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Thrombotic microangiopathy associated with proteasome inhibitors

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Abstract

The ubiquitin proteasome pathway plays a key role in cell cycle, function and survival. Bortezomib (BTZ) and Carfilzomib (CFZ) are the first two inhibitors of the proteasome pathway, indicated in treatment of patients with multiple myeloma. In the past few years, there have been few case reports that have highlighted the association between proteasome inhibitors (BTZ and CFZ) with acute kidney injury (AKI). In most of these case reports and initial trials, the underlying mechanism of AKI has been unclear. In this article, we discuss the association and pathogenesis of proteasome inhibitors-associated AKI. We also report the first case of CFZ-associated AKI with kidney biopsy evidence of thrombotic microangiopathy and the presence of microangiopathic hemolytic anemia.

Key words: onconephrology

Proteasome inhibitors

The ubiquitin proteasome pathway plays a key role in the targeted destruction of cellular proteins and is central for cell cycle, function and survival, making proteasome inhibition an attractive target in cancer [1–4]. Bortezomib (BTZ), the first inhibitor of the proteasome pathway, was approved in 2003 for the treatment of multiple myeloma (MM) [5, 6]. It was initially approved only for MM post-transplant relapse or as a second-line treatment in patients unsuitable for transplantation. In 2008, it was approved for induction therapy in MM. It is a boron molecule that reversibly inhibits threonine residue of 26S proteasome. Carfilzomib (CFZ) is a second generation proteasome inhibitor that was approved in 2012 for patients with MM who had relapsed and were refractory to BTZ and at least one thalidomide derivative [7–9]. CFZ inhibits the beta-5 proteasome subunit by forming an irreversible adduct through two covalent bonds, allowing more sustained and more specific inhibition than the single reversible adduct formed by BTZ [10]. Unlike BTZ, CFZ is not associated with peripheral neuropathy but has a higher incidence of cardiopulmonary and kidney toxicity. BTZ not only inhibits beta-5 proteasome but also some neuroprotective molecules and serine proteases that are involved in kidney injury, which explains the higher incidence of peripheral neuropathy but lower incidence of kidney dysfunction compared with CFZ [11].

Proteasome inhibitors and acute kidney injury

BTZ's approval for initial treatment of MM was based on the Phase 2 trials CREST and SUMMIT [6, 12, 13]. In these early trials, acute kidney injury (AKI) or thrombotic microangiopathy (TMA) was not reported as a drug-related adverse event [14]. CFZ's initial approval for the treatment of MM was largely based on a Phase 2 trial by Siegel *et al.* [15, 16]. In this study, an increase in serum creatinine (sCr) was reported in 25% of the patients (sCr 1.5 times from baseline to requiring dialysis), out of which 5% had AKI, which was defined as SCr >3 times baseline. Most of these patients required drug interruption, discontinuation or dose reduction. In another integrated safety profile study of CFZ, there was a 24.1% incidence of an increased sCr, out of which 17.7% were thought to be treatment related. It was interesting to note that, in this

Table 1. Case reports of AKI associated with use of proteasome inhibitors [17–24]

Study/year	Drug used	Age/sex	AKI	TMA	Time to onset of AKI/TMA	Renal biopsy	BMT	Underlying disease
Wanchoo et al. [17]	CFZ	78/M	+	n/a	Day 2	No	No	MM
Shely et al. [18]	CFZ	55/M	+	n/a	Day 5	No	No	MM
Hobeika et al. [20]	CFZ	62/M	−	+	Day 42	Yes	Yes	MM
Jhaveri et al. [19]	CFZ	68/M	+	n/a	Day 9	No	No	MM
Salmenniemi et al. [23]	BTZ	52/F	+	+	Day 11 at the fifth treatment	No	Yes	MM
Mehta et al. [21]	BTZ	70/F	+	+	Day 2 after ninth dose	No	No	MM
Moore et al. [22]	BTZ	57/F	+	+	Day 2	No	Yes	MM
Morita et al. [24]	BTZ	54/M	+	+	Day 8	No	Yes	MM

M, Male; F, Female; AKI, acute kidney injury; TMA, thrombotic microangiopathy; BMT, bone marrow transplant.

study, fever, anemia and thrombocytopenia were also reported in 30, 46 and 29% of patients, respectively.

