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Inhibition of histone deacetylase 6 (HDAC6) protects against vincristine-induced peripheral neuropathies and inhibits tumor growth

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ABSTRACT

As cancer is becoming more and more a chronic disease, a large proportion of patients is confronted with devastating side effects of certain anti-cancer drugs. The most common neurological complications are painful peripheral neuropathies. Chemotherapeutics that interfere with microtubules, including plant-derived vinca-alkaloids such as vincristine, can cause these chemotherapy-induced peripheral neuropathies (CIPN). Available treatments focus on symptom alleviation and pain reduction rather than prevention of the neuropathy. The aim of this study was to investigate the potential of specific histone deacetylase 6 (HDAC6) inhibitors as a preventive therapy for CIPN using multiple rodent models for vincristine-induced peripheral neuropathies (VIPN). HDAC6 inhibition increased the levels of acetylated α-tubulin in tissues of rodents undergoing vincristine-based chemotherapy, which correlates to a reduced severity of the neurological symptoms, both at the electrophysiological and the behavioral level. Mechanistically, disturbances in axonal transport of mitochondria is considered as an important contributing factor in the pathophysiology of VIPN. As vincristine interferes with the polymerization of microtubules, we investigated whether disturbances in axonal transport could contribute to VIPN. We observed that increasing α-tubulin acetylation through HDAC6 inhibition restores vincristine-induced defects of axonal transport in cultured dorsal root ganglion neurons. Finally, we assured that HDAC6-inhibition offers neuroprotection without interfering with the anti-cancer efficacy of vincristine using a mouse model for acute lymphoblastic leukemia. Taken together, our results emphasize the therapeutic potential of HDAC6 inhibitors with beneficial effects both on vincristine-induced neurotoxicity, as well as on tumor proliferation.

1. Introduction

Chemotherapy-induced peripheral neuropathies are the most common neurological side effects of anti-cancer treatments with an incidence of up to 80% (Cavaletti et al., 2011; Seretny et al., 2014). Chemotherapeutics that interfere with microtubules, including the plant-derived taxanes and vinca-alkaloids, cause these peripheral neuropathies (Jaggi and Singh, 2012; Kannarkat et al., 2007). Vincristine is such a chemotherapeutic drug that belongs to the family of vinca-alkaloids and despite being the most neurotoxic drug of its class, vincristine is remarkably effective and therefore still widely used to treat hematological cancers (Gennery, 1985). Vincristine-induced neurotoxicity primarily affects large sensory nerves leading to dysesthesia and paresthesia, reduced electrophysiological response of the sural nerve and decreased intra-epidermal nerve fiber densities (Grisold et al., 2012). In more severe forms, motor problems also arise (Baron, 2006; Park et al., 2013). Symptoms of vincristine-induced peripheral neuropathies (VIPN) are dose dependent, arise progressively in a

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stocking-glove type distribution and persist even after the treatment has been ceased (= coasting) (Schneider et al., 2015). Due to the enormous impact on the quality of life, the threshold for clinical toxicity depends on VIPN and could require excessive pain treatment or early cessation of the anti-cancer therapy (Gennery, 1985). To date, little is known about the pathophysiology of VIPN and no effective cure is available. Instead, management is limited to pain relief with anti-epileptics, anti-depressants and opioids, which can induce a myriad of side effects (Zareba, 2009). Therefore, a high medical need for an effective treatment for chemotherapy-induced peripheral neuropathies still exists (Cavaletti and Marmiroli, 2010; Majithia et al., 2016).

Vincristine exerts its anti-cancer effect by inhibiting the assembly of microtubules. These highly dynamic structures, composed of α- and β-tubulin heterodimers, play an important role in multiple cellular processes, including cytoskeleton homeostasis, mitosis, cell migration and intracellular trafficking (Lawson and Carazo Salas, 2013). Microtubule dynamics is also of pivotal importance in post-mitotic neurons (Conde et al., 2009; Stanton et al., 2011). These polarized cells rely on intact microtubules for neuronal architecture, but they also function as tracks for axonal transport between the cell body and synapses. Moreover, disturbances in axonal transport are a common hallmark in a variety of neurodegenerative disorders and peripheral neuropathies (Cashman and Höke, 2015; d'Ydewalle et al., 2011; De Vos and Hafezparast, 2017; Holzbaur and Scherer, 2011; Prior et al., 2017). As vincristine alters microtubule dynamics, axonal transport in neurons might also be affected and could contribute to the neurotoxicity observed in patients.

One of the key mechanisms regulating axonal transport in neurons are post-translational modifications of α- and β-tubulin. Especially acetylation at the ε-amino group of lys-40 of α-tubulin influences axonal transport (Dompierre et al., 2007). Increased levels of acetylation functions as a recruitment cue and improves the docking of motor proteins to microtubules (Reed et al., 2006). As such, α-tubulin acetylation is tightly regulated by α-tubulin acetyltransferases (αTATs) and histone deacetylase 6 (HDAC6), a member of the class IIb histone deacetylases. Unlike conventional histone deacetylases, HDAC6 is mostly localized in the cytoplasm, has a duplication of its catalytic deacetylase domain and a poly-ubiquitin associated zinc finger domain (Grozinger et al., 1999). These unique features allow HDAC6 to interact with substrates other than histones, including α-tubulin (Hubbert et al., 2002; Zhang et al., 2003). Although there is a correlation between microtubule dynamics and acetylation of α-tubulin (Almeida-Souza et al., 2011), it is still unclear how one impacts the other and how HDAC6 is involved in this process.

