Short communication

Tubular cell apoptosis and cidofovir-induced acute renal failure

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Cidofovir is an antiviral drug with activity against a wide array of DNA viruses including poxvirus. The therapeutic use of cidofovir is marred by a dose-limiting side effect, nephrotoxicity, leading to proximal tubular cell injury and acute renal failure. Treatment with cidofovir requires the routine use of prophylactic measures. A correct knowledge of the cellular and molecular mechanisms of cidofovir toxicity may lead to the development of alternative prophylactic strategies. We recently cared for a patient with irreversible acute renal failure due to cidofovir. Renal biopsy showed tubular cell apoptosis. Cidofovir induced apoptosis in primary cultures of human proximal tubular cells in a temporal (peak apoptosis at 7 days) and concentration (10–40 µg/ml) pattern consistent with that of clinical toxicity. Apoptosis was identified by the presence of hypodiploid cells, by the exposure of annexin V binding sites and by morphological features and was associated with the appearance of active caspase-3 fragments. Cell death was specific as it was also present in a human proximal tubular epithelial cell line (HK-2), but not in a human kidney fibroblast cell line, and was prevented by probenecid. An inhibitor of caspase-3 (DEVD) prevented cidofovir apoptosis. The survival factors present in serum, insulin-like growth factor-1 and hepatocyte growth factor, were also protective. The present data suggest that apoptosis induction is a mechanism contributing to cidofovir nephrotoxicity. The prophylactic administration of factors with survival activity for tubular epithelium should be further explored in cidofovir renal injury.

Introduction

Cidofovir is an antiviral nucleoside phosphonate analogue that has activity against a wide array of DNA viruses including herpes virus, adenovirus, papillomavirus, polyomavirus and poxvirus [1]. Among the latter, cidofovir has activity against smallpox and could become an essential drug in cases of bioterrorism [2,3]. However, cidofovir is marred by nephrotoxicity, a dose-limiting side effect. Cidofovir nephrotoxicity is mainly observed in proximal tubular epithelium and leads to Fanconi syndrome and acute renal failure [4]. Treatment with cidofovir requires the routine use of prophylactic measures to prevent nephrotoxicity. These include hydration, the use of probenecid and the avoidance of other nephrotoxic agents. However, there are patients in whom these measures cannot be used, but cidofovir should still be administered as a life-saving drug [5,6]. The selective proximal tubular toxicity of cidofovir results from the presence of the renal organic anion transporter 1 (OAT1) in the basolateral membrane of these cells. OAT1-mediated uptake of cidofovir leads to selective accumulation and toxicity in renal proximal tubular cells [7]. Probenecid inhibits OAT1, prevents the uptake of cidofovir by proximal tubular epithelium and decreases the incidence of nephrotoxicity [7]. However, 56% of patients have side effects ascribed to probenecid, which are dose-limiting in 7% [4]. In this regard, a correct knowledge of the cellular and molecular mechanisms of cidofovir toxicity may lead to the development of alternative prophylactic strategies. We present here a
case of irreversible acute renal failure induced by cidofovir in which prominent loss as well as apoptosis of proximal tubular epithelial cells were noted. In addition, we report that cidofovir selectively induces apoptosis in human proximal tubular epithelial cells. Probenecid, caspase inhibition or survival factors prevent apoptosis induced by cidofovir in the tubular epithelium.