In the past few years, there have also been a few case reports that have highlighted the association between proteasome inhibitors (BTZ and CFZ) with AKI [17–24]. In most of these case reports and initial trials, underlying mechanism of AKI has been unclear. Vasoconstriction of renal arteries leading to AKI has been hypothesized by Wanchoo et al., and the use of N-acetyl-L-cysteine partially mitigated the renal injury upon re-challenge of CFZ [17].

Thrombotic microangiopathy associated with proteasome inhibitors

There have been four cases of BTZ-associated AKI with evidence of underlying TMA (Table 1). Kidney biopsy was not performed in any of these cases. Hobeika et al. [20] recently published a case of a patient presenting with worsening hypertension and proteinuria after CFZ exposure. Kidney biopsy showed features of TMA and associated podocytopathy, with foot process effacement. This presentation was not associated with microangiopathic hemolytic anemia (MAHA). A similar pathology has also been described in TMA associated with vascular endothelial growth factor (VEGF) inhibitor exposure [25, 26]. We report the first case of CFZ-associated AKI with kidney biopsy evidence of TMA and the presence of MAHA.

Case Report: A 63-year-old Caucasian male with MM IgG kappa, ISS stage II, status post 2 autologous bone marrow transplants (last one ∼6 months prior to this presentation) was found to have AKI [sCr of 6.3 mg/dL (557 µmol/L)] during his routine visit to receive his second dose of CFZ. Two weeks prior to this presentation, he was started on chemotherapy with CFZ. His sCr was 0.7 mg/dL (53 µmol/L) at this time with no evidence of proteinuria. His past medical history was also significant for hypertension, dyslipidemia and urate nephrolithiasis. He was initially diagnosed with MM ∼18 months previously when he had presented with a right pleural-based mass, which was associated with a destructive lesion of an adjacent rib. An fine needle aspiration of the lesion was performed at that time, which showed sheets of plasma cells consistent with MM. His kappa-to-lambda ratio (κ/λ) one year previously was 75.84 and was 1.58 at the time of this presentation.

During his current presentation, he reported worsening lower extremity swelling and weight gain with decreasing urine output. He denied having diarrhea, shortness of breath, chest discomfort, orthostatic symptoms or any exposure to any nephrotoxins including contrast agents. His current medications included acyclovir, dexamethasone, fluconazole, fludrocortisone, gabapentin, loratadine, omeprazole, pentamidine inhaled, propranolol, simvastatin and trazodone. His temperature was 98.6°F

Table 2. Laboratory values of patient mentioned in the case report

	4 weeks before presentation	Labs at presentation	3 weeks after presentation
Hb (mg/dL)	11.3 (113 g/L)	5.7 (57 g/L)	8.2 (82 g/L)
Platelets	176	35	123
SCr (mg/dL)	0.7 (62 µmol/L)	6.3 (557 µmol/L)	2.9 (257 µmol/L)
UPCR	0.1	1.5	0.4
LDH (U/L)	170 (1.7 µkat/L)	777 (7.77 µkat/L)	180 (1.8 µkat/L)
Haptoglobin		<10	62
Schistocytes		2+	

Hb, hemoglobin; SCr, serum creatinine; UPC, urine protein-to-creatinine ratio; LDH, lactate dehydrogenase.

(37°C), blood pressure was 141/80 mm of Hg (no evidence of orthostasis), pulse 94/min and oxygen saturation 97% on room air. Physical exam revealed mild diffuse bilateral wheezing and lower extremity edema up to the knees. His significant laboratory results are shown in Table 2. Antiphospholipid antibodies and anti-neutrophil cytoplasmic antibody serologies were negative. Serum complements (C3 and C4) were within normal range. Urinalysis was significant for 2+blood and 2+proteins without casts or crystals. Urine microscopy was bland. Urine sodium was 33 mEq/L (33 mmol/L), and urine Cr was 97.1 mg/dL (8583 µmol/L) with a urine protein-to-creatinine ratio (UPCR) of 1.5. Renal ultrasound was unremarkable without any evidence of hydronephrosis. A presumptive diagnosis of thrombotic thrombocytopenic purpura / hemolytic-uremic syndrome symptoms was made, and empiric plasmapheresis was started. The patient was not re-challenged with CFZ again.

