dendritic cells are specialized for T-cell activation [30–33]. However, in addition to the well-known function of dendritic cells in adaptive immunity, dendritic cells also participate in the early innate immune response [34]. Thus dendritic cells bridge innate and adaptive immunity in renal IRI. Dendritic cells are activated by TNFα and IFN-γ or are activated through interaction with NKT cells via CD40-CD40L. Activated dendritic cells secrete IL-12, IL-2 and IFN-α/β and in addition, present glycolipids via CD1d, which activates NKT cells. NKT cells can then mediate injury directly or indirectly through the release of inflammatory factors that regulate T helper (Th) cell differentiation and activation of T cells.

Several studies have demonstrated that $CD4^+$ T cells are involved in kidney IRI [22,35–38]. When subjected to IRI, kidneys from C57BL/6 mice demonstrated within 30 min of reperfusion a significant increase in $CD4^+$ cells (145% of control) from single-cell kidney suspensions as measured by flow cytometry [39]. A significant fraction of $CD4^+$ T cells expressed activation markers. However, conventional $CD4^+$ T cells are thought to play an obligatory role in antigen specific, cognate immunity that requires 2–4 days for T cell processing, a time course that cannot explain the rapid, innate immune response following IRI. By contrast, NKT cells are a T cell sublineage [40] known to participate in innate immunity and may contribute to the early events in IRI. NKT cells participate in early innate immune response to IRI of acute islet allograft, liver IRI [41] and kidney IRI [39]. Kidney IRI in mice administered antibodies to block CD1d (a molecule on antigen presenting cells that present glycolipids and activate NKT cells), or to deplete NKT cells or in mice deficient of NKT cells, was markedly attenuated. These effects were associated with a significant decrease in renal infiltration and, in activation of NKT cells, and a decrease in IFN-gamma-producing neutrophils. The results support the essential role of NKT cells and neutrophils in the innate immune response of renal IRI by mediating neutrophil infiltration and production of IFN-gamma.

Sphingosine 1-phosphate (S1P) receptors and analogs

Sphingolipids, essential components of the cell membrane, are classified into sphingomyelins, ceramides and glycosphingolipids. They have potent and sometimes divergent, effects on cellular homeostasis. S1P is a specific ligand for a family of five G protein coupled endothelial differentiation gene (Edg) receptors, S1P1-5 (formerly Edg1, 5, 3, 6, 8) that evoke diverse cellular signaling responses. S1P receptors regulate different biological processes depending on their pattern of expression and the different heterotrimeric G proteins present. S1P binds to receptors or acts as an intracellular second messenger to stimulate cell survival, inhibit cell apoptosis, inhibit cell adhesion and movement [42–48]. However, accumulation of cell membrane lipid by-products including sphingomyelin/ceramide/sphingosine pathway is involved in acute renal tubular cell injury [49]. FTY720, a sphingosine analog, is a potent immunomodulator that has been tested for prevention of rejection in kidney transplantation [50,51]. Although this investigational drug failed in phase III renal transplantation trials, it is currently in phase III trials as monotherapy for relapsing remitting multiple sclerosis. FTY720 is a substrate for sphingosine kinase (SPHK) 2 and its phosphorylation *in vivo* is necessary for efficacy [45,46,48,52]. Phosphorylated FTY720 (FTY720-P) is a receptor-nonselective S1P analog that activates four of the five known receptors for S1P. FTY720 induces lymphopenia, which is because of enhanced retention in secondary lymphoid organs, without causing cytotoxicity or suppressing growth potential [53–57]. FTY720 induced apoptosis is mediated by the mitochondrial cascade.

Mouse CD4+ T cells predominantly express $S1P_1$ and $S1P_4$ receptors. Naïve and memory T cells recognize and respond to S1P mainly through $S1P_1$ receptors. At low concentrations (10–100 nM), S1P enhances the chemotaxis of CD4+ cells, whereas 0.3–3 μM S1P inhibits the chemotaxis [58]. S1P suppresses the initiation of T cell proliferation and secretion of IFN-γ and IL-4, without affecting IL-2 [59]. S1P also regulates vascular permeability [60], which is an important process of hypoxia. IFN-γ-stimulated macrophages were strongly inhibited by FTY720. Alterations in both ceramide and sphingosine expression occur during the renal tubular ischemic damage [61].

S1P1 receptors in AKI

Our data [62] show that $S1P_1$ receptors are expressed at the highest level with the following rank order: $S1P_1 > S1P_3 > S1P_2 > S1P_4$ in the mouse kidney; and a time-dependent increase after IR that begins after 2 h with the maximum expression after 6 h.

