Effects of Proteasome Inhibition on Vascular Function

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The proteasome inhibitors (PI) bortezomib (BTZ) and carfilzomib (CFZ) are cornerstone therapies for the treatment of multiple myeloma (MM). BTZ, a first generation and reversible PI, has never demonstrated any discernible vascular toxicity. In contrast, multiple studies have demonstrated an association with vascular toxicity with CFZ, an irreversible PI, including hypertension. However the mechanisms underlying CFZ-induced vascular effects remain unknown. We hypothesized that CFZ causes endothelial toxicity and reno-vascular pathology, leading to vascular dysfunction and hypertension.

Human umbilical vein cells were treated with increasing concentrations of BTZ and CFZ (1 nM to 10 uM) for 1 hour. Cell viability by MTS assay after 24 hours was significantly reduced with CFZ in a dose-dependent manner (20% viability at 10 uM) compared to BTZ (80% viability at 10 uM). In order to assess the effects of PI treatment *in vivo*, we first confirmed proteasome inhibition after intraperitoneal and intravenous injection of CFZ and BTZ in wild-type C57BL/6 mice. Chymotrypsin-like peptidase activity was inhibited acutely, 4 hours after injection, for both CFZ and BTZ; however, only CFZ demonstrated inhibition after chronic therapy (twice weekly injections for up to 4 weeks). *Ex vivo* murine mesenteric arteriole function was assessed after 2 and 4 weeks of twice weekly CFZ and BTZ treatment. Both endothelium-dependent (in response to acetylcholine) and –independent (with sodium nitroprusside) vascular relaxation were preserved after CFZ and BTZ treatment, when compared to vehicle control. Immunohistochemistry (IHC) of aorta and kidney sections after 2 weeks of CFZ treatment showed no change in adventitial collagen deposition. However, T-cell CD3 staining was increased, and staining for F4/80, a macrophage marker, was decreased in kidney sections of CFZ-treated animals as compared with animal controls. There was no change in CD3 and F4/80 staining in aortic sections of CFZ-treated animals.

These studies suggest decreased endothelial cell viability after CFZ treatment. Increased IHC staining for CD3 in CFZ-treated murine kidneys suggest a possible immune-mediated mechanism for hypertension and vascular events and warrants further research.

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