The proteasome inhibitor Bortezomib aggravates renal ischemia-reperfusion injury

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Submitted 25 September 2008; accepted in final form 15 May 2009

Huber JM, Tagwerker A, Heininger D, Mayer G, Rosenkranz AR, Eller K. The proteasome inhibitor Bortezomib aggravates renal ischemia-reperfusion injury. Am J Physiol Renal Physiol 297: F451–F460, 2009. First published May 20, 2009; doi:10.1152/ajprenal.90576.2008.—Bortezomib is a well-established treatment option for patients with multiple myeloma (MM). It is a selective and reversible inhibitor of the proteasome that is responsible for the degradation of many regulatory proteins that are involved in apoptosis, cell-cycle regulation, or transcription. Because patients with MM are prone to develop acute renal failure, we evaluated the influence of Bortezomib on renal ischemia-reperfusion injury (IRI). Mice were subjected to renal IRI by having the renal pedicles clamped for 30 min followed by reperfusion for 3, 24, and 48 h. Mice were either pretreated with 0.5 mg/kg body wt Bortezomib or vehicle intravenously 12 h before induction of IRI. Serum creatinine and tubular necrosis were significantly increased in Bortezomib compared with vehicle-treated mice. The inflammatory response was found to be significantly decreased in Bortezomib-treated mice as reflected by a decreased infiltration of CD4+ T cells and a significantly decreased Th1 cytokine expression in the kidneys. In contrast, apoptosis was significantly increased in kidneys of Bortezomib-treated mice compared with vehicle-treated controls. Increased numbers of TUNEL-positive cells/mm² and increased mRNA expression of proapoptotic factors were detected in kidneys of Bortezomib-treated mice. Of note, p21, a cell senescence marker, was also significantly increased in kidneys of Bortezomib-treated mice. In summary, we provide evidence that Bortezomib worsens the outcome of renal IRI by leading to increased apoptosis of tubular cells despite decreased infiltrating T cells and proinflammatory mediators.

Inflammation; T cells; apoptosis; senescence

THE UBQUITIN-PROTEASOME SYSTEM is the major intracellular degradation pathway in eukaryotic cells and leads to degradation of key proteins including p53 and NF-κB, which are involved in apoptosis, inflammation, senescence, and angiogenesis (46). Bortezomib is a first-in-class selective and reversible inhibitor of the 20S proteasome that has been approved for the treatment of multiple myeloma (MM) in patients who have received at least one prior treatment and who progressed on their last therapy. The overall response rates in the phase II trials accounted for 33–50% (21, 38). Recent reports provided compelling evidence that Bortezomib has the potential to improve renal failure, which is associated with MM (7, 22, 33). The most common cause of renal failure is due to tubular damage by light chains, so called cast nephropathy or myeloma kidney. In general, patients with MM are prone to acute kidney injury (AKI) on the basis of divergent forms of insult to the kidney including ischemia (3, 24). Nevertheless, the mechanisms leading to the observed improvement in kidney function by Bortezomib are unknown.

Renal ischemia-reperfusion injury (IRI) is associated with a cascade of cellular events leading to tissue damage and subsequent increased clinical morbidity in the setting of AKI or decreased allograft survival in the setting of kidney transplantation (19, 45). Several key players in renal IRI, namely apoptosis (40), senescence (16, 31) and inflammation (37), have been shown to be regulated by the proteasome inhibitor Bortezomib (2, 6, 12, 39). This drug influences cell-cycle regulators such as p21 that are involved in senescence and proteins involved in apoptosis, such as p53, p21, and bax (1, 28). On the other hand, it inhibits the NF-κB pathway, which is involved in many inflammatory reactions (reviewed in Ref. 12). Proteasome-inhibition has been shown to be beneficial in other models of IRI, namely cerebral (35, 49), myocardial IRI (5, 18, 36), and skeletal muscle IRI (34), by acting anti-inflammatory.

In contrast, we here provide evidence that proteasome inhibition by Bortezomib aggravates renal IRI by increasing apoptosis despite its potency of limiting the inflammatory response.

