Systemic Inhibition of Cathepsin S Attenuates Hypersensitivity in Animal Models of Neuropathic and Inflammatory Pain

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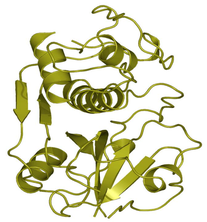


Introduction

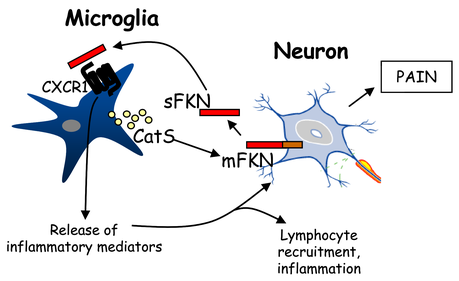
The activation of spinal microglia and astrocytes plays a role in the development and maintenance of neuropathic pain states subsequent to CNS or PNS injury. Recently, it has been demonstrated that cathepsin S (CatS), a lysosomal cysteine protease expressed and released by activated microglia, is pronociceptive by causing the cleavage and release of membrane-bound fractalkine (Clark *et al.*, 2007).

We assessed the effect of acute and prolonged treatment with reversible and highly selective cathepsin S inhibitors VBY-891 and VBY-036, on tactile allodynia and thermal hyperalgesia in a mouse partial sciatic nerve ligation model of neuropathic pain. Mechanical and thermal thresholds were assessed at baseline and on days 3, 7, and 10 after surgery. We also evaluated VBY-036 in preclinical models of inflammatory pain. We also assessed cathepsin S inhibitors for sedative effects in a locomotor activity test in mice. All Cathespin S inhibitors evaluated were non-sedating (data not shown).

The results of these studies in multiple models of neuropathic and inflammatory pain further illustrate that cathepsin S a compelling new target in pain.

Cathepsin S Biology

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



VBY-891, VBY-036 Summary

	VBY-891		VBY-036	
Enzyme	K_i (app) (uM)	Fold Selectivity vs CatS	K_i (app) (uM)	Fold Selectivity vs CatS
Cathepsin S	0.000312	1	0.000113	1
Cathepsin L	0.37	1185	0.16	1415
Cathepsin B	0.57	1826	0.25	2212
Cathepsin F	0.43	1791	0.3	2654
Cathepsin K (huRab)	0.11	458	0.1	884

No activity (IC_{50} >50uM) for either compound was detected on a series of cellular cysteine, serine, or aspartic proteases including cathepsin E, ELA1, ELA2, BACE1, Chymase, Chymotrypsin, FVIIa, FXa, pKallekrein, thrombin, trypsin, tryptase.

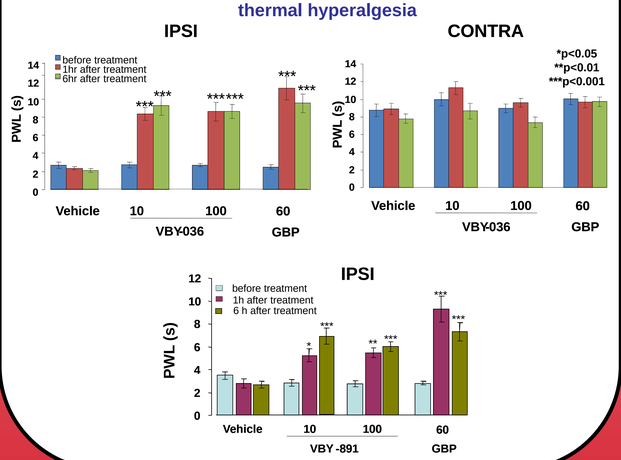
Table 1. VBY-891 and VBY-036 are potent, selective, and reversible inhibitors of cathepin S, with inhibition constants (Ki) of 312 and 113 pM, respectively.