Plasmapheresis was continued while ADAMTS13 levels were pending. ADAMTS13 results (>50%) were available on Day 10, and thus plasmapheresis was stopped after five sessions. The patient never required dialysis, and over the next week, his sCr started to improve with improving urine out. The patient underwent an ultrasound guided kidney biopsy 1 week after presentation, when platelets were >65 000 mm³. The biopsy revealed glomerular and rare arteriolar TMA with mild acute tubular necrosis (Figure 1A and B). There was no diagnostic evidence of renal involvement by myeloma or paraproteins. His hemoglobin, platelets, lactate dehydrogenase (LDH) and haptoglobin were normalized over the next 3 weeks. The patient was treated supportively for the rest of his stay. His sCr continued to improve, and last known value 45 days after the initial presentation was 1.8 mg/dL (159 µmol/L). Workup for atypical HUS (e.g. complement factor H autoantibodies) was not done, though normal complement level and improvement in TMA argue against it.
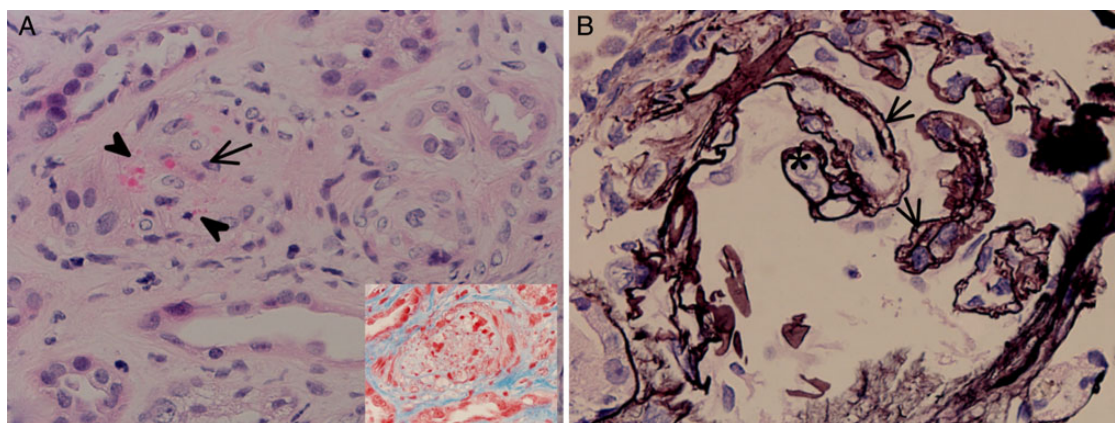
Fig. 1. Features of ongoing TMA under treatment. TMA-type changes. These changes were very focal in the biopsy, and most arteries and glomeruli were essentially unremarkable or showed nonspecific 'ischemia/recovery' changes. (**A**) Focal small interlobular artery/pre-arterioles: luminal narrowing with pale mucoid edema (arrow), and endothelial cell reaction, with erythrocyte fragmentation and 'entrapment' (arrowheads) within the intimal layer, which shows myointimal widening and clearing of cytoplasm in the myocyte cell layer (H&E stain 40× magnifications). Adjacent renal tubules show features of acute tubular injury. The inset figure shows a trichrome stain (60× magnification), which enhances the fragmented erythrocytes (the hallmark finding of microvascular hemolysis) in red and a pale intima with no significant collagen deposition (which would have stained blue). (**B**) Glomerulus: capillary loop 'double-contours' [arrow, sharp silver positive (black) linear staining on each of the capillary loop sides, which does not stain in between them], which indicate glomerular basement membranes reduplication response to endothelial damage (note the 'plump' endothelial cell occlusion, asterisk). Also noted is ischemic wrinkling ('collapse'—arrowhead) of other glomerular capillary loops (Jones methenamine silver stain, 60× magnification). BC (Bowman's capsule).