Case report

A 35-year-old male with stage C3 HIV infection was admitted due to a 1-week history of persistent vomiting. He was receiving chronic suppressive treatment with cidofovir for cytomegalovirus (CMV) retinitis. At the start of therapy, serum creatinine (0.9 mg/dl, 79.5 µmol/l), phosphate and uric acid were normal. Four 5 mg/kg cidofovir infusions were administered with probenecid (4 g over 8 h) and saline (2 l) prophylaxis, at 3-week intervals. The last dose was administered 2 weeks before admission. Concurrent medications included amprenavir, ritonavir, lamivudine, zidovudine, tenofovir, sulphone, clarithromycin and ethambutol and all preceded cidofovir therapy. Prior to the last cidofovir infusion, serum creatinine was 1.4 mg/dl (124 µmol/l), and there was biochemical evidence of Fanconi syndrome. In the emergency room, serum creatinine was 12.6 mg/dl (1114 µmol/l). Urine analysis demonstrated proteinuria 150 mg/dl and glycosuria 300 mg/dl in the presence of normoglycaemia. Renal biopsy showed remarkable tubular lesions. There was patchy involvement characterized by dilated lumen and complete absence of epithelium in numerous tubules with the presence of apoptotic and sloughed cells in the lumen (Figure 1). There were occasional atrophic tubuli and minimal mononuclear inflammatory interstitial infiltration. Glomeruli and vessels were normal. The patient required renal replacement therapy until his death 4 months later.

Materials and methods

Cells

Primary cultures of human proximal tubular epithelial cell (Cambrex, East Rutherford, NJ, USA) were grown
Cidofovir nephrotoxicity

Results

Cidofovir induces apoptosis in human proximal tubular epithelial cells

Apoptosis was identified by the presence of hypodiploid cells and by morphological features, that is, nuclear shrinkage, condensation and fragmentation as well as decreased cell size (Figure 2A and 3B). In addition, cidofovir increased the number of propidium iodide negative, annexin V positive apoptotic cells with preserved cell membrane permeability (cidofovir 40 µg/ml 19.1 ±1.6% vs vehicle 5.9 ±1.3%; P=0.002) while the number of necrotic propidium iodide positive cells remained under 1.3% in both groups. Cidofovir induced apoptotic death in primary human proximal tubular epithelial cells in a dose-dependent manner (Figure 2A,B). Cidofovir also induced apoptosis in the human proximal tubular epithelial cell line HK-2 (Figure 2C). In contrast, cidofovir was non-toxic to a human renal fibroblast cell line (Figure 2D). The dose range that induced apoptosis was similar to the cidofovir concentration reached in serum of patients during therapeutic dosing (Figure 2B,C). Therapeutic serum cidofovir concentration is 11–20 µg/ml (38–70 µM). An increased rate of apoptosis was already evident following 5 days of incubation and further increased at 7 days.

Modulation of cidofovir-induced apoptosis

Cidofovir-induced apoptosis resulted in the appearance of active caspase-3 fragments (Figure 3A). In this regard, caspase-3 inhibition by DEVD prevented cidofovir apoptosis (Figure 3B). The survival factors present in serum, or the inhibition of the cellular uptake of cidofovir by probenecid, prevented cidofovir-induced apoptosis (Figure 3C). Specific factors with survival factor activity for tubular cells, such as IGF-1 and HGF also decreased the rate of cidofovir-induced apoptosis (Figure 3C).

Conclusions

The frequency of renal adverse effects reported in patients treated with cidofovir in clinical trials is high: proteinuria or renal failure lead to dose reduction in 25–30% of patients [4]. Renal dysfunction is usually at least partially reversible. As illustrated in previous case reports, subtle reversible decrements in renal function may occur following cidofovir administration [6]. Renal dysfunction peaks 1 week after cidofovir administration [6]. This time course is consistent with that of cidofovir-induced apoptosis in cultured tubular epithelium. In addition, two previous cases of irreversible cidofovir renal injury had been reported [5,6]. In one patient, development of allergy to probenecid...
precluded its prophylactic use [5]. In the other case, the predisposing factors were less clear [6]. A mild decrement in renal function might have been present given the patient’s age, condition (AIDS) and serum creatinine level. However, an estimate of the creatinine clearance by the Cockcroft–Gault formula was not provided. In addition, the patient, as in the present case, was receiving ritonavir.

