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Inhibitors of calpain activation (PD150606 and E-64) and renal ischemia-reperfusion injury

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Abstract

Calpain activation has been implicated in the development of ischemia-reperfusion (I-R) injury. Here we investigate the effects of two inhibitors of calpain activity, PD150606 and E-64, on the renal dysfunction and injury caused by I-R of rat kidneys in vivo. Male Wistar rats were administered PD150606 or E-64 (3 mg/kg i.p.) or vehicle (10%, v/v, DMSO) 30 min prior to I-R. Rats were subjected to bilateral renal ischemia (45 min) followed by reperfusion (6 h). Serum and urinary biochemical indicators of renal dysfunction and injury were measured; serum creatinine (for glomerular dysfunction), fractional excretion of Na^+ (FE_{Na}, for tubular dysfunction) and urinary *N*-acetyl-β-D-glucosaminidase (NAG, for tubular injury). Additionally, kidney tissues were used for histological analysis of renal injury, immunohistochemical analysis of intercellular adhesion molecule-1 (ICAM-1) expression and nitrotyrosine formation. Renal myeloperoxidase (MPO) activity (for polymorphonuclear leukocyte infiltration) and malondialdehyde (MDA) levels (for tissue lipid peroxidation) were determined. Both PD150606 and E-64 significantly reduced the increases in serum creatinine, FE_{Na} and NAG caused by renal I-R, indicating attenuation of renal dysfunction and injury and reduced histological evidence of renal damage caused by I-R. Both PD150606 and E-64 markedly reduced the evidence of oxidative stress (ICAM-1 expression, MPO activity, MDA levels) and nitrosative stress (nitrotyrosine formation) in rat kidneys subjected to I-R. These findings provide the first evidence that calpain inhibitors can reduce the renal dysfunction and injury caused by I-R of the kidney and may be useful in enhancing the tolerance of the kidney against renal injury associated with aortovascular surgery or renal transplantation.
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1. Introduction

Despite significant advances in critical care medicine, ARF remains a major clinical problem, causing considerable morbidity and mortality that has not decreased significantly over the last 50 years [1]. As previous pharmacological interventions against ARF have proven to be largely negative in the clinical setting, the development of novel therapeutic interventions against ARF has remained a topic of intense research interest [1]. Renal ischemia is a major cause of ARF, initiating a complex and interrelated sequence of events resulting in injury to, and the eventual death of renal cells via both apoptotic and necrotic renal cell death pathways [2,3]. Furthermore, although essential for the survival of ischemic renal tissue,

Abbreviations: ARF, acute renal failure; FE_{Na}, fractional excretion of Na^+; ICAM-1, intercellular adhesion molecule-1; I-R, ischemia-reperfusion; MDA, malondialdehyde; MPO, myeloperoxidase; NAG, *N*-acetyl-β-D-glucosaminidase; NF-κB, nuclear factor-kappaB; PMN, polymorphonuclear leukocyte

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renal reperfusion causes additional damage (reperfusion injury) and together, I-R of the kidney leads to ischemic ARF [4].

Two groups of cysteine proteases, caspases and calpains, are involved in the development of the acute renal injury caused by I-R of the kidney [3]. Calpains, which are calcium-dependent non-lysosomal cysteine proteases, participate in several normal physiological cellular processes including signal transduction involving calcium signalling, remodelling of cytoskeletal-membrane attachment and apoptosis [5–7]. Two major isoforms of calpain were originally identified: calpain 1 (or μ-calpain, CAPN1) and calpain 2 (m-calpain, CAPN2), which require low (μM) and high (mM) calcium concentrations for activation, respectively [5]. More recently, 14 mammalian calpain genes and proteins have been characterised, many of which are implicated in pathological conditions [5–8]. For example, calpains 1 and 2 have been associated with stroke, traumatic brain injury, Alzheimer's disease and the development of cataracts, mutations of calpain 3 are implicated in limb-girdle muscular dystrophy and cataracts, the calpain 9 gene is down-regulated in gastric cancer and calpains 8 and 10 are implicated in the pathophysiology of type 2 diabetes mellitus [5]. Thus, excessive calpain activation, subsequent to intracellular calcium accumulation, has been identified in a variety of disorders. Several studies have demonstrated that calpain activation is involved in the development of I-R injury in several organs including the brain [9,10], heart [11–13] and liver [14,15]. I-R also inhibits the activity of the endogenous calpain inhibitor, calpastatin [16], thereby contributing to excessive calpain activation. Excessive activation of calpain has been implicated in the pathophysiology of ischemic ARF [17–19] and in vitro studies have demonstrated that calpain activation is involved in the cellular injury and death caused by renal hypoxia and nephrotoxic agents [20–23]. There is also evidence that calpain is activated in vivo in the ischemic rat kidney [24].