We postulate CFZ as a potential etiology of TMA. Discontinuation of CFZ along with supportive management was associated with renal recovery in our patient. This patient presented with AKI secondary to TMA associated with MAHA (elevated LDH, low haptoglobin, schistocytes on peripheral blood smear) and thrombocytopenia. Although causality is difficult to establish, initiation of drug followed by rapid onset of AKI without any known precipitating factors and recovery after stopping the drug makes it very convincing to postulate that CFZ induced MAHA with TMA in kidney and this resulted in the development of AKI. The patient in this report had no other obvious cause of AKI including volume depletion, tumor lysis syndrome or administration of iodinated contrast medium. Active infection (sepsis) or the presence of disseminated intravascular coagulation was ruled out with negative blood, urine and respiratory cultures along with a normal fibrinogen level (211 mg/dL). He was not exposed to any other therapeutic agents except CFZ, pomalidomide and dexamethasone 2 months before presentation and was in the middle of his second cycle. This patient did have a history of two hematopoietic stem cell transplants (HSCT) 6 and 9 months prior to the presentation. HSCT has been associated with MAHA and renal TMA and is called transplant-associated thrombotic microangiopathy (TA-TMA) [27–29]. TA-TMA is believed to be caused by direct microvascular toxicity initiated by endothelial injury [30]. The patient in our report was 6 months out of HSCT and had spontaneous recovery after discontinuation of CFZ, and thus, his presentation was unlikely to be TA-TMA.

Proposed pathogenesis of proteasome inhibitor-associated thrombotic microangiopathy

Syndromes of TMA present clinically with anemia, thrombocytopenia and signs of intravascular hemolysis, e.g. high LDH and low haptoglobin. All of the causes and pathological mechanism create a pro-thrombotic state and potentiate formation of microthrombi leading to end-organ damage. TMA can be hereditary and acquired [31, 32] (Table 3).

Table 3. Causes of TMA [33, 34]

Hereditary causes	• ADAMTS 13 deficiency • Loss of function mutation in the gene controlling alternative complement pathway regulatory proteins, e.g. Factor H • Mutation in MMACHC and DGKE genes
Acquired causes	• Antibodies against ADAMTS13 • Shiga toxin activating Gb3 • Antibodies against Factor H • Drug-induced TMA (VEGF inhibition and/or drug-dependent antibodies)

ADAMTS 13, A disintegrin and metalloproteinase with a thrombospondin type 1 motif, member 13—also known as von Willebrand factor-cleaving protease (VWFCP); MMACHC, methylmalonic aciduria and homocystinuria type C protein; DGKE, diglyceride kinase ppsilon; Gb3, globotriaosylceramide; TMA, thrombotic microangiopathy; VEGF, vascular endothelial growth factor.

VEGF inhibition has been proposed as one of the potential pathological factors responsible for TMA associated with AKI [25, 26]. VEGF is critical in maintenance and growth of normal vasculature. It is produced by podocytes in high concentration and is considered to diffuse across the glomerular basement membrane against the urinary flow and with its concentration gradient to bind to its receptors on glomerular endothelial cells. Podocytes and glomerular endothelial cells use VEGF-VEGF-R2 signaling for transmembrane communication, which is essential for structural and functional integrity of glomerular endothelium and fenestrations [35]. Any disruption of this mechanism by inhibiting its production (proteasome inhibitors), using bevacizumab (anti-VEGF antibodies), pazopanib (inhibits VEGF 1–3) and sunitinib (VEGF tyrosine kinase inhibitor) can potentially lead to kidney damage specifically TMA and podocytopathy causing proteinuria and hypertension [25, 26, 35].