We previously observed that inhibition of the deacetylating function of HDAC6 rescues the phenotype of several mouse models for inherited peripheral neuropathies (Benoy et al., 2016, 2015; d'Ydewalle et al., 2011). HDAC6 inhibition effectively restored deficits in axonal transport in dorsal root ganglion (DRG) neurons isolated from Charcot-Marie-Tooth disease (CMT) type 2 mouse models (Benoy et al., 2016, 2015; d'Ydewalle et al., 2011; Shen et al., 2016). Due to the phenotypic resemblance of patients diagnosed with CMT and those with VIPN, we hypothesized that vincristine could induce axonal problems by interfering with microtubule dynamics. Interestingly, mitochondrial dysfunction also contributes to cisplatin-induced peripheral neuropathy, a different form of CIPN. Cisplatin treatment results in the depletion of the mitochondrial GTPase mitofusin-2 (MFN2), a protein associated with CMT2A (Bobylev et al., 2017; Züchner et al., 2004). The importance of mitochondrial dysfunction in peripheral neuropathy was further emphasized by the fact that cisplatin not only binds nuclear but also mitochondrial DNA (mtDNA), resulting in mitotoxicity in neurons (Podratz et al., 2017). In a recent study, mitochondrial bioenergetics, their axonal transport and the cisplatin-induced phenotype was completely restored using ACY-1083, another HDAC6-specific inhibitor (Krukowski et al., 2017). Therefore, the aim of this study was to investigate whether inhibition of HDAC6 could increase the acetylation of α-tubulin, rescue axonal transport deficits and prevent the development of VIPN.

The use of HDAC6 inhibitors is also a hot topic in cancer research. Specific HDAC6 inhibitors have been developed for the treatment of multiple myeloma and are currently tested in clinical trials (Santo et al., 2012). Generally, most research focuses on neuroprotection regardless of cancer, or on the anti-cancer efficacy, neglecting the neurotoxicity. As HDAC6 has been implicated in neurodegeneration as well as in cancer, we investigated both the neuroprotective effects and assured the anti-cancer efficacy of vincristine in combination with HDAC6 inhibitors. We found that HDAC6 inhibition has beneficial effects on both aspects.

2. Results

2.1. Pharmacological inhibition of HDAC6 protects against VIPN

Using a mouse model for VIPN, we observed that weekly administration of vincristine (500 μg/kg, ip) resulted in a progressive sensory peripheral neuropathy, characterized by decreased sensory nerve action potential (SNAP) amplitudes of the caudal nerve that stabilized after 4 weeks of treatment (Supplemental Fig. S1A). Nerve conduction velocities were unaffected, suggesting vincristine primarily affects the axons of peripheral nerves (Supplemental Fig. S1B). On the behavioral level, we used the Electronic Von Frey (EVF) test to determine the response to mechanical stimuli. The electrophysiological changes correlated with a hypersensitivity when applying pressure to the hind paw of mice treated with vincristine (Supplemental Fig. S1C). Vincristine toxicity was limited to the sensory nervous system as innervation of the neuromuscular junctions at the gastrocnemius muscle was normal and as no changes in rotarod performance, CMAP amplitudes or latencies were observed during the treatment (Supplemental Fig. S1, D–H).

Next, we investigated whether HDAC6 inhibition had an effect on the pathophysiology of VIPN using a small molecule, ACY-738, that specifically inhibits the deacetylating function of HDAC6 (Jochems et al., 2014). The severity of the sensory axonopathy was significantly reduced in mice that were co-treated with vincristine and ACY-738. Both the decrease in SNAP amplitudes and the mechanical hypersensitivity were significantly, although partially, restored (Fig. 1, A and B). We validated these findings using a second HDAC6-specific inhibitor, Tubastatin A (Butler et al., 2010), emphasizing the specific contribution of HDAC6 to the assessment of VIPN (Fig. 1, A and B). Also in a rat model for VIPN, we confirmed that inhibition of HDAC6 significantly reduced the symptoms induced by vincristine, both at the electrophysiological and at the behavioral level with 23.5% and 43.1% respectively (Supplemental Fig. S2).

As VIPN is considered a “dying-back” neuropathy (Silva et al., 2006), we evaluated the effect of HDAC6 inhibition on vincristine-induced defects at the most distal part of the peripheral nervous system. Analyzing skin biopsies of mice treated with vincristine alone or in combination with a HDAC6 inhibitor for the presence of the pan-neuronal marker, protein gene product 9.5 (PGP9.5), shows that HDAC6 inhibition restored PGP9.5 expression in the glabrous skin of the hind paw (Fig. 1, C and D). Histological examination confirmed that HDAC6-inhibition completely preserved the intra-epidermal nerve fiber density in animals treated with vincristine (Fig. 1, E–I and Supplemental Movies S1–S4). All together, these results show that HDAC6 inhibitors can reduce the severity of vincristine-induced neurotoxicity *in vivo*.

2.2. HDAC6 inhibition increases acetylated α-tubulin in vivo

Next, we determined the level of acetylated α-tubulin, the major substrate for HDAC6 (Hubbert et al., 2002; Zhang et al., 2003), in tissues isolated from mice that underwent chemotherapy with vincristine alone or in combination with ACY-738. Vincristine slightly reduced α-tubulin acetylation exclusively in sensory tissues (saphenous nerve and DRGs), but not in the sciatic nerve or spinal cord (Fig. 2). These findings