The patient in the present case developed evidence of Fanconi syndrome and irreversible acute renal failure. The concomitant use of other potentially nephrotoxic agents and the administration of cidofovir when the serum creatinine had risen may have contributed to this evolution. Tenofovir was prescribed; at that time no reports of tenofovir nephrotoxicity had been published. Tenofovir is an acyclic nucleoside phosphonate with reverse transcriptase activity approved for the treatment of HIV. As cidofovir, tenofovir is transported into proximal tubular cells by OAT1. Despite this fact, tenofovir had initially been reported to be non-nephrotoxic in clinical trials [11] and in cultured proximal tubular epithelial cells [12]. However, two recent cases of tenofovir-induced acute renal failure have been reported [13,14]. One was an HIV patient with pre-existing chronic renal disease [13]. The other patient was cirrhotic, HIV positive and taking, amongst other drugs, ritonavir [14]. In both cases there was recovery of renal function following drug withdrawal [13,14]. Ritonavir is also a potentially nephrotoxic drug [15,16]. While ritonavir may have contributed to renal toxicity, it is unlikely to have been the main injurious agent, as ritonavir nephrotoxicity usually occurs early following introduction of the drug (3–21 days) [15,16].

Cidofovir is eliminated by the kidney and excreted extensively as unchanged drug in the urine via both glomerular filtration and active tubular secretion [17]. Over 90% of an intravenous dose is recovered unchanged in the urine over 24 h. Cidofovir accumulates in renal proximal tubular cells, where OAT1 mediates its uptake through the basolateral membrane [7]. Cidofovir accumulation mediated by this mechanism leads to selective proximal tubular cell toxicity. Probenecid prevents the uptake of cidofovir by proximal tubular epithelium. This decreases the renal clearance of cidofovir by blocking its active tubular secretion. In addition, it decreases the incidence of nephrotoxicity [17]. However, 56% of patients have side effects ascribed to probenecid and they are dose-limiting in 7% [4]. In addition, as exemplified by the present case report, given the increasing complexity of the condition of patients receiving cidofovir, it is not always feasible to keep them off potentially nephrotoxic drugs.

A better understanding of the molecular mechanism of cidofovir-induced tubular cell injury could help
develop alternative preventive strategies. These mechanisms are poorly understood. In rats and guinea pigs, apoptosis of proximal tubular epithelium is prominent following administration of cidofovir for 5 days [18]. In the patient in the present study, extensive denudement of the tubular epithelium was present. Loss of tubular epithelium is a common manifestation of renal tubular cell death in human samples [19]. In addition, apoptotic cells were noted among the persisting epithelial cells. In cell culture experiments, cidofovir induced proximal tubular cell apoptosis in a temporal and concentration pattern consistent to that of in vivo cidofovir toxicity [6]. Cell death was specific as it was also present in a human proximal tubular epithelial cell line but not in a fibroblast cell line. In addition, prevention of entry of cidofovir into the cell by probenecid also prevented apoptosis. These experiments also reveal possible future prophylactic strategies. The bi-weekly injection of cidofovir makes this drug especially suitable for such strategies.

Caspases are intracellular proteases that are activated during apoptosis [20]. DEVD is an inhibitor of caspases activated during the effector phase of apoptosis, such as caspase-3. Caspase inhibition resulted in protection from cidofovir-induced apoptosis. In addition, survival factors, such as those present in serum, also protected tubular cells. Indeed cytokines with survival factor activity for renal tubular epithelium, such as IGF-1 and HGF [20,21], are also protected from apoptosis induced by cidofovir. Despite their success in animal models [20,21], the therapeutic administration of such cytokines to humans with already established acute renal failure has been disappointing [22].

The present data suggest that apoptosis induction is a mechanism contributing to cidofovir nephrotoxicity. The prophylactic (as opposed to therapeutic) administration of factors with survival factor activity for tubular epithelium should be further explored in systems such as cidofovir nephrotoxic renal injury. Nevertheless, we must emphasize that, currently, the need to follow the indicated dosing guidelines is the most important strategy for reducing cidofovir-related nephrotoxicity.

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