# Gabapentin antitumor and antihyperalgesic effects in a preclinical model of cancer pain.

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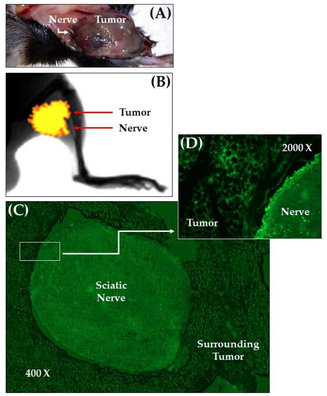
**Background:** Cancer pain may be the consequence of physical nerve compression by a growing tumor, which in turn may trigger different hyperalgesic molecular mechanisms involving calcium deregulation.

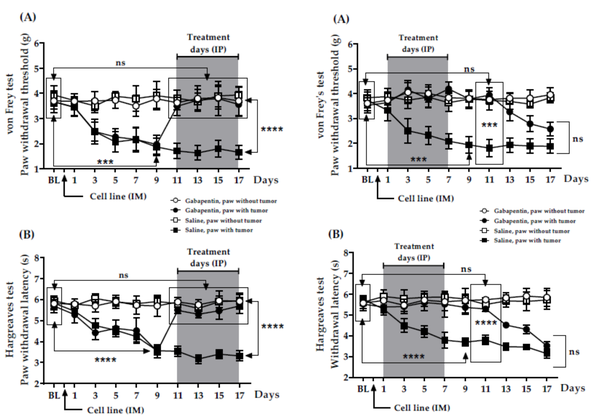
**Objective:** We employed a murine model to study whether gabapentin, acting in Cav-alpha2-delta subunits of voltage-gated calcium channels of melanoma cells and primary afferents, exerts simultaneous antihyperalgesic and antitumor effects.

**Methods:** A fluorescent melanoma cell line (B16–BL6/Zs green) was inoculated IM into the proximity of the sciatic nerve in male C57BL/6 mice. The tumor gradually compressed the nerve, causing hypersensitivity, evaluated by means of the von Frey and Hargreaves behavioral tests. Tumor growth was characterized via *in vivo* imaging techniques. Every other day, gabapentin (100 mg/Kg), or saline, was IP administered to each animal. In the therapeutic protocol, gabapentin was administered once the tumor had induced increased nociception. In the preventive protocol, gabapentin was administered before the appearance of the positive signs. Additionally, *in vitro* experiments were performed to determine gabapentin's effects on cell-line proliferation, the secretion of the chemokine CCL2, and calcium influx.

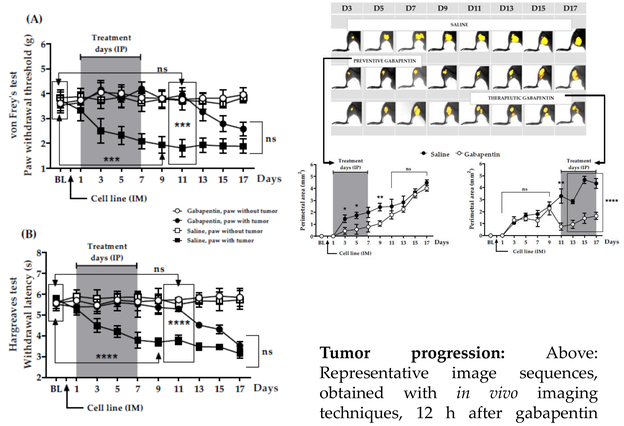
**Results:** In the therapeutically treated animals, baseline responses to noxious stimuli were recovered, and tumors were significantly reduced. Similarly, gabapentin reduced tumor growth during the preventive treatment, but a relapse was noticed when the administration stopped. Gabapentin also inhibited cell proliferation, the secretion of CCL2, and calcium influx.




**Tumor–nerve details:** (**A**) Tumor mass engulfing the sciatic nerve. (**B**) *In vivo* image showing the tumor fluorescence signal co-registered with an anatomical X-ray plane of the inoculated hind paw. (**C**) Fluorescent microscope image (400 X) of a transversal section of the sciatic nerve surrounded by the tumor. (**D**) Insert at higher magnification (2000 X) showing the fluorescent B16–BL6/Zs green cells around the sciatic nerve. Images were taken 17 days after inoculation of the cell line (endpoint).



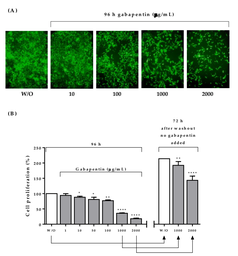

**Therapeutic protocol:** Gabapentin (100 mg/Kg), or saline, was administered IP (days 11–17, grey box) to evaluate their effects on nociceptive responses caused by tumor progression: (A) von Frey test; (B) Hargreaves test. BL: Baseline (response value before IM inoculation of the cell line and before starting treatment). Contralateral non-inoculated limbs were used as internal controls. ns: non-significant. ***$p \leq 0.0002$, ****$p \leq 0.0001$, $n$=10 mice/group. Each point represents the mean ± SEM of the sample.