MATERIALS AND METHODS

Animals and renal ischemia model. Experiments were performed on male C57BL/6 mice weighing 18–22 g at the age of 10–12 wk. To evaluate the optimal dose of Bortezomib to use for in vivo applications in the kidney, we injected intravenously different doses of Bortezomib (0, 0.1, 0.25, 0.5, and 0.75 mg/kg body wt) and evaluated the markers of the unfolded protein response after 12 h. The dose of 0.5 mg/kg body wt was revealed to be the minimal dose for our application (Fig. 2). We chose the timing of Bortezomib injection to get optimal inhibition of the proteasome. The timing is comparable to Neubert et al. (32). Therefore, mice received 0.5 mg/kg body wt Bortezomib (LC Laboratories, Woburn, MA) or vehicle, respectively, 12 h before experimental IRI. Bortezomib was dissolved in DMSO at a concentration of 3.8 mg/ml (10 mmol/l), and aliquots were stored at −80°C. Stock solution was diluted in 100 μl 0.9% (wt/vol) NaCl to a final concentration of 0.5 mg/kg body wt. The vehicle contains 2.5 μl DMSO in 100 μl 0.9% (wt/vol) NaCl. Mice were anesthetized, an incision was made on the central abdomen, and microvascular clamps were applied to bilateral renal pedicles. After 30 min of renal ischemia, clamps were removed and the incision was closed. During the procedure, body temperature of mice was controlled at 37.5°C using an adjustable heating pad. Animals were euthanized after 3, 24, or 48 h. Sham-operated animals received either Bortezomib or vehicle and underwent the same surgical procedure without having the renal pedicles clamped. They were euthanized after 48 h. We had no drop outs or deaths before the 48-h observation period. The investigator performing the clamping of renal pedicles was blinded for the experimental groups and was not directly involved in the evaluation of the
experiments. All animal experiments were approved by Austrian veterinary authorities (BMBWK-66.011/0126-BrGT/2006).

Assessment of renal function. Blood samples were obtained from mice 3, 24, and 48 h after ischemia, and serum creatinine levels were measured as markers of renal function. Serum creatinine was measured using a creatinine autoanalyzer (Beckman Coulter, Fullerton, CA).

Assessment of tubular necrosis. Kidney tissue was fixed in buffered 4% formalin overnight and then embedded in paraffin wax. The kidneys were sectioned at 4 μm and stained with periodic acid-Schiff (PAS) or hematoxylin and eosin (HE) using a standard protocol. Tubular injury was assessed in PAS-stained sections using a semiquantitative scale as recently described (37a). Briefly, the percentage of cortical tubules showing epithelial necrosis was scored: 0 = no tubular necrosis; 1 = <10%; 2 = 10–25%; 3 = 26–50%; 4 = 51–75%; 5 = >75% tubular necrosis. The scoring was performed by one blinded pathologist.

Purification of the 20S proteasome and measurement of proteasome activity. The 20S subunits were purified as described (13). Briefly, a part of the explanted kidneys (≈50 mg) were homogenized in 1.5 volumes buffer A (20 mM Tris, 20 mM KCl, 10 mM magnesium acetate, 2 mM DTT, and 10% glycerol, pH 7.6). The soluble protein fraction was isolated by centrifugation at 30,000 g for 30 min at 4°C. The pellet was discarded, and the supernatant of this first centrifugation step was centrifuged again at 100,000 g for 6 h at 4°C. The pellet of this second centrifugation step was washed twice and resuspended in 200 μl fresh buffer A. The protein concentrations were determined using the BCA Protein Assay Kit (Pierce, Rockford, IL). Proteasome activity was measured by the release of 7-amino-4-methylcoumarine after cleavage from the labeled substrate Suc-LLVY-AMC according to the protocol recommended by the manufacturer (cat. no. APT280; Chemicon International, Hampshire, UK). The product was measured at an emission wavelength of 465 nm (λexcitation = 360 nm).

