Metformin and other metabolic inhibitors attenuate neuropathic pain and tumor growth in mice with paraneoplastic syndrome and CIPN

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Abstract

Chemotherapy-induced peripheral neuropathy (CIPN) and paraneoplastic neurological syndrome are two conditions that can cause significant pain and discomfort in cancer patients. CIPN is a common side effect of certain chemotherapeutics and can result in numbness, tingling, and pain. Paraneoplastic neurological syndrome, on the other hand, is a rare disorder that occurs when cancer-fighting antibodies attack parts of the nervous system. Both neuropathies can persist which can adversely affect the quality of life and the rehabilitation of cancer patients. Unfortunately, therapies that can alleviate tumor or chemotherapy-induced neuropathic pain that do not interfere with tumor growth do not currently exist. The main goal of this study was to identify a therapeutic strategy that can achieve both anti-tumor and analgesic effects. The chemotherapeutic, bortezomib, has been shown to induce aerobic glycolysis in sensory neurons which lead to bortezomib-induce neuropathic pain. Aerobic glycolysis is also a hallmark of cancer cells, suggesting a common metabolic vulnerability. Paraneoplastic neuropathies are commonly associated with lung cancers. Hence, we used Lewis Lung Carcinoma cells (LLC1) to develop a mouse model of paraneoplastic neuropathy. We hypothesized that blocking metabolic pathways could alleviate CIPN and paraneoplastic neuropathic pain without compromising on tumor control. To test our hypothesis, we demonstrated that mice implanted with LLC1 developed significant allodynia. Treatment with bortezomib attenuated tumor growth but exacerbated the neuropathic pain. However, co-treatment with metformin, which blocks bortezomib-induced aerobic glycolysis in sensory neurons and prevents CIPN, attenuated both tumor growth and neuropathic pain. Similarly, inhibition of lactate dehydrogenase and pyruvate dehydrogenase kinase with oxamate and dichloroacetate respectively, also reduced tumor growth and pain. These results suggest that targeting metabolic pathways is a promising strategy to improve oncologic outcomes and alleviate neuropathic pain in cancer patients.

Materials and Methods

Experimental animals and Tumor implantation

Pathogen-free, 4–6 weeks old adult male and female C57BL/6J mice were obtained from Jackson Laboratories were housed in temperature-controlled rooms with standard rodent chow and water available ad libitum. Animals were randomly assigned to treatment or control groups for the behavioral experiments. Animals were housed five per cage. All behavioral experiments were performed by experimenters who were blinded to the experimental groups and treatments. Tumor growth was initiated by 1x10^6 LLC1 cells were resuspended in 100 µl 1:1 ratio mixture of Geltrex or Matrigel and Dulbecco's phosphate buffered saline (DPBS) were subcutaneously injected into the right flank of the C57BL/6J mice. For baseline mechanical withdrawal thresholds of the left hind paw were measured after habituation for 1 h using the up-down method. Starting on the day 7, the tactile withdrawal thresholds were tested. Tumor growth was monitored by measuring the perpendicular diameters (length/width) of tumor size by using digital vernier caliper (0-200 mm) for every 2 or 3days, and the tumor volume was calculated. On day 16, the mice were sacrificed, DRG and the tumors were harvested for western blot analysis.

Drug treatments

In all the experiments, C57Bl/6J mice were treated with intraperitoneal (IP) injections of 0.2 mg/kg/day of bortezomib for total dose of 1mg/kg, Oxamate (500 mg/kg/day), Dichloroacetate (DCA) (100 mg/kg/day) and metformin (150 mg/kg/day) for five consecutive days was either combination or alone. The vehicle treated group received intraperitoneal saline for five consecutive days.

