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Neurotoxicity to DRG neurons varies between rodent strains treated with cisplatin and bortezomib

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ABSTRACT

Chemotherapy-induced peripheral neuropathy (CIPN) is a major dose limiting side effect that can lead to long-term morbidity. Approximately one-third of patients receiving chemotherapy with taxanes, vinca alkaloids, platinum compounds or proteasome inhibitors develop this toxic side effect. It is not possible to predict who will get CIPN, however, genetic susceptibility may play a role. We explored this hypothesis using an established *in vitro* dorsal root ganglia neurite outgrowth (DRG-NOG) assay to assess possible genetic influences for cisplatin- and bortezomib-induced neurotoxicity. Almost all previous *in vitro* studies have used rats or mice. We compared DRG-NOG between four genetically defined, inbred mouse strains (C57BL/6 J, DBA/2 J, BALB/cJ, and C3H/HeJ) and one rat strain (Sprague Dawley). Our studies found differences in cisplatin and bortezomib-induced neurotoxicity between mouse and rat strains and between the different mouse strains. C57BL/6 J and Balb/cJ DRG-NOG was more sensitive to cisplatin than DBA/2 J and C3H/HeJ DRG-NOG, and all mouse strains were more sensitive to cisplatin than rat. Bortezomib induced a biphasic dose response in DBA/2 J and C3H/H3J mice. C57BL/6 J DRG-NOG was most sensitive and Balb/cJ DRG-NOG was least sensitive to bortezomib. Our animal data supports the hypothesis that genetic background may play a role in CIPN and care must be taken when rodent models are used to better understand the contribution of genetics in patient susceptibility to CIPN.

1. Introduction

The genealogy of the laboratory mouse has been well documented with most strains of inbred mice originating from the colony of Abbie Lathrop [1]. These mice were distributed and independent inbred colonies were generated around the world. A mouse strain is considered inbred when it has been bred brother to sister for 20 generation and can be traced back to one breeder pair at the 20th or subsequent generation (Goios et al., 2007). Outbred mice, on the other hand are a closed population of mice maintained for high heterozygosity for at least four generations [2]. Despite a common inbred mouse ancestry, significant genome sequences and their genetic variations have been found and cataloged in 17 different mouse strains from various inbred colonies. Next-gen sequencing found a total of 56.7 M single nucleotide polymorphisms (SNPs), approximately 9.45 M small insertions and deletions and 711,920 structural variations within the different mouse strains [3]. MtDNA sequencing confirmed a high sequence similarity consistent with a common female ancestor, with 15 base substitutions between 11 mouse strains sequenced [4]. Genetic variability between mouse strains in combination with genetic homogeneity within mouse strains makes inbred mice good models to study genetic influences on drug response.

Genetic variations between mouse strains can influence response to drug treatment and is associated with different behavioral phenotypes. In streptozotocin (STZ) treated mice, genetic strain variations showed a significant difference in renal injury. DBA/2 J and KK/H1J mice showed significantly greater renal injury then C57BL/6 J, AJ and MRL/MpJ mice [5]. In models of chemotherapy-induced neurotoxicity, differences in behavior responses were observed between paclitaxel treated mouse strains. Ten different mouse strains were exposed to paclitaxel for 7 days and tested for mechanical allodynia using the Von Frey assay. DBA/2 J mice with high response and C57BL/6 mice with low response to paclitaxel treatment were further studied for thermal hyperalgesia and cold allodynia. DBA/2 J had a significantly longer response time than C57BL/6 J when tested for response to heat, however, there was no difference in response to cold [6].

Chemotherapy induced peripheral neuropathy (CIPN) is a serious side effect of cancer treatment for many patients and can affect long-term quality of life. Cisplatin and bortezomib are effective agents for the treatment of germ line cancers and multiple myeloma that induce peripheral neuropathy in 20–40% of patients. Peripheral neuropathy is

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linked to the cumulative dose of cisplatin administered to patients and for bortezomib development of neuropathy appears to be dose-related [7]. It has been reported that patients with mild or subclinical inherited neuropathies have exaggerated neurotoxic responses to chemotherapy drugs [8]. However, it is not well understood why some patients get neuropathy while others do not.

In experimental models, the mechanisms of neurotoxicity appear to be different between cisplatin and bortezomib. Cisplatin kills dorsal root ganglion (DRG) neurons, *in vitro*, by binding both nuclear and mitochondrial DNA, inducing DNA damage and apoptosis [9–15]. Bortezomib inhibits proteasome function, in cultured DRG neurons, inducing accumulation of polymerized tubulin and inhibition of mitochondrial axonal transport [15]. Both cisplatin and bortezomib have also been shown to have significant effects on the mitochondria. Cisplatin binds to mtDNA inhibiting mtDNA replication and transcription leading to mitochondrial vacuolization and degradation, *in vitro* and *in vivo* [14]. Bortezomib has been shown to induce deficits in mitochondrial complex I and II function and significantly decreased ATP production *in vivo* [16]. None of these studies have looked at genetic variation in relationship to developing CIPN.