Immunohistochemical studies. Immunohistochemistry was performed on frozen sections as described recently (17). Briefly, macrophages were stained using a F4/80 rat anti-mouse macrophage antibody (clone: CI:A3–1; Serotec, Oxford, UK), and a semiquantitative scoring system was performed as follows: 0 = no cells stained positive, 1+ = 5 to 10 cells, 2+ = 10 to 50 cells, 3+ = 50 to 200 cells, and 4+ = over 200 cells stained positive per low-power field. For the detection of CD4+ T cells, a rat anti-mouse CD4 monoclonal antibody (clone YTS191.1; Serotec) was used. In all cases, an IgG2a isotype antibody (clone G155–178; Pharmingen, San Diego, CA) served as a negative control. Biotin-conjugated goat anti-rat IgG antibody (Jackson ImmunoResearch Laboratories, Cambridgeshire, UK) was used as a secondary antibody, followed by incubation with an avidin-biotin complex (Vector Laboratories, Burlingame, CA) and subsequent development with 0.4% 3-amin-9-ethylcarbazole (Sigma, St. Louis, MO) for 4 min and counterstain-
ing with Gill’s Hematoxylin No. 3 (Polysciences, Warrington, PA). Quantification of T cells was done by counting the number of cells in six adjacent high-power fields of renal cortex and medulla. For the detection of cleaved caspase 3, the same technique was used. As the primary antibody, a rabbit anti-cleaved caspase 3 antibody (Cell Signaling, Danvers, MA) was used. Biotin-conjugated goat anti-rabbit IgG antibody (ImmuNoResearch Laboratories) was used as a secondary antibody. Quantification was done by counting the number of positive cells in six adjacent high-power fields in the inner cortex and outer medulla, which are mostly affected by renal IRI.

Quantification of mRNA by real-time PCR. Total RNA was isolated from tissue using TRIreagent (Molecular Research Center; Cincinnati, OH) according to the protocol recommended by the manufacturer. Reverse transcription was carried out with 4 μg of total RNA using SuperScript III Reverse Transcriptase (Invitrogen, Carlsbad, CA) and random primers (Invitrogen) according to the protocol recommended by the manufacturer. The cDNA was subjected to real-time PCR amplification using either TaqMan Gene Expression Assays (Applied Biosystems, Foster City, CA) for bip (Mm00457866_m1), chop (Mm00492097_m1), GATA-3 (Mm00484683_m1), IFN-γ (Mm00450960_m1), IL-10 (Mm00439616_m1), mpo (Mm01298424_m1), p21 (Mm00432448_m1), T-box expressed in T cells (T-bet) (Mm00457866_m1), or trail-R2 (Mm00547866_m1) in combination with TaqMan Universal PCR Master Mix (Applied Biosystems) or Platinum SYBR Green qPCR SuperMix-UDG (Invitrogen) together with the respective primer pairs: bax: forward, CTG AGC TCA CCT TGG AGG, reverse, GCC ATC CAC AAA GAT G; fas: forward, TGT GCT GGT TGC TGT GC; TNF-α: forward, GAG GTC TGG GCC ATA GAA CT.

In situ cell death detection. TUNEL was performed using a commercially available staining kit (Roche, Basel, Switzerland) with the following changes to the recommended protocol. Glass slides were pretreated with Vectabond (Vector Laboratories) according to the manufacturer’s protocol. After rehydration, tissue sections were treated with 10 μg/ml Proteinase K (Roche), and TUNEL mixture was applied onto each section. The slides were incubated in a humidified chamber for 60 min at 37°C in the dark. Finally, slides were rinsed three times in PBS. Positive controls were generated by incubating samples from control kidneys at room temperature for 10 min with 100 μl of a 1,000 U/ml solution of DNase I (Invitrogen) in DNase I buffer (50 mM Tris-HCl, pH 7.5, 1 mg/ml BSA) to induce strand breaks. Sections were then washed three times in PBS. Negative controls were created by incubating sections with label solution alone. Nuclei were counterstained with DAPI (Molecular Probes Europe, Leiden, The Netherlands) at a concentration of 300 nM. TUNEL-positive tubular cells per mm² in cortical tubules were quantified.

Table 1. Difference between Bortezomib-treated mice and vehicle-treated sham-operated controls

<table>
<thead>
<tr>
<th>Bortezomib</th>
<th>Vehicle</th>
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<tbody>
<tr>
<td>Creatinine, mg/dl</td>
<td>0.12±0.03</td>
</tr>
<tr>
<td>bax</td>
<td>0.799±0.206</td>
</tr>
<tr>
<td>puma</td>
<td>0.354±0.020</td>
</tr>
<tr>
<td>fas</td>
<td>1.172±0.217</td>
</tr>
<tr>
<td>p21</td>
<td>0.225±0.113</td>
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Data are expressed as mean ± SD. Mice received either 0.5 mg/kg body wt Bortezomib or vehicle intravenously, and sham operations were performed after a 12-h delay (n = 4 per group). After 48 h, mice were euthanized, and serum creatinine was evaluated. Additionally, expression of bax, puma, fas, and p21 was evaluated in kidneys by real-time PCR, n.s., not significant.

