외국연수회원발표

Fractalkine Receptor Inhibition is Protective Against Ischemic Acute Renal Failure in Mice

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Abstract-1

Fractalkine (CX3CL1) is expressed on injured endothelial cells and is a potent chemoattractant and adhesion molecule for macrophages carrying the fractalkine receptor (CX3CR1). The aim of this study was to investigate the role of CX3CL1, and its ligand CX3CR1, in ischemic acute renal failure (ARF) in mice. On immunoblotting, CX3CL1 protein expression in the kideny increased markedly in ischemic ARF. On immunofluorescence staining, the intensity of CX3CL1 staining in blood vessels was significantly more prominent in ischemic ARF compared to controls. A specific anti-CX3CR1 antibody (25 μg IP 1hr before induction of ischemia) was functionally and histologically protective against ischemic ARF. CX3CR1 is predominently expressed on macrophages. Macrophage infiltration in the kidney in ischemic ARF was significantly decreased after anti-CX3CR1 antibody treatment. To determine the role of macrophage in ischemic ARF, macrophages in the kidney were depleted using liposomal-encapsulated clodronate (LEC). LEC resulted in significant functional and histological protection against ischemic ARF. In summary, in ischemic ARF, 1) there is upregulating of CX3CL1 protein in the kidney, specially in blood vessels, 2) CX3CL1 inhibition using a specific antibody is partially protective and is associated with reduced macrophage infiltration in the kidney, 3) macrophage depletion in the kideny is protective.

Abstract-2

The potent antineoplastic agent cisplatin is a frequent cause of acute renal failure (ARF). We have recently demonstrated that cisplatin can induce endothelial cell injury in vitro. There has been growing evidence of the role of inflammatory cells, cytokines and leukocyte-endothelial interactions in the pathogenesis of cisplatin-induced ARF. Fractalkine (CX3CL1) is expressed on activated endothelial cells and can function as both a potent chemoattractant molecule and an adhesion molecule for inflammatory cells carrying the fractalkine receptor (CX3CR1). The aim of this study was to investigate endothelial injury, fractalkine and its receptor expression in cisplatin-induced ARF. Cisplatin (30 mg/kg) was injected intraperitoneally into wild-type C57BL/6 mice. Simultaneously, western blotting, RT-PCR and immunofluorescence staining for fractalkine and its receptor were investigated. Circulating von Willebrand Factor Ag (vWF) concentration was measured as a marker of endothelial injury. 1) After injection with cisplatin, serum creatinine (mg/dL) and BUN (mg/dL) were unchanged compared to vehicle-treated mice on day 1, day 2. Renal dysfunction was the most severe on day 3 (2.0 ± 0.6, 161.2 ± 49.4 in cisplatin-treated mice vs 0.3 ± 0.1, 15.5 ± 2.1 vehicle-treated mice). 2) vWF in plasma (ng/mL) was increased on day 2 after cisplatin demonstrating the presence of systemic endothelial cell injury (2.98 ± 1.91 in day 0, 7.38 ± 5.56 in day 1, 40.15 ± 13.46 in day 2 ($n=4$, $p<0.05$ vs day 0, 1, 3), 9.15 ± 8.21 in day. 3) On immunoblotting of whole kidney, CX3CL1 protein in cisplatin-treated

group was increased compared to vehicle-treated group from day 1 after cisplatin injection. CX3CL1 protein expression was 7.5-fold increased on day 1 ($n=3$, $p<0.05$), 7-fold on day 2 ($n=3$, $p<0.05$) and 8-fold on day 3 ($n=5$, $p<0.05$) versus vehicle group on band densitometric analysis. 4) On immunofluorescence staining, the intensity of CX3CL1 staining in blood vessels was significantly more prominent in cisplatin-induced ARF compared to controls on day 2 after cisplatin administration. 5) The anti-CX3CR1 antibody was not protective against cisplatin-induced ARF. In summary, plasma vWF, a marker of endothelial injury, is markedly increased in cisplatin-induced ARF. CX3CL1 expression in cisplatin-induced ARF occurs on day 1 after cisplatin administration and precedes the development of ARF. In addition, there is upregulation of CX3CL1 protein in the kidney, specifically in blood vessels. However, the anti-CX3CR1 is not protective in cisplatin-induced ARF in mice.