**Therapeutic potential for leukocyte elastase in chronic pain states harboring a neuropathic component**

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**Supplementary information:**

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| **Suppl. Fig.1.** E**ffects of systemic delivery of Sivelestat in the Spared nerve injury (SNI) model of neuropathic pain.**1. Paw withdrawal responses to von Frey force of 1.4 g before SNI (Basal) and at 1, 3, 6 and 24h following 20 mg/Kg dosage of i.p. Sivelestat or Vehicle on day 8 following SNI.
2. Paw withdrawal responses to von Frey force of 1.4 g before SNI (Basal) and at 1, 3, 6 and 24h following 20 mg/Kg dosage of i.p. Sivelestat or Vehicle at 4W following SNI.

**\*** denotesP ≤ 0.05 as compared to basal,† represents P ≤ 0.05 as compared to the respective vehicle treated group, Two-way ANOVA of repeated measures followed by Turkey’s post *hoc t*est; n = 7 mice per group. |

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| **Suppl. Fig.2.** **Dose-dependent effects of systemic delivery of Sivelestat on neuropathic pain.** Analysis of SNI-induced neuropathic mechanical hypersensitivity following intraperitoneal application of LE inhibitor, Sivelestat, as compared to the vehicle-injected group. A single dose of 0.2 or 2.0 or 20 or 50 mg/kg body weight Sivelestat was injected i.p. on day 8 (POD8) or day 28 (POD28) post-SNI (Blue arrow). In all panels, **\*** denotesP ≤ 0.05 as compared to basal,† represents P ≤ 0.05 as compared to the vehicle treated group at respective time-point, Two-way ANOVA of repeated measures followed by Tukey’s post *hoc t*est; *n* = at least 6 mice per group.1. Mechanical response thresholds calculated as von Frey filament strength required to achieve 60% withdrawal frequency before SNI (Basal) or at 1, 3, 6 and 24h following each dosage of i.p. Sivelestat or Vehicle on day 8 following SNI.
2. Mechanical response thresholds calculated as von Frey filament strength required to achieve 60% withdrawal frequency before SNI (Basal) or at 1, 3, 6 and 24h following each dosage of i.p. Sivelestat or Vehicle on day 28 following SNI.
3. An integral of responsivity to mechanical stimuli over all von Frey forces tested (0.02 g to 1.0 g) in the Sivelestat-injected group as compared to a vehicle-injected group of mice, represented as the area under the curve (AUC) before SNI (Basal) or at 1, 3, 6 and 24h following each dosage of i.p. Sivelestat or Vehicle on day 8 following SNI.
4. An integral of responsivity to mechanical stimuli over all von Frey forces tested (0.02 g to 1.0 g), represented as the area under the curve (AUC) before SNI (Basal) or at 1, 3, 6 and 24h following each dosage of i.p. Sivelestat or Vehicle on day 28 following SNI.
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| **Suppl. Fig.3.** **Effects of systemic delivery of Sivelestat on neuropathic pain.** Analysis of SNI-induced neuropathic mechanical hypersensitivity in the paw contralateral to SNI operated paw following intraperitoneal application of LE inhibitor, Sivelestat, as compared to the vehicle-injected group. A single dose of 0.2 or 2.0 or 20 or 50 mg/kg body weight Sivelestat was injected i.p. on day 8 (POD8) or day 28 (POD28) post-SNI (Blue arrow).1. Paw withdrawal responses to von Frey force of 0.16 g before SNI (Basal) and at 1, 3, 6 and 24h following each dosage of i.p. Sivelestat or Vehicle on day 8 following SNI.
2. Mechanical response thresholds calculated as von Frey filament strength required to achieve 60% withdrawal frequency before SNI (Basal) or at 1, 3, 6 and 24h following each dosage of i.p. Sivelestat or Vehicle on day 8 following SNI.
3. An integral of responsivity to mechanical stimuli over all von Frey forces tested (0.02 g to 1.0 g), represented as the area under the curve (AUC) before SNI (Basal) or at 1, 3, 6 and 24h following each dosage of i.p. Sivelestat or Vehicle on day 8 following SNI.
4. Paw withdrawal responses to von Frey force of 0.16 g before SNI (Basal) and at 1, 3, 6 and 24h following each dosage of i.p. Sivelestat or Vehicle on day 28 following SNI.
5. Mechanical response thresholds calculated as von Frey filament strength required to achieve 60% withdrawal frequency before SNI (Basal) or at 1, 3, 6 and 24h following each dosage of i.p. Sivelestat or Vehicle on day 28 following SNI.
6. An integral of responsivity to mechanical stimuli over all von Frey forces tested (0.02 g to 1.0 g), represented as the area under the curve (AUC) before SNI (Basal) or at 1, 3, 6 and 24h following each dosage of i.p. Sivelestat or Vehicle on day 28 following SNI.
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| **Suppl. Fig. 4. Sex-specific effects of systemic delivery of Sivelestat on neuropathic pain**. Analysis of SNI-induced neuropathic mechanical hypersensitivity in the paw ipsilateral to SNI operated paw following intraperitoneal application of LE inhibitor, Sivelestat, as compared to the vehicle-injected group. A single dose of 20 mg/kg body weight Sivelestat was injected i.p. on day 8 (POD8) or day 28 (POD28) post-SNI (Blue arrow). n = 12 mice in the male group and 6 mice in the female group. A. Paw withdrawal responses to von Frey force of 0.16 g before SNI (Basal) and at 1, 3, 6 and 24h following i.p. Sivelestat or Vehicle on day 8 post-SNI.B. Paw withdrawal responses to von Frey force of 0.16 g before SNI (Basal) and at 1, 3, 6 and 24h following i.p. Sivelestat or Vehicle on day 28 post-SNI. |