Fig. 2. The proteasome activity is inhibited and the unfolded protein response (UPR) is activated by Bortezomib. Mice were treated with different concentrations of Bortezomib (n = 4 per group). A: after 12 h, the purification of the 20S proteasome subunit of kidneys was performed, and the inhibitory function of Bortezomib was evaluated. Proteasome activity was measured by the release of 7-amino-4-methylcoumarine after cleavage from the labeled substrate Suc-LVVY-AMC. A dose of 0.5 and 0.75 mg/kg body wt significantly inhibited the proteasome activity in the kidney compared with vehicle-treated controls. Data are presented as means ± SD. Additionally, the UPR was evaluated by measuring the UPR markers bip (B) and chop (C) in the kidneys by real-time PCR. Both were significantly increased in kidneys of mice treated with 0.5 and 0.75 mg/kg body wt. The fold increase compared with the ΔCt of healthy mice is given. (*P < 0.05; n.s. not significant.)
Statistical analysis. The Mann-Whitney U-test was performed, and \( P < 0.05 \) was considered as significant. In the case of evaluating scores, we used the adjacent Chi-square test. Statistical analyses were done with SPSS 13.0.1 for Windows (SPSS, Chicago, IL).

RESULTS

Renal IRI is aggravated by Bortezomib treatment. Mice were either treated with 0.5 mg/kg body wt of Bortezomib or vehicle intravenously. After 12 h, mice were subjected to renal

Table 2. The Th1 response is decreased by Bortezomib

<table>
<thead>
<tr>
<th></th>
<th>3 h</th>
<th>24 h</th>
<th>48 h</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>B</td>
<td>V</td>
<td>B</td>
</tr>
<tr>
<td>T-bet</td>
<td>1.00±0.35</td>
<td>1.04±0.26</td>
<td>0.29±0.20*</td>
</tr>
<tr>
<td>IFN-(\gamma)</td>
<td>0.97±0.31</td>
<td>1.10±0.50</td>
<td>0.84±0.55</td>
</tr>
<tr>
<td>TNF-(\alpha)</td>
<td>0.84±0.33</td>
<td>1.19±0.54</td>
<td>0.96±0.49</td>
</tr>
<tr>
<td>GATA-3</td>
<td>1.07±0.35</td>
<td>1.02±0.20</td>
<td>0.87±0.24</td>
</tr>
<tr>
<td>IL-10</td>
<td>0.85±0.94</td>
<td>0.60±0.64</td>
<td>0.98±0.61</td>
</tr>
</tbody>
</table>

Data are expressed as mean ± SD. Mice were treated with Bortezomib (B) or vehicle (V) 12 h before induction of ischemia. Kidneys were allowed to reperfuse for 3 h \( (n = 12 \) Bortezomib group, \( n = 13 \) vehicle group), 24 h \( (n = 12 \) Bortezomib group, \( n = 14 \) vehicle group), or 48 h \( (n = 9 \) Bortezomib group, \( n = 8 \) vehicle group). Evaluation of the Th1 marker T-bet and the Th1-specific cytokines IFN-\(\gamma\) and TNF-\(\alpha\) as well as the Th2 marker GATA-3 and the Th2-specific cytokine IL-10 was performed by quantitative PCR. *\( P < 0.05 \) as compared with vehicle-treated mice after the respective reperfusion interval.
IRI by having renal pedicles clamped for 30 min followed by reperfusion of 3, 24, or 48 h. Significantly increased serum creatinine levels were observed in Bortezomib- compared with vehicle-treated mice after 3, 24, and 48 h of reperfusion, respectively (Fig. 1A). Tubular necrosis score was significantly elevated in Bortezomib-treated mice compared with vehicle-treated mice after 48 h (Fig. 1B). Of note, no difference in serum creatinine and tubular necrosis was detected between Bortezomib- and vehicle-treated sham-operated controls (Table 1).

To test whether Bortezomib effectively works in the kidney, we evaluated the inhibition of proteasome activity and the activation of the terminal unfolded protein response (UPR) by measuring the expression of bip and chop, both markers of activation of the UPR (32). We tested different doses of Bortezomib and found 0.5 mg/kg body wt to be the minimal dose to significantly inhibit the proteasome activity (Fig. 2A) and to significantly increase the expression of bip and chop in the kidneys immediately before inducing renal IRI (Fig. 2, B and C).