Results

1. Cathepsin S Inhibitors Are Efficacious In A Model of Neuropathic Pain: Mouse PNL

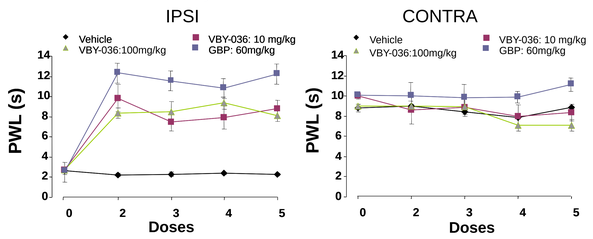
- Mouse Partial Sciatic Nerve Ligation Model (Seltzer et al, 1990)
- Left sciatic nerve was partially ligated (ipsilateral paw, IPSI)
- Right paw served as an internal control (contralateral paw, CONTRA)
- Mechanical and thermal thresholds assessed at baseline and on days 3, 7, and 10 after surgery
- VBY compounds were administered on days 11-15 post-surgery, with sc dosing at 10 and 100 mg/kg [VBY compounds can be dosed orally or sc]

Efficacy of VBY-036 and VBY-891 after Single Dose, on neuropathic thermal hyperalgesia

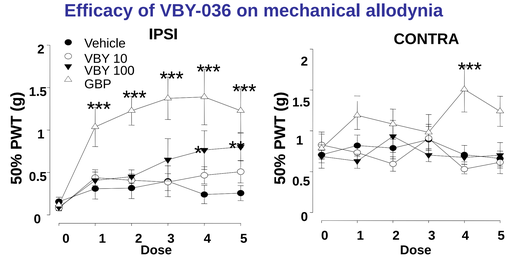


Results

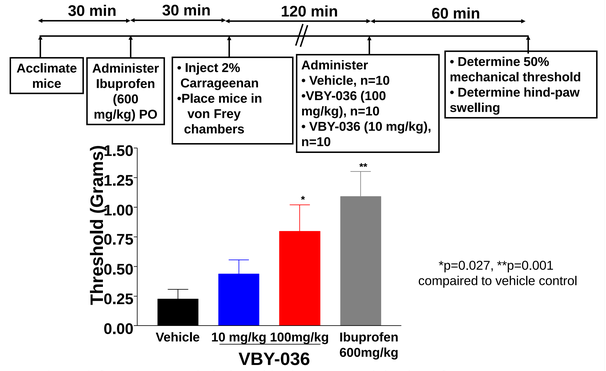
Efficacy of VBY-036 after Multi-dose



Efficacy of VBY-036 on mechanical allodynia



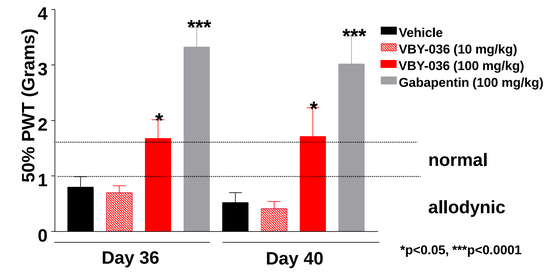
2. VBY-036 Efficacy Acute Inflammatory Pain



- Acute inflammatory pain induced by intraplantar injection of carrageenan
- VBY-036 reversed mechanical hyperagesia but did not reduce paw swelling in this assay suggesting a direct effect on hyperalgesia
- Assay control Ibuprofen was administered 1h prior to carrageenan and also significantly reduced paw swelling
- Similar results obtained in a zymosan-induced thermal hyperalgesia model (not shown)

Results

3. VBY-036 Reverses Chronic Inflammatory Pain: Arthritis Model



- Arthritis was induced in mice by immunization with collagen in adjuvant and treatment was administered on days 36-40, after arthritis was fully established
- VBY-036 reversed established mechanical allodynia, and efficacy is maintained following 5 days of dosing
- Gabapentin also reversed allodynia with an increase in 50% PWT above normal, suggestive of known sedative effects
- Treatment did not reduce paw swelling (not shown)

Conclusions

1. The selective cathepsin S inhibitors VBY-891 and VBY-036 reversed established thermal hyperalgesia and allodynia in the PNL model of neuropathic pain. There was no tolerance after repeated dosing and compounds remained efficacious.
2. VBY-036 also demonstrated efficacy in acute inflammatory pain models, and also reversed established allodynia in a model of chronic inflammatory pain.
3. Cathepsin S inhibitors were non-sedating in a locomotor activity test in mice (not shown).
4. Cathepsin S inhibitors may be efficacious therapeutics for the treatment of human neuropathic and inflammatory pain, with a novel mechanism of action.
5. VBY-891 is progressing into human clinical studies.

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.

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