Measurement of neurite outgrowth (NOG) from embryonic DRG neurons *in vitro* is an established model to study the neurotoxic effects of various agents including chemotherapy drugs and can be used for genetic screens. [11–14,17–19]. It is a rapid and reproducible way to look at general neurotoxicity of compounds. Our studies were designed to look at the effects of cisplatin and bortezomib on NOG and determine whether genetic variations between mouse strains would alter the sensitivity of the DRG neurons.

2. Materials and methods

2.1. Animals

A total of 25 timed-pregnant mice from 4 strains, C57BL/6 J, DBA/2 J, BALB/cJ, and C3H/HeJ (Jackson Laboratory, Bar Harbor, ME) and 3 timed pregnant Sprague Dawley rats (Harlan, Indianapolis, IN) were used for the experiments. All animal studies were in accordance with the Association for Assessment and Accreditation of Laboratory Animal Care International. Mouse strains were chosen from different hereditary lineages and NOG compared to each other as well as compared to the rat model we routinely use in our laboratory (Fig. 1).

2.2. Surgical procedure

On embryonic day 13, timed pregnant female mice were anesthetized with sodium pentobarbital. E-13 mouse embryos were removed from the uterus and placenta then placed into L-15 medium (Life Technologies, Grand Island, NY). The pups were euthanized by decapitation and the spinal column was removed using a dissecting microscope (Carl Zeiss, Jena, Germany). Dorsal root ganglia (DRG) attached to the spinal cord cervical, thoracic, lumbar and sacral segments were removed and placed into a separate dish. 30–40 DRG were isolated from each pup. All surgical procedures were performed using aseptic precautions and sterile technique under a laminar flow clean hood. Embryonic day 15 rat pups were processed in the same manner.

2.3. Cell culture

Whole DRG explants were cultured on 35-mm collagen coated, plastic dishes with AN2 medium (MEM plus 10% calf bovine serum, 200 mM L-glutamine, 20% glucose) in the presence of 10 ng/ml NGF and incubated at 37 °C. Initial plating was done in a small volume of medium for 1–2 h to allow for attachment followed by additional medium up to 1 ml.

2.4. Neurite outgrowth assay

In each dish 3–4 DRGs were plated into AN2 medium media with or without 1, 5, 10, and 50 μg/ml cisplatin or 25, 50, 100 and 200 nM bortezomib. Each rodent strain had 3–4 replicate dishes with a total of 17–35 DRG explants per condition. Cultures were incubated for 48 h at 37 °C. NOG was evaluated by acquiring images using a Nikon digital camera (Nikon, Melville, NY) and measuring the length of the longest neurite of each DRG using ImageJ software (NIH, Bethesda, MD, USA) at 48 h [18,19]. Shown are images of neurite outgrowth of rat, C3H/HeJ and C57BL/6 J DRG neurons under control conditions, (Fig. 2A, B, G) treated with 5 μg/ml cisplatin (Fig. 2C, D, E) or 50 nM bortezomib (Fig. 2E, F).

2.5. Data analysis and statistics:

Data was analyzed and graphed using Prism Software (Graph Pad, La Jolla, CA). Statistical analysis was done using one way ANOVA and Bonferroni's multiple comparisons test. To calculate the half maximal inhibitory concentration (IC50), the dose response curve of cisplatin

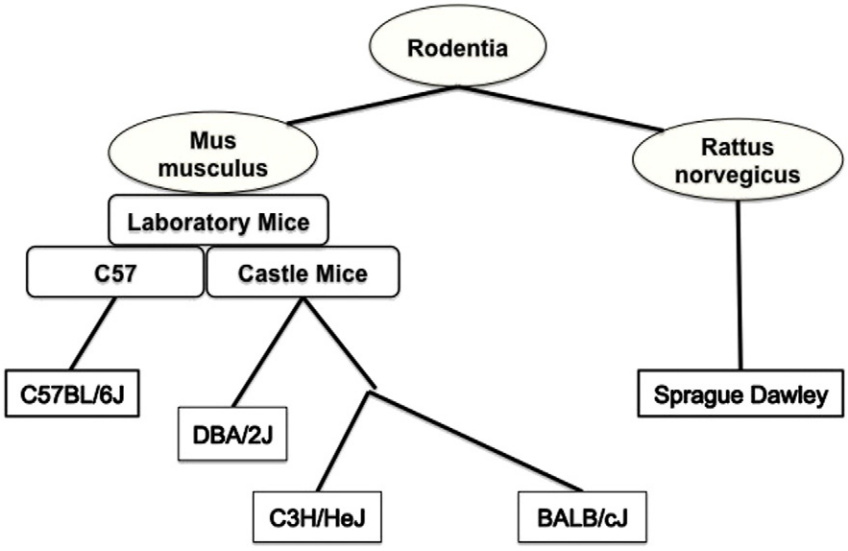

Fig. 1. Diagram of the genetic relationship between different mouse strains and rat. Abby Lathrop from 1903 to 1915 bred mice from which the C57 related and Castle mouse lines were generated. DBA/2 J, C3H/HeJ and Balb/cJ are different strains derived from the Castle mouse line and C57BL/6 J are from the C57 related line [1]. Sprague Dawley Rats are a common outbred laboratory rat developed by the Sprague Dawley Animal Company (Madison, WI).