The inflammatory response is inhibited by Bortezomib treatment. Next, we determined the infiltration of neutrophils, macrophages, and CD4⁺ T cells into kidneys of mice subjected to renal IRI that were treated with either Bortezomib or vehicle. No differences were found in the neutrophil and macrophage kidney infiltration between the two groups (data not shown). Interestingly, decreased levels of kidney-infiltrating CD4⁺ T cells were observed in kidney sections of Bortezomib-treated mice compared with vehicle-treated mice after 3, 24, and 48 h of reperfusion (Fig. 3). The difference reached significance after 48 h of reperfusion.

To evaluate a possible predominance of CD4⁺ T cell subtypes, we performed real-time PCR for transcription factors specific for the Th1 and Th2 lineage, namely T-box expressed in T cells (T-bet) and GATA binding protein 3 (GATA-3), respectively. The expression of T-bet in kidneys of Bortezomib-treated mice was decreased after 3 h and reached significantly decreased levels after 24 and 48 h of reperfusion compared with vehicle-treated mice (Table 2). In line, the Th1 cytokines IFN-γ and TNF-α were also significantly deceased in Bortezomib-treated mice 48 h after ischemia (Table 2). In contrast, the Th2-specific transcription factor GATA-3 and its transcript IL-10 were equally regulated in Bortezomib- and vehicle-treated mice at all tested endpoints (Table 2).

Bortezomib increases apoptosis in renal IRI. The initiation of cell loss through the process of apoptosis plays a prominent role in the pathogenesis of renal IRI (41). Therefore, several apoptosis-relevant factors including members of the intrinsic pathway of apoptosis as bcl-2-associated X protein (bax) and p53-upregulated mediator of apoptosis (puma) and a contributor of the extrinsic pathway of apoptosis, namely fas, were evaluated in kidneys by real-time PCR. The intrinsic pathway markers bax and puma were significantly increased in kidneys of Bortezomib-treated mice compared to vehicle-treated mice 3 and 24 h after inducing renal IRI (Fig. 4, A and B). Fas was found to be significantly increased in kidneys of Bortezomib-treated mice compared with respective controls at all tested endpoints (Fig. 4C). Of note, no significant difference in the transcription of these proapoptotic genes was detected in Bortezomib-treated mice compared with vehicle-treated sham-operated controls (Table 1).
For localization and quantification of apoptotic cells within the kidney, TUNEL assay was performed on renal tissue sections. Increased levels of apoptotic cells were counted in sections of Bortezomib-treated mice compared with vehicle-treated mice after 24 h of reperfusion (Fig. 5). This was conferred by evaluating the number of cells expressing cleaved caspase 3 (Fig. 6).

Expression of the CDK inhibitor p21. The cyclin-dependent kinase inhibitor p21 is described to be upregulated during renal IRI (16) and to be regulated by the proteasome inhibitor

![Graph](image)

Fig. 5. Bortezomib increases apoptosis in renal IRI. Mice were treated with Bortezomib (shaded box) or vehicle (open box) 12 h before induction of ischemia. Kidneys were allowed to reperfuse for 3 h (n = 12 Bortezomib group, n = 13 vehicle group) and 24 h (n = 13 Bortezomib group, n = 14 vehicle group). TUNEL assay was performed on paraffin-embedded sections. Apoptotic cells were quantified and displayed as TUNEL-positive cells/ mm². Significantly increased levels of apoptotic cells were detected in kidneys of Bortezomib-treated mice compared with vehicle-treated mice after 24 h of reperfusion (*P < 0.05). Additionally, representative pictures of TUNEL stainings of vehicle-treated mice (B-D) and Bortezomib-treated mice (E-G) 24 h after induction of renal IRI are shown. We performed double staining to show the TUNEL-positive nuclei (white arrows). Nuclei were stained with DAPI, and overlays with TUNEL-positive cells (FITC) are shown (magnification ×400).
Bortezomib (6, 27). Therefore, we used our experimental setting of renal IRI to determine the regulation of p21 transcription and found significantly increased levels of p21 mRNA in kidneys of Bortezomib-treated mice compared with respective controls 3, 24, and 48 h after induction of renal IRI (Fig. 7).

Of note, no difference in the expression of p21 was observed between Bortezomib-treated mice and vehicle-treated sham-operated controls (Table 1).