Many calpain inhibitors have been developed over the previous decade [25–27] and subsequently, the beneficial effects of calpain inhibition during I-R of many organs have been reported. Specifically, agents such as PD150606, E-64, calpain inhibitor-1, NS-7, A-705239 and calpastatin have been used to investigate the role of excessive calpain activation in the brain and heart [11,23,28–33]. Furthermore, the ability of calpain inhibitor-1 to reduce the organ injury caused by hemorrhagic shock (which involves I-R of several major organs including the kidney) has also been reported [34]. With respect to the kidney, PD150606, SJA7019, SJA7029, *Z*-Leu-Phe-COOH, *Z*-Leu-Abu-CONH-CH_2-CH(OH)-Ph and *Z*-Leu-Phe-CONH-Et have been shown to protect rabbit renal proximal tubular cells against antimycin A-induced cell death in vitro [23,35,36]. The α-mercaptoacrylic acid derivative PD150606 [3-(4-iodophenyl)-2-mercapto-(*Z*)-2-propenoic acid] is a non-peptide, cell-permeable, uncompetitive and selective calpain inhibitor which binds to the Ca^{2+}-binding domain of calpains 1 and 2 with high affinity only when the substrate is bound to protease [26,29,37]. Although the effectiveness of PD150606 in in vivo models of ischemic disease has yet to be fully elucidated, one investigation has recently shown that intracerebroventricular administration of 100 μM PD150606 into rat brains subjected to ischemic insult was able to effectively inhibit hippocampal calpain activity [38]. In contrast, several in vitro investigations have revealed the beneficial effects of inhibition of calpain activation by PD150606. For example, PD150606 was able to provide inhibit calpain activation in two intact cell systems and thereby provide neuroprotection [29,38]. At 100 μM, PD150606 was able to effectively reduce calpain activation and reduce cell death in rat and rabbit renal proximal tubules subjected to hypoxia or nephrotoxins [21,39,40]. E-64 [*trans*-epoxysuccinyl-L-leucylamido(4-guanidino)-butane] is both structurally and pharmacologically different to PD150606 [26]. E-64 is an irreversible, general inhibitor of cysteine proteases which, at 40 μM, was able to effectively reduce fodrin breakdown in slices of rat cerebral cortex subsequent to calpain activation caused by hypoxia/hypoglycaemia and thereby provide neuroprotection [28]. E-64 was also able to effectively reduce calpain activation and reduce cell death in rabbit renal proximal tubules suspensions exposed to diverse nephrotoxins such as antimycin A and bromohydroquinone [40] and reduce proteinuria in an in vivo experimental model of glomerulonephritis [41]. Although not as cell permeable as PD150606, there is some evidence that the uptake and subsequent effectiveness of E-64 may be related to a generalised increase in membrane permeability [42] which can prevail during renal I-R [4,43]. Furthermore, E-64d, an esterified and more cell-permeable analogue of E-64, has been shown to reduce calpain activation in rat myocardial tissues after global ischemia [12].

Few studies have investigated the effects of calpain inhibitors on the renal dysfunction and injury caused by I-R of the kidney in vivo. We have previously demonstrated that calpain inhibitor-1 can reduce renal dysfunction and injury caused by I-R of the rat kidney via attenuation of the expression of pro-inflammatory genes primarily via inhibition of the transcription factor NF-κB [34,44]. The present study was designed to evaluate the effectiveness of more potent and specific calpain inhibitors, specifically, the structurally and chemically distinct inhibitors PD150606 and E-64, in an established in vivo rat model of renal I-R injury [45]. Subsequently the ability of these calpain inhibitors to reduce the oxidative and nitrosative stress associated with renal I-R was elucidated using a combination of established biochemical and immunohistological assays.

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