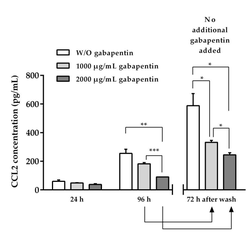

**Preventive protocol:** Gabapentin (100 mg/Kg), or saline, was administered IP (days 1–7, grey box) to evaluate their effects on nociceptive responsiveness caused by tumor progression:
(A) von Frey test; (B) Hargreaves test. BL: Baseline (response value before IM inoculation of the cell line and before starting treatment). Contralateral non-inoculated limbs were used as internal controls. ns: non-significant. ***p≤0.0009, ****p<0.0001, n=10 mice/group. Each point represents the mean ± SEM of the sample.



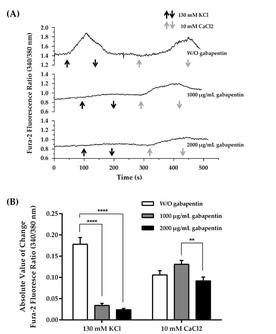

**Tumor progression:** Above: Representative image sequences, obtained with *in vivo* imaging techniques, 12 h after gabapentin administration, indicating tumor progression in three different intact mice receiving either IP saline (first row), preventive IP gabapentin (second row), or therapeutic IP gabapentin (third row) after the inoculation of the cell line. Below: Perimetral area ($mm^2$), corresponding to all tumors growing in animals receiving either saline or gabapentin (100 mg/Kg). Left: Preventive treatment. Right: Therapeutic treatment. BL: Baseline (response value before IM inoculation of the cell line and before starting treatment). ns: non-significant. *p≤0.0314, **p≤0.0024, ****p≤0.0001, n=10 mice/group. Each point represents the mean ± SEM of the sample




**Cell proliferation:** (A) Representative inverted fluorescent microscope images (200X) of the melanoma cell line B16–BL6/Zs green incubated for 96 h under different gabapentin doses (10, 100, 1000, or 2000 μg/mL). (B) Proliferation assay using MTT to verify viability. The results are expressed as a percentage of cellular proliferation after 96 h of incubation with different doses of gabapentin (1, 10, 50, 100, 1000, or 2000 μg/mL) compared with non-treated cells (100% proliferation), and after 72 h of gabapentin washout (no additional gabapentin added). Each column represents the mean ± SEM of the sample. *p≤0.05, **p=0.0046, ****p<0.0001.




**CCL2 concentration**: Effect of gabapentin on the CCL2 secretion in B16–BL6/Zs green murine melanoma cells. Cultures were incubated with gabapentin (1000 or 2000 μg/mL) for 24 and 96 h, and 72 h after gabapentin washout (no additional gabapentin added). ELISA analyzed supernatants obtained from 3 independent assays (n=3, each by triplicate), which were used to determine the chemokine concentration. Each column represents the mean ± SEM. *p≤ 0.04, **p≤0.005, ***p≤0.0005.




**Calcium influx:** Effect of gabapentin treatment on calcium influx in B16–BL6 murine melanoma cells: (A) Representative records showing changes in the time course of Fura-2 fluorescence ratio (340/380 nm) induced by 130 mM KCl, or 10 mM $CaCl_2$, in control and gabapentin-treated cells (1000 or 2000 μg/mL). Cells were preincubated with gabapentin for 24 h before starting the experimental procedure, and maintained throughout the recordings. When exposing the cells to 130 mM KCl (black arrows), gabapentin caused calcium influx inhibition. Once the basal calcium level was recovered in the control condition (1mM $CaCl_2$), the subsequent exposure to 10 mM $CaCl_2$ (grey arrows) induced an increase in calcium levels in all conditions. (B) Summarized data showing the absolute value of change (experimental condition baseline) of transient calcium responses after 130 mM KCl, or 10 mM $CaCl_2$ exposure, in control and gabapentin-treated cells, expressed as Fura-2 fluorescence ratio (340/380 nm). Bars represent the mean ± SEM of data from control cells (n=32) and gabapentin-treated cells (n=17 under 1000 μg/mL, and n=28 under 2000 μg/mL). **p=0.0061; ****p<0.0001 vs. control.

**For more details: Int. J.Mol. Sci. 2021, 22, 9671.**
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**Conclusions:** Using gabapentin to modulate pathophysiological calcium signaling in cancer might represent an effective multivalent therapeutic strategy to control tumor development and progression, as well as tumor-induced hypersensitivity, indicating its active role as a pharmacological regulator of calcium remodeling in pathological conditions.

**Poster #139**