CHARACTERIZATION *IN VIVO* OF TWO DIFFERENT MOLECULAR MECHANISMS INVOLVED IN THE DEVELOPMENT OF BORTEZOMIB-INDUCED PERIPHERAL NEUROPATHY

Cristina Meregalli[1], Federica La Russa[1], Alessia Chiorazzi[1], Barbara Sala[1], Annalisa Canta[1], Guido Cavaletti[1]

[1]Department of Neuroscience and Biomedical Technologies, University of Milan-Bicocca, Monza, Italy.

Bortezomib (BTZ) is a chemotherapy drug with clinical efficacy in patients with relapsed/refractory, relapsed and newly diagnosed multiple mieloma (MM). It acts by inhibiting protein degradation by the proteasoma, but its clinical use is limited by a dose-limiting neurotoxicy and by the development of tumor resistance. BTZ-induced peripheral neuropathy may occur in cancer patients from a variety of mechanisms, among which proteasoma inhibition and the alteration of microtubule's stability. To study *in vivo* these two different mechanisms involved in the onset of neuropathy, we used a well-characterized rat model of BTZ-induced peripheral neuropathy. Wistar rat were administered with BTZ 0.20 mg/kg, three times/week for eight weeks. At the end of the treatment period the relationship between neurotoxicity and the profile of the proteasoma inhibition was evaluated in different tissues. The level of 20S proteasoma inhibition was assessed by the proteasoma activity assay into blood mononuclear cells (PBMC), sciatic nerve and brain by fluorimetric assay at different time points. Moreover, we examined microtubule polymerization in sciatic nerve by comparing the distribution of acetylated tubulin (a post translational marker of stabilized microtubule) between polymerized (P) and soluble (S) fractions by western blot experiments.

After eight weeks of treatment, we observed BTZ-related neurophysiologic alterations and neuropathological damages in the axons of sciatic nerves and in sensory neurons and satellite cells of dorsal root ganglia, demonstrating the onset of a peripheral neuropathy. The recovery of the proteasoma activity was observed within 24 hours from the drug administration if BTZ was injected in a single acute dose; while the proteasoma activity remained suppressed if the drug was chronically administered. This effect was probably due to a cumulative effect of chronic administration of BTZ on its biological target. Besides, in this study we increase in the amount of acetylated-tubulin in the polymerized fraction in BTZ-treated sciatic nerves as compared with control animals. This preclinical work provide a potential explanation for the development of BTZ-induced peripheral neuropathy, through the bortezomib's ability to induce a chronic proteasoma inhibition and to suppress the cytoskeleton dynamics.

In conclusion, this model showed a toxic effect on peripheral nervous system induced by a prolonged BTZ-induced of proteasoma inhibition and stabilized microtubule. Therefore this model can be useful for the study of "*de novo*" proteasoma synthesis that can be useful for the recovery of its activity. Moreover this work will enable us to better characterize the potential role of microtubule stabilization in the neurotoxicity of bortezomib.

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