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| **Suppl. Fig.5.** **Effects of systemic delivery of Sivelestat on gait in sham-operated mice.** Analysis of SNI-induced gait following intraperitoneal application of LE inhibitor, Sivelestat, as compared to the vehicle-injected group. A single dose of 50 mg/kg body weight Sivelestat was injected i.p. on day 28 following sham surgery (Blue arrow). 1. Duration of paw contact before Sham surgery and at 4h following i.p. Sivelestat or Vehicle injection on day 28 following Sham surgery from paws ipsilateral or contralateral to the Sham surgery.
2. Area of maximum intensity before SNI and at 4h following i.p. Sivelestat or Vehicle injection on day 28 following Sham surgery from paws ipsilateral or contralateral to the SNI surgery.
3. Area of total paw contact before SNI and at 4h following i.p. Sivelestat or Vehicle injection on day 28 following Sham surgery from paws ipsilateral or contralateral to the SNI surgery.
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| **Suppl. Fig.6. Effect of systemic delivery of Sivelestat or Pregabalin on basal mechanical sensitivity in sham-operated mice.** Analysis of SNI-induced neuropathic mechanical hypersensitivity in the sham-operated mice following the intraperitoneal application of LE inhibitor, Sivelestat or Pregabalin as compared to the vehicle-injected group. A single dose of 20 mg/kg body weight Sivelestat or Pregabalin was injected i.p. on day 8 (POD8) or day 28 (POD28) post-SNI (Blue arrow). In all panels, **\*** denotesP ≤ 0.05 as compared to basal,† represents P ≤ 0.05 as compared to the vehicle treated group at respective time-point, Two-way ANOVA of repeated measures followed by Tukey’s post *hoc t*est; *n* = at least 6 mice per group.1. Paw withdrawal responses to von Frey force of 0.16g at 1, 3, 6 and 24h following each dosage of i.p. Sivelestat or Pregabalin on day 8 following Sham surgery.
2. Mechanical response thresholds calculated as von Frey filament strength required to achieve 60% withdrawal frequency before SNI (Basal) or at 1, 3, 6 and 24h following each dosage of i.p. Sivelestat or Pregabalin or Vehicle on day 8 following Sham surgery.
3. Paw withdrawal responses to von Frey force of 0.16g at 1, 3, 6 and 24h following each dosage of i.p. Sivelestat or Pregabalin on day 28 following Sham surgery.

 Mechanical response thresholds calculated as von Frey filament strength required to achieve 60% withdrawal frequency before SNI (Basal) or at 1, 3, 6 and 24h following each dosage of i.p. Sivelestat or Pregabalin or Vehicle on day 28 following Sham surgery. |

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| **Suppl. Fig.7. The impact of systemic delivery of Sivelestat on neuropathic pain of cancer origin**Analysis of cancer-induced mechanical hypersensitivity in the paw contralateral to tumor cell implanted paw following intraperitoneal application of Leucocyte elastase inhibitor, Sivelestat, as compared to the vehicle-injected group. A single dose of 20 mg/kg body weight Sivelestat or an equal volume of vehicle was injected i.p. on day 28 (PID28) following tumor cell implantation in the femur (Blue arrow). 1. Paw withdrawal responses to von Frey force of 0.16 g before SNI (Basal) and at 1, 3, 6 and 24h following 20 mg/kg body weight i.p. Sivelestat or Vehicle on day 28 following tumor cell implantation.
2. Mechanical response thresholds calculated as von Frey filament strength required to achieve 60% withdrawal frequency before SNI (Basal) or at 1, 3, 6 and 24h following 20 mg/kg body weight i.p. Sivelestat or Vehicle on day 28 following tumor cell implantation.

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