The proteasome inhibitor Bortezomib has been shown to interfere with nearly all pathways involved in the pathogenesis of renal IRI. Because MM patients are prone to many forms of AKI and Bortezomib is very potent to reverse MM-associated cast nephropathy, we were interested in its effects on renal IRI. Most interestingly, mice treated with Bortezomib displayed aggravated renal IRI in the presented study by increasing apoptosis and senescence despite limitations of the inflammatory response.

So far, Bortezomib has been shown to have the potential to limit antigen presentation by dendritic cells (43), the proliferation of B cells (32), and the activation of Th1 cells including the production of Th1 cytokines (2, 4). Because T cells have been shown to play a prominent role in the pathogenesis of renal IRI (17, 37), we focused on evaluating T cell infiltration and T cell-specific cytokine expression in the kidneys. In line with the published in vitro data (2), we found a significant decrease in the CD4+ T cell infiltration into kidneys of Bort-
Fig. 7. Expression of the CDK inhibitor p21 is elevated by Bortezomib. Mice were treated with Bortezomib (shaded box) or vehicle (open box) 12 h before induction of ischemia. Kidneys were allowed to reperfuse for 3 h (n = 12 Bortezomib group, n = 13 vehicle group), 24 h (n = 13 Bortezomib group, n = 14 vehicle group), or 48 h (n = 11 Bortezomib group, n = 10 vehicle group). Levels of p21 mRNA were evaluated by quantitative PCR. Bortezomib-treated mice displayed significantly increased p21 mRNA levels at all tested endpoints compared with vehicle-treated mice (*P < 0.05).

Pezomib-treated mice displayed significantly increased p21 mRNA levels at all group). Levels of p21 mRNA were evaluated by quantitative PCR. Bortezomib group, n = 11 vehicle group). The stabilization of p53, is also known to be stabilized by Bortezomib (48) and was found to be significantly increased in our Bortezomib-treated mice after all reperfusion time points. p21 is significantly upregulated in renal IRI (9, 16) and seems to be a promising marker for increased damage of tubular cells (16). Thus its role in the pathogenesis is inconclusive, and data are divergent. Although Meygesi and coworkers (30) provided compelling evidence by using p21-knockout animals that up-regulation of p21 is beneficial for the outcome of renal IRI (30), functional studies using inhibitors showed opposite effects (16, 23). To date, it is not known whether the observed upregulation of p21 in the Bortezomib-treated mice is a protective or harmful mechanism of renal tubular cells.

Contrary to our data, the group of Matsumura (20, 44) has provided data on the effect of other proteasome inhibitors, namely N-benzylxyacarbonyl-Ile-Glu(o-t-Bu)-Ala-leucinal and lactacycin, on the outcome of renal IRI. By using a different model of renal IRI (45-min ischemia and contralateral nephrectomy) and different concentrations of the proteasome inhibitors, they found the proteasome inhibitors to be beneficial by decreasing endothelin-1 expression without evaluating apoptosis or infiltration of inflammatory cells (20, 44). The differences observed in our model of renal IRI compared with other models of IRI (5, 18, 35) might be due to the multiple effects of Bortezomib. Differences to models of cerebral IRI (35) might be explained by the fact that Bortezomib can only in very limited amounts cross the blood-brain barrier (14) and thereby act only systemically anti-inflammatory rather than locally proapoptotic. It might further be speculated that Bortezomib can induce apoptosis in a different amount depending on the cell population affected. Thus ischemic skeletal and heart muscle cells (5, 18) might be less susceptible to Bortezomib compared with ischemic tubular cells.

The clinical implications of our data are that when starting Bortezomib it has to be taken into account that patients are not at risk developing or having an AKI because of ischemia. The effects of Bortezomib might be comparable to Rapamycin that has been shown to delay the recovery of AKI in renal transplant patients (29, 42), probably because of limiting tubular recovery (25). Moreover, recent clinical studies have proven a beneficial effect of Bortezomib on the renal function in MM patients with cast nephropathy (7, 22, 33). Thus the mechanism leading to improvement of renal function in multiple myeloma by Bortezomib seems to be the result of a decrease of the B cell clone rather than a beneficial effect on harmed tubular cells.

Together, Bortezomib has beneficial but also detrimental effects on renal IRI because it acts in a way that is anti-inflammatory but also proapoptotic. The proapoptotic effects of Bortezomib seem to outweigh its anti-inflammatory capacities thereby increasing renal failure in IRI.

**REFERENCES**


**GRANTS**

This work was sponsored by an educational grant from Janssen-Cilag.

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