A Visualized Guideline for Cancer Chemotherapy Induced Neuropathic Pain in Mice Using Cisplatin

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Abstract

Neuropathic pain associated with cancer chemotherapy remains an important source of trouble for different cancer therapy regimens. Cisplatin is a platinum based agent widely prescribed in clinics and induces dose-dependent painful sensory neuropathy; modeling of its toxicity in animal models is, therefore, of interest. Among various species of rodent models, mice are used frequently to reproduce the neuropathic condition. Special considerations are required for using mice in this procedure. Different steps, considerations and approaches including cisplatin intravenous injection as well as behavioral assessment of cisplatin sensory neuropathy are explained in this paper.

Keywords: Cisplatine, Neuropathy, Intravenous injection, Mice

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Introduction

Neuropathic pain is characterize by spontaneous burning pain which is associated with allodynia and hyperalgesia that can be caused by divergent conditions including: diabetes [1], alcohol consumption [2], entrapment neuropathy [3] as well as cancer chemotherapy agents [4]. Cancer chemotherapeutic compounds are mainly accompanied with some adverse effects such as nausea, vomiting, loss of appetite and induces different kinds of toxicities including: nephrotoxicity, ototoxicity and neurotoxicity [4, 5]. Neuropathy remains the most important dose limiting toxicity in different cancer chemotherapeutic agents including: cisplatin, oxaliplatin, carboplatin, vincristine, taxol and suramin [2, 6, 7].

Cisplatin is the prototype platinum agent that is classified as an alkylating agent and is used for several forms of solid tumors especially lung, testicular, colorectal, ovarian and bladder cancer for decades. One of the typical phenotypes of cisplatin toxicity is neurotoxicity manifested in pain symptoms especially in distal extremities that are believed to be dose-dependent painful sensory neuropathy [5]. Little is known about the reason and mechanism(s) involved in the development of alkylating agents especially cisplatin induced neuropathy. Given complications of cisplatin-induced neuropathy, there is a pressing need to find exact mechanism(s) and development of novel strategies. To achieve this aim, more studies must be designed using standard methods. Cisplatin induced neuropathic pain has some characteristics including reproducibility and validity, making it an excellent animal model for the study of neuropathy induced by chemotherapeutic agents, particularly alkylating agents [2, 4, 5, 7-10]. The visualized and step by step description of experimental procedures outlined in this paper includes a description of cisplatin preparation, required instruments and animal’s handling techniques, aimed to cope with current problems in conception and proper implementation of these experimental procedures.

Protocol

Assembly of mice intravenous injection unit

To assemble the injection unit, a 30-gauge dental needle is attached to a 50 µl Hamilton syringe by a 15 cm length polyethylene 10 tube (Figure 1, A and B). Once it is assembled (Figure 1C), the entire length of needle and polyethylene tube should be heparinized, to avoid clot formation within the injection unit.

Preparation of cisplatin

Each animal should be weighted (Figure 2) prior to the injection in order to calculate cisplatin volume to be administrated for each mouse. To induce cisplatin neuropathy, 2 mg/kg should be administered intravenously. Cisplatin volume is adjusted at a constant volume of 1 ml/kg for intravenous injection.
Animal preparation
Since intravenous administration can be difficult in practice, restraining is usually required. Restrain the mouse using restraint device (Figure 3), then swab the tail surface using 70% ethanol for the dual purpose of cleaning and dilating tail venues (Figure 4, Figure 5, A and B).

Cisplatin administration
Grasp the tail with your left hand and rotate the tail to visualize veins (Figure 6, A and B). Two lateral veins are located superficially on each side of the tail, characterized by their darker color than that of the skin (Figure 5, C and D). In 2/3 the way down the tail, insert the needle, bevel up, approximately parallel to vein toward the animal’s head. The exact approach and correct place of the needle is approved by blood flash in the polyethylene tube. To avoid vein collapse, the syringe should not be aspirate. Slowly start the injection. If there is any resistance and/or blister, stop the injection and remove the needle, then reinsert gently above previous site. Vein lumen will be cleared if the procedure is correct (Figure 7).

Post administration procedure
Remove the needle after injection. Inspect the injection site for possible bleeding. In case of bleeding, pressure should be applied with a gauze or piece of cotton (Figure 8). Once the injection
procedure is finished, return the animal to the cage. Based on earlier studies, 96 hours post cisplatin administration, mice are considered with peripheral neuropathy that can be confirmed by standard nociception tests [4, 5, 9].

Evaluation of cisplatin induced peripheral neuropathy using cold plate test

There are various kinds of behavioral tests used for pain research. Some of these tests are believed to be accurate research tools for neuropathic pain such as: Randall-Selitto paw-withdrawal test, von Frey hair test, Heat and cold stimulation [9, 11].

One of the important tests to evaluate neuropathic pain is cold plate test. The temperature of cold plate is kept at 2°C±0.2°C; then, the animal is placed within the transparent Plexiglas cylinder on the cold plate and the time that it takes to lift on its hind paw should be recorded using a stopwatch (Figure 9). 30 second cut-off time should be set to avoid tissue damage.

Materials

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<td>Stopwatch</td>
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References

8. Flatters SJ, Bennett GJ: Ethosuximide reverses paclitaxel- and...

