

Systemic Inhibition of Cathepsin S Attenuates Vincristine-Induced Neuropathic Hypersensitivity

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Introduction

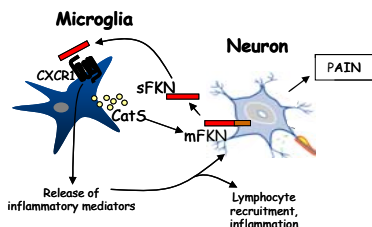
The activation of spinal microglia and astrocytes plays a role in the development and maintenance of neuropathic pain states subsequent to CNS or PNS injury. Recently, it has been demonstrated that cathepsin S (CatS), a lysosomal cysteine protease found in antigen presenting cells such as CNS microglia, is pro-nociceptive via the cleavage of the neuronal chemokine fractalkine (FKN) (Clark *et al.*, 2007).

Here, we assessed the effect of acute and prolonged treatment with a reversible and highly selective cathepsin S inhibitor, VB-285, on mechanical allodynia in a vincristine model of chemotherapy-induced pain.

Painful peripheral neuropathy occurs with chemotherapeutic agents in the taxane class, as well as with the vinca alkaloid and platinum-complex classes and neuropathic pain constitutes a dose-limiting side effect of these anti-neoplastic drugs.

Cathepsin S Biology

- > A compelling new target for neuropathic pain
- > Cysteine protease released by macrophages/microglia in neuropathic states
- > Cleaves fractalkine (FKN) from the surface of sensory neurons
 - FKN activates microglia
- > Important for the maintenance of neuropathic pain



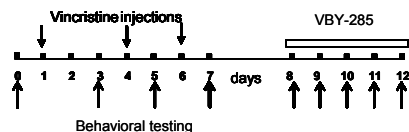
VB-285 Summary

Enzyme	K _i (app) (nM)	Fold Selectivity vs CatS
Cathepsin S	0.01	1
Cathepsin L	2.2	220
Cathepsin B	2.8	280
Cathepsin F	3.4	340
Cathepsin K (huRab)	2.4	240

Table 1. VB-285 is a potent, selective, and reversible inhibitor of cathepsin S with an inhibition constant (K_i) of 0.01 nM.

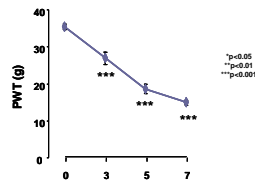
Methods

Male Wistar rats were injected three times on non-consecutive days with the chemotherapy agent vincristine sulphate (200 µg/kg, total cumulative dose 600 µg/kg). By 7 days after the first injection rats had developed robust mechanical allodynia.



- VB-285 or gabapentin was administered days 8-12, after neuropathy was fully established in groups of 8 rats.
- The rat hind paw thresholds to mechanical stimulation were monitored by using a Dynamic Planar Aesthesiometer (Ugo Basile, Italy). Each rat paw withdrawal threshold (PWT) was calculated as the average of three consecutive tests performed at 5 minute intervals by an experimenter blind to the treatments. A cut-off of 50g was imposed to prevent tissue damage.
- FKN content in CSF was quantified by ELISA (RnD RayBio® Rat Fractalkine ELISA Kit)
- Mechanical withdrawal thresholds were analyzed by Repeated Measure (RM) Two way ANOVA followed by Tukey test. ELISA data were analyzed by One Way ANOVA followed by Tukey test

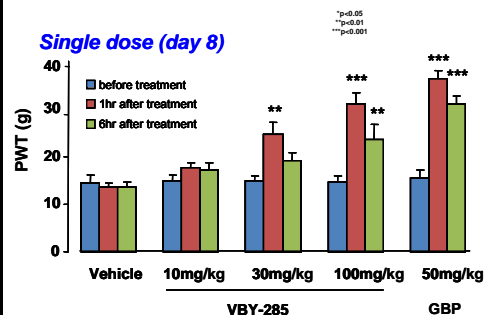
1. Vincristine induces severe allodynia



Results

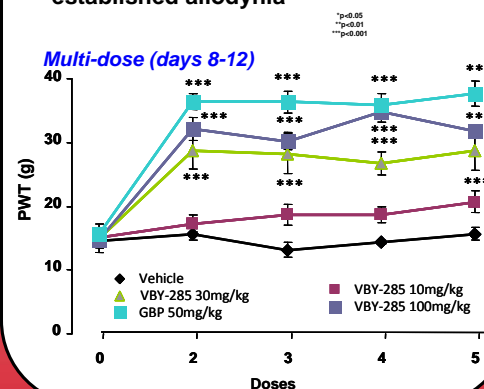
2. Single administration of VB-285 reverses established allodynia

Single dose (day 8)



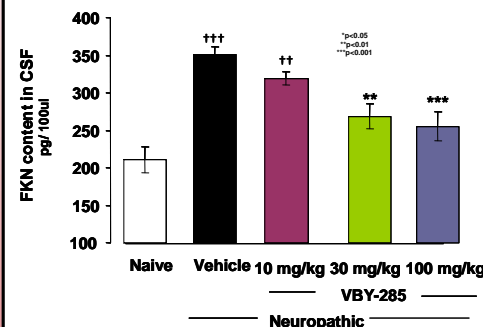
3. Multiple doses of VB-285 reverse established allodynia

Multi-dose (days 8-12)



Results

4: VB-285 inhibits the increase in fractalkine in the cerebral spinal fluid



Conclusions

1. The selective cathepsin S inhibitor VB-285 reversed allodynia in a rat model of vincristine-induced neuropathic hypersensitivity
2. VB-285 reversed the increase in FKN in the CSF
 - This is the first demonstration of a cathepsin S inhibitor impacting FKN levels in the CNS
 - FKN could be used as a biomarker to predict efficacy and/or stratify patients in the clinic
3. Cathepsin S inhibitors may be efficacious therapeutics for the treatment of human neuropathic pain, with a novel mechanism of action

Reference: Clark AK, Yip PK, Grist J, Gentry C, Staniland AA, Marchand F, Dehvari M, Wotherspoon G, Winter J, Ullah J, Bevan S, & Malcangio M (2007). Inhibition of spinal microglial cathepsin S for the reversal of neuropathic pain. *Proc Natl Acad Sci U S A* 104, 10655-10660.