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Tanespimycin Protects Cultured Rat Dorsal Root Ganglia from Bortezmib Toxicity

John Megill[1], Oliver Flint[1], Jae Kwagh[1], Faye Wang[1], Christopher Storck[1], Frederic Moulin[1], Jan Oberdoerster[2], David Berman[1], Bruce Car[1]

1Bristol-Myers Squibb Research and Development, PO Box 5400, Princeton, NJ 08543
2 Bristol-Myers Squibb Research and Development, 6000 Thompson Road, East Syracuse NY, 13057

Tanespimycin, an inhibitor of Hsp90, is in phase 3 clinical trials in combination with bortezomib in patients with relapsed/refractory multiple myeloma. Bortezomib -induced peripheral neuropathy is the key dose-limiting toxicity in patients. In a rat model tanespimycin reversed bortezomib -induced peripheral neuropathy. An in vitro method was developed to explore the mechanism of tanespimycin-mediated neuroprotection in primary rat dorsal root ganglion (DRG) cells.

Differentiated DRG cultures (d17 rat fetuses) were treated for 24 hours with tanespimycin (10 - 5000 nM), or bortezomib (1 - 1000 nM) alone, or in combination. ATP, caspase 3/7 induction, calpain activity, proteasomal chymotrypsin-like activity, and HSp70 induction (in-cell Western) were used as measures of cell viability, apoptosis, neuron-specific protease activity, proteasome function, and Hsp90 inhibition, respectively. Neuronal morphology was evaluated by light and electron microscopy. At concentrations ≥100 nM, both bortezomib and tanespimycin induced 5-fold or greater increases in caspase activity. Cell viability (ATP) was reduced to 20% control values. bortezomib (≥100 nM) reduced DRG neurite extensions. When DRG cells were co-exposed to both tanespimycin (>500 nM) and bortezomib, concentration-dependent decreases in viability were abrogated and neurite extensions were preserved. Tanespimycin, but not bortezomib, induced both calpain and proteasome activity (5- and 3-fold, respectively, and these increases were reversed by combinations of tanespimycin and bortezomib. Hsp70 induction by tanespimycin (100 nM) was more than doubled in combination with bortezomib (10 nM).

In primary cultures of rat DRG neurons, Tanespimycin ameliorated bortezomib -induced apoptosis and loss of viability, and restored neuronal morphology. These neuroprotective effects of Tanespimycin are consistent with the effects observed in a rat model of bortezomib-induced peripheral neuropathy and with the lack of severe peripheral neuropathy observed in patients in the phase 1/2 study of Tanespimycin combined with bortezomib in multiple myeloma. Possible mechanisms for the protective effect of tanespimycin on bortezomib-induced neuronal toxicity in multiple myeloma include super-induction of Hsp70 and abrogation of calpain activity.

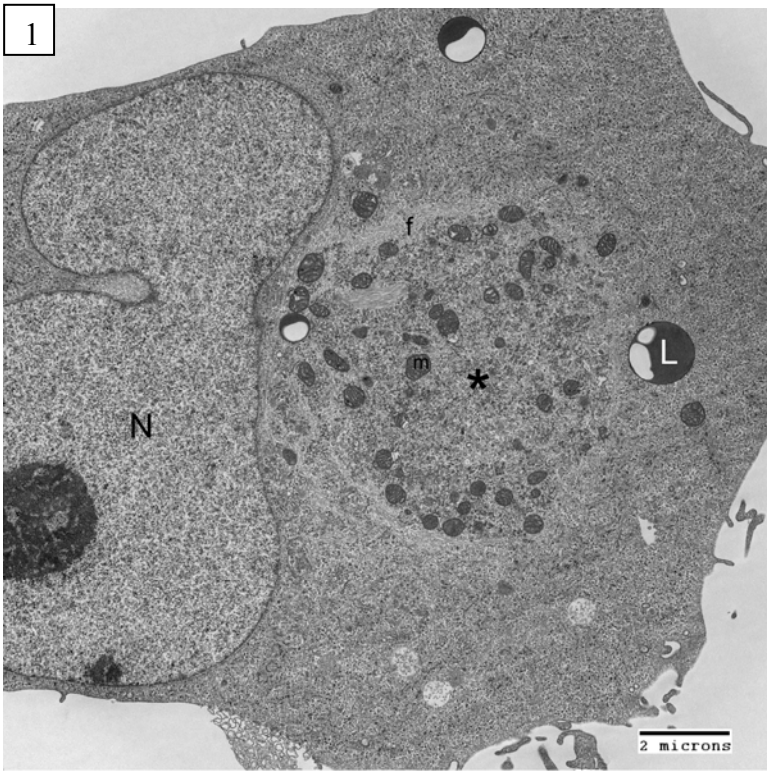
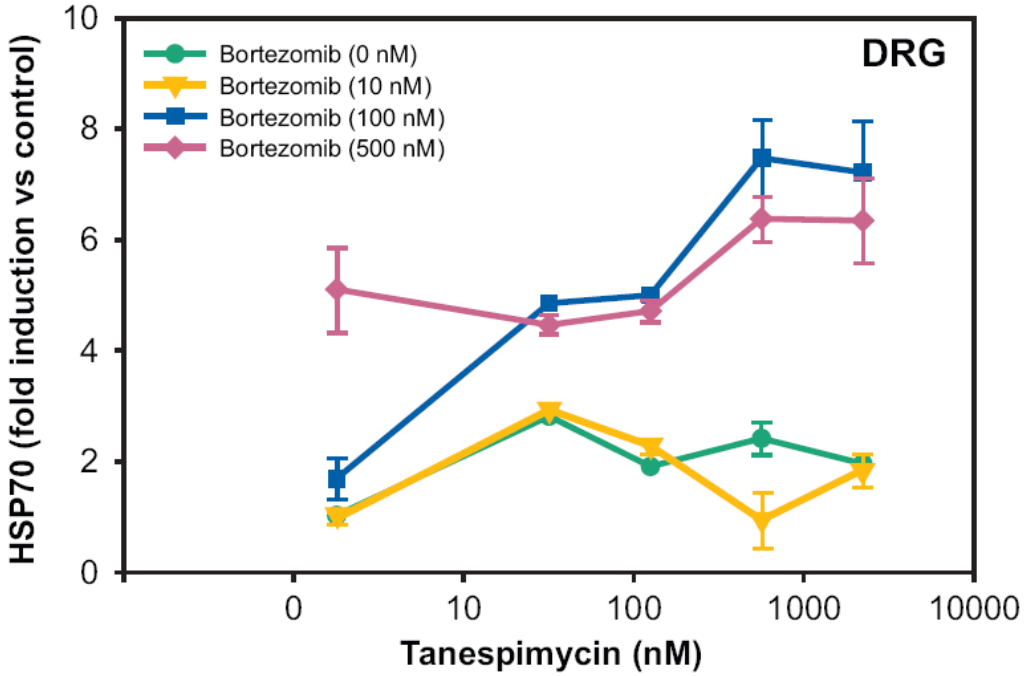


Figure 1. Dorsal root ganglion cells treated with 100 nM Bortezomib. The nucleus (N) was deformed by an aggresome(*) that contained most of the mitochondria (m) of the cell as well as other cytoplasmic organelles, and was surrounded by cytoskeletal filaments (f). Some lipid droplets ((L) were also evident. Bar = 2 microns.



DRG, dorsal root ganglion.

Figure 2. Induction of HSP70 in DRG Cells and Myeloma Cells by Combinations of Tanespimycin and Bortezomib

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