Given the potent effect of S1P analogs to induce peripheral lymphopenia and importance of T lymphocytes in the pathogenesis of IRI we sought to determine the effect of $S1P_1$ receptor activation on kidney IRI. FTY720 was given i.p. to C57BL/6 mice, and animals were subjected to ischemia for 32 min followed by reperfusion for 24 h. Kidney IRI led to marked increase in plasma creatinine, leukocyte infiltration and vascular permeability. FTY720 significantly decreased plasma creatinine in a dose response manner with a maximal reduction of ~73 and ~69% with doses of 240 μg/kg and 48 μg/kg, respectively (Fig. 1). FTY720 treatment also led to a decrease in leukocyte infiltration, vascular permeability and peripheral blood lymphocyte counts. The protective effect of FTY720 was reversed with VPC44116, a selective $S1P_1$ receptor antagonist [63]. Furthermore, SEW2871; a selective $S1P_1$ agonist, significantly decreased plasma creatinine in a dose response manner with a maximal reduction of ~70% at a dose of 10 mg/kg (Fig. 1). Analysis of kidneys by light microscopy revealed minimal histologic signs of ischemic injury with FTY720 or SEW2871 treatment compared to vehicle group. Using RT-PCR, we found a time-dependent increase in the $S1P_1$ mRNA expression following IRI that begins after 2 h with the maximum expression at ~4 h. Additional studies have confirmed these initial findings [64,65]. Although we believe that FTY720 through

$S1P_1$ receptors induces inhibition of initiation of early events of T cell function, it may also induce direct protective effects on tubules.

Conclusions

The treatment of AKI is an important priority given the high morbidity and mortality associated with this disorder. Furthermore less recognized is its contribution to the prevalence of ESRD. Current information from existing databases indicated the incidence continues to rise and thus will continue to remain a serious medical problem. S1P1 analogs appear to be particular attractive for the treatment of AKI given. Certain S1P1 analogs are in clinical trials thus its efficacy can readily be tested for AKI. Given the current emphasis on trial design, rapid clinical testing is feasible.

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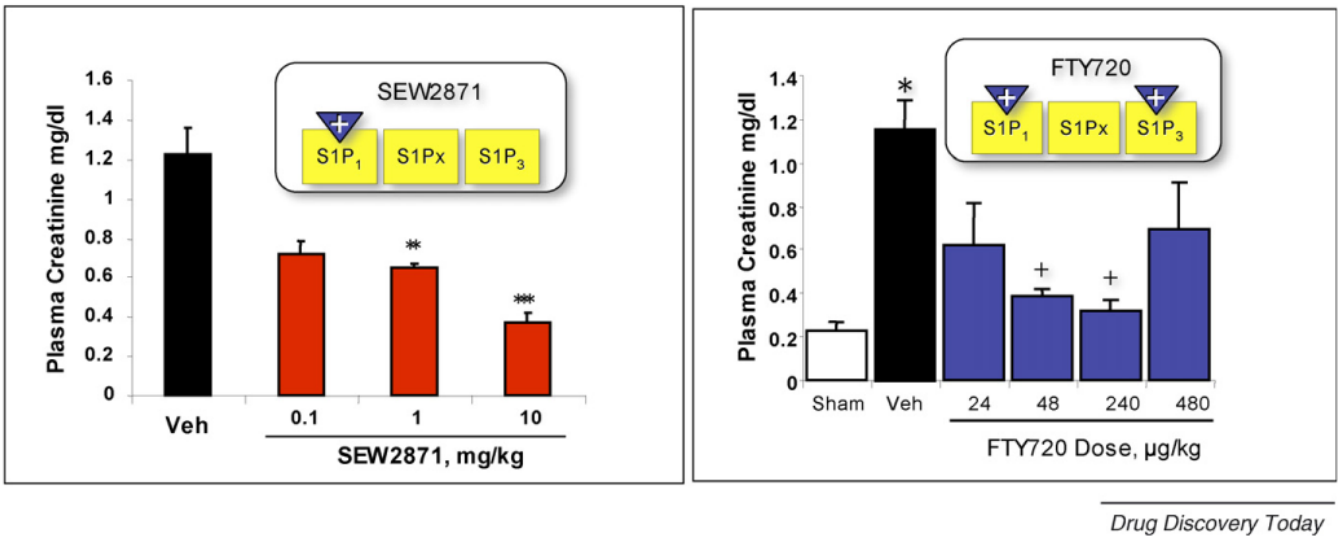

Fig. 1. S1P1 Selective Activation Reduces Ischemia-Reperfusion Injury

Effect of S1P1 analogs on plasma creatinine after renal IRI. (Left panel) Mouse kidneys were subjected to 32 min ischemia and 24 h reperfusion and treated with vehicle or SEW2871 (0.1, 1 and 10 mg/kg/dose). Values are means ± S.E.M.; $n = 5$ for each group. $^{*}P < 0.05$, $P < 0.01$, $^{***}P < 0.001$ compared to vehicle treatment. (Right panel) Mouse kidneys were subjected to 32 min ischemia and 24 h reperfusion and treated with vehicle or FTY720 (24, 48, 240 and 480 µg/kg/dose). Blood was obtained following 24 h of reperfusion and plasma creatinine was assayed. Values are means ± S.E.M.; $n = 4$ for each group. $^{*}P < 0.005$ compared to sham; $^{+}P < 0.005$ compared with vehicle treatment (data redrawn from [62]).