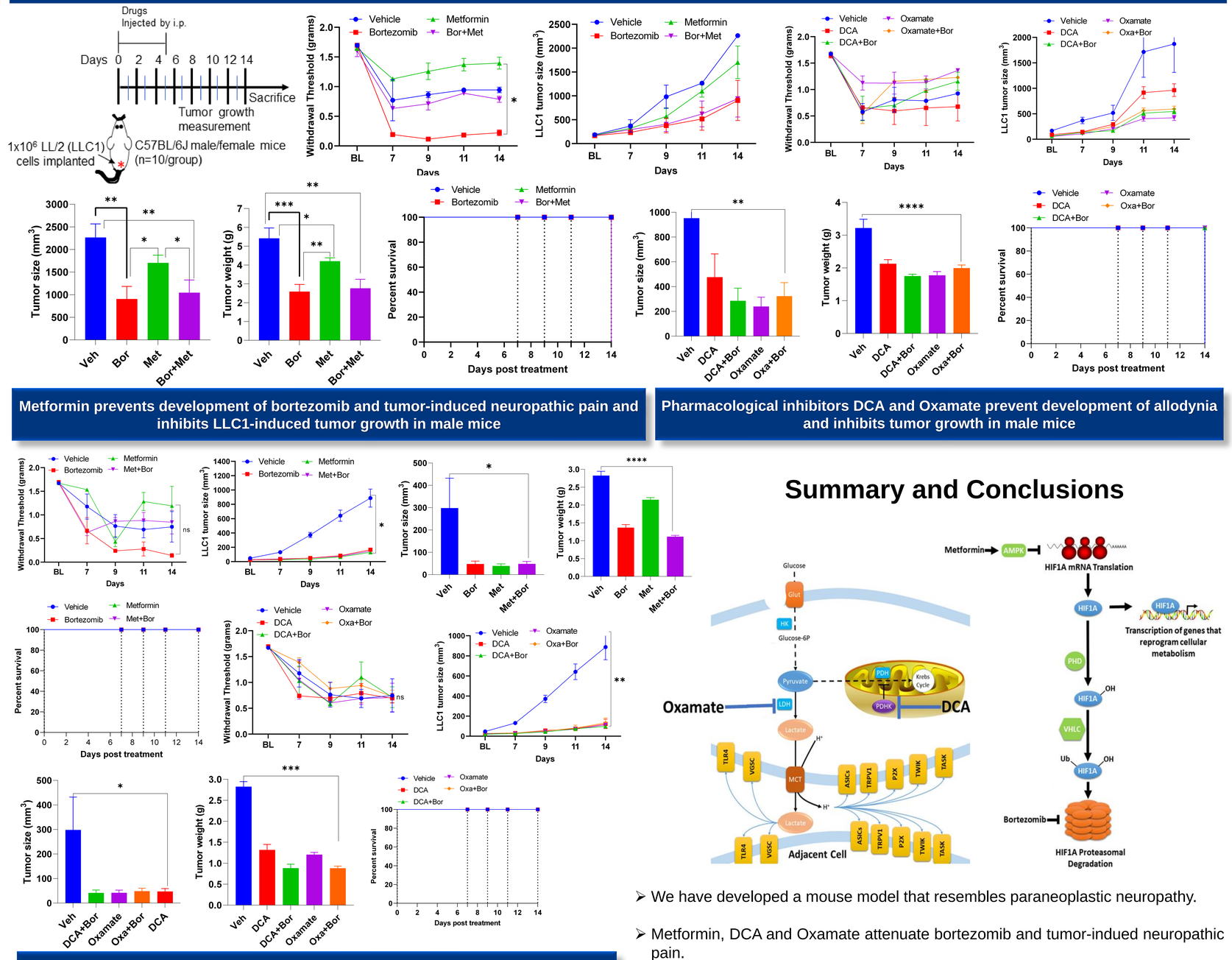
Mechanical testing

Male and Female C57Bl/6J mice were placed in acrylic boxes with wire mesh floors, and baseline mechanical withdrawal thresholds of the left hind paw were measured after habituation for 1 h using the up-down method. After determining the baseline withdrawal thresholds of mice hind paw using von Frey filaments, the mice were intraperitoneally (IP) injected with metformin, DCA, Oxamate drugs. For untreated mice (vehicle), DPBS were injected at indicated days either combination or alone. Starting on the day 7, the tactile withdrawal thresholds were tested.

Statistical analysis and data presentation

Data are based on the means and the standard error of the means (±SEM). Graph plotting and statistical analysis used GraphPad Prism Version 8 (Graph Pad Software, Inc. San Diego, CA, USA). When analyzing evoked pain behavior data, two-way repeated-measures (RM) analysis of variance (ANOVA) followed by post-hoc pairwise comparisons with Bonferroni correction was used. Western blot data were analyzed using the unpaired *t*-test. A priori level of significance at 95% confidence level was considered at $P < 0.05$.

Results



Summary and Conclusions

- We have developed a mouse model that resembles paraneoplastic neuropathy.
- Metformin, DCA and Oxamate attenuate bortezomib and tumor-indued neuropathic pain.
- These drugs also attenuate tumor growth.