Proteasome inhibitors affect VEGF pathways via NF-κB pathways [33, 34, 36] (Figure 2). *NF-κB* is a protein involved in the regulation of genetic transcription. NF-κB activation in response to various
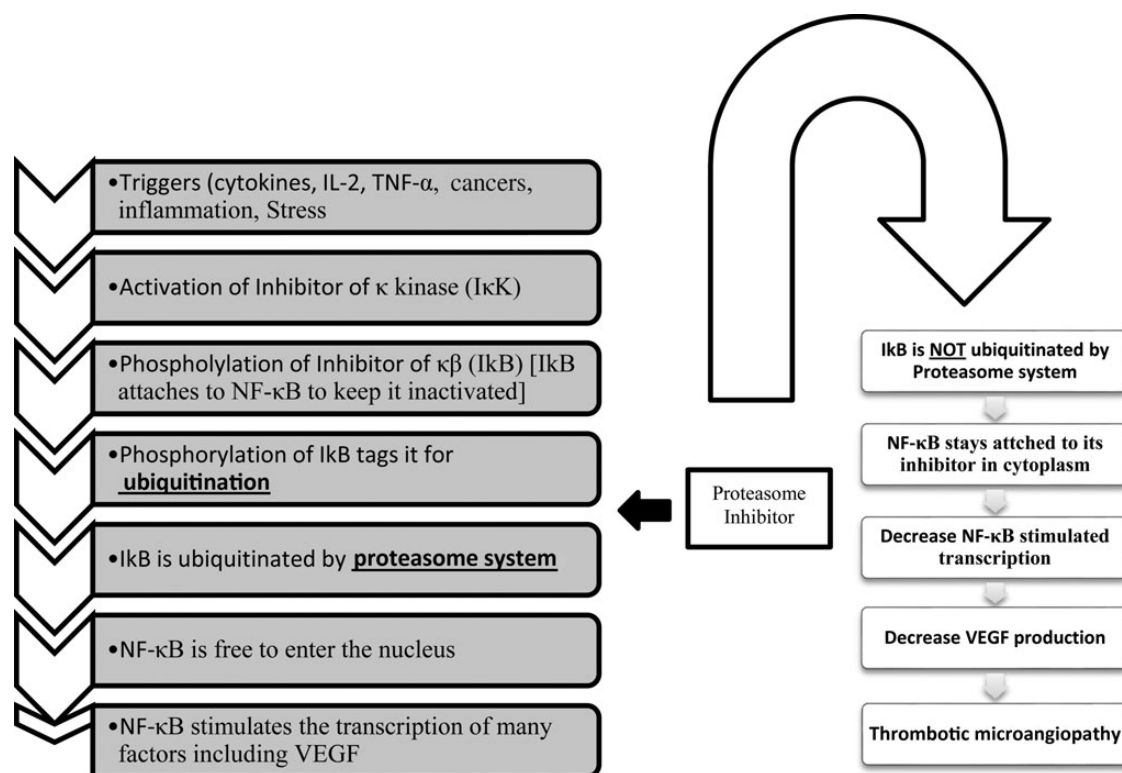
Fig. 2. Proposed pathogenesis of proteasome inhibitor-associated thrombotic microangiopathy.

extracellular signals (including reactive oxygen species, TNFα, IL-1β, bacterial polysaccharides and radiation) is initiated by degradation of IκB (inhibitor of NF-κB). IκB kinase (IKK), when activated by extracellular signals described above, causes phosphorylation of IκB leading to ubiquitination of IκB. After IκB degradation, NF-κB is free to enter the nucleus and influence DNA transcription [37]. An increase in the activity of NF-κB promotes VEGF expression [38]. Proteasome inhibitors prevent ubiquitination of IκB even after phosphorylation by IKK; this in turn prevents NF-κB from entering the nucleus and effecting transcription. Thus, proteasome inhibitors decrease NF-κB levels in the nucleus leading to decreased VEGF production [39–41], potentially predisposing to TMA.

Acknowledgements

Conflicts of interest statement

None declared.

(See related articles by Thakkar *et al*. Onconephrology abstracts and publication trends: time to collaborate. *Clin Kidney J* (2015) 8: 629–631 and by Stallone *et al*. Management and prevention of post-transplant malignancies in kidney transplant recipients. *Clin Kidney J* (2015) 8: 637–644.)

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