

## **Electrophysiological, behavioral and molecular characterization of the neuropathic pain in bortezomib-induced peripheral neuropathy.**

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Bortezomib (BZ) is a potent and selective first-in-class proteasome inhibitor that is mainly used to treat relapsed, refractory multiple myeloma. Painful peripheral neuropathy (PPN) is a significant side effect of BZ-based chemotherapy and, although it is one of the major reasons for a dose reduction and discontinuation of life-saving therapy, its mechanisms remain poorly understood. Recently, BZ has been shown to cause metabolic changes that result from drug accumulation in the dorsal root ganglia (DRG). Moreover, deficits in the major peripheral nerve fibres may contribute to the pathogenesis of the distal sensory axonal PPN. The effects of BZ and the involvement of changes in the spinal dorsal horn have yet to be elucidated. Here we characterized the effects of 4 weeks of intravenous BZ treatment in BALB/c mice. We examined the development of peripheral neuropathy by measuring caudal and digital nerve conduction velocities (NCV) and histology to detect pathological changes in the DRG and sciatic nerve. We also assessed changes in the mechanical/thermal thresholds and the effects of the drug on sensory fiber function by measuring current perception threshold (CPT) with a Neurometer. To investigate relevant spinal cord structures involved in BZ-induced PPN, we conducted electrophysiological recordings of spinal dorsal horn (SDH) neurons to examine their electrical activity, immunofluorescence assays to detect neuronal apoptotic events and a morphological analysis of spinal cord and roots. BZ treatment produced an impairment of the caudal and digital NCV. DRG of BZ-treated mice often had evidence of sensory neuron and satellite cell degeneration. Moreover BZ caused severe axonal degeneration of myelinated and unmyelinated fibres. BZ-induced mechanical allodynia also developed and CPT analysis demonstrated a partial impairment of the A $\delta$  and C fibers function. The electrophysiological recordings in the SDH revealed that, despite the inability of BZ to cross the blood-brain barrier, it induced an increase in wide dynamic range neuron activity, particularly after light stimulations of the hind paw. Lastly, morphological alterations in the dorsal roots and in the dorsal column of the spinal cord were evident. Our results demonstrate that chronic treatment with BZ produces a painful neuropathy in the mouse and that both peripheral and central nervous system structures are affected. Therefore, this model will enable us to conduct further mechanistic studies of BZ-related antineoplastic activity, peripheral neurotoxicity and pain. Further, this model can be used as a reference in the preclinical discovery of new neuroprotective as well as of analgesic compounds.

This work was partially funded by “AISAL, Associazione per le Scienze degli Animali da Laboratorio” and by P30 NR011396 to S.G.D.