Reduced Mechanical Allodynia and Neuronal Responses in the DRG in the Presence of SHP-1 Overexpression

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INTRODUCTION: Transient Receptor Potential Vanilloid 1 (TRPV1) has been an attractive drug target for the treatment of musculoskeletal (MSK) pain. However, the development of TRPV1 agonists and antagonists has been halted by serious on-target adverse effects such as impaired cutaneous noxious heat sensation. It has been reported that the Src homology region 2 domain-containing phosphatase 1 (SHP-1) dephosphorylates (deactivates) TRPV1 in dorsal root ganglia (DRG) and delays the development of cancer-induced bone pain. SHP-1 has also been shown to alleviate Complete Freund Adjuvant (CFA)-induced inflammatory pain by TRPV1 deactivation. The objective of this study was to determine if SHP-1 overexpression has an impact on TRPV1-mediated neuronal responses and capsaicin-induced acute pain behavior in mice. Our long-term goal is to explore if TRPV1 modulation by SHP-1 may provide an effective treatment for MSK pain without the limitations of direct TRPV1 antagonists.

METHODS: Mice with systemic SHP-1 overexpression were previously created in our laboratory (SHP-1 transgenic, Shp1-Tg). *Shp1* gene expression was compared in the DRG of wild type (WT) and Shp1-Tg mice by RT-qPCR. To obtain spatial information about SHP-1 (encoded by the *Ptpn6* gene) and *Trpv1* gene expression in the DRG, L3-L5 DRG samples from 12 weeks old female mice of both genotypes were fixed in formalin, embedded in OCT, and analyzed by RNA in situ hybridization (RNAscope), using probes for *Ptpn6*, *Trpv1* and *Scn10a* (encoding $Na_V1.8$ voltage-gated sodium channels). TRPV1 protein expression was detected in the DRG by Western blot. Neuronal responses were evaluated using *in vitro* calcium imaging after capsaicin stimulation (200 nM) of cultured DRG neurons. To test acute inflammatory pain, WT and Shp1-Tg mice were injected with 12 ug capsaicin or vehicle in the left footpad. Nocifensive behavior was recorded and evaluated as the time spent licking the paw during the first 5 minutes after injection. Mechanical allodynia was determined by von Frey filaments by an investigator blinded for the groups, 4 hours post-capsaicin injection. Paw thickness was measured with a caliper. The tyrosine phosphorylation status of TRPV1 was determined in the DRG ex vivo by immunoprecipitation and western blot. The study was approved by IACUC (#23-032). Data were tested for statistical significance using GraphPad Prism 9.4.0. Data of mechanical allodynia was log transformed before statistical analysis. Group sizes were determined based on preliminary data obtained by similar methods. $p<0.05$ was considered statistically significant.

RESULTS: RNAscope revealed significantly higher *Ptpn6/Shp1* expression in the DRG of Shp1-Tg mice (Fig. 1C) (p=0.0156). Lower ΔCq values were detected in the DRG of Shp1-Tg mice compared to WTs (p=0.004), confirming higher *Shp1* gene expression in transgenic mice (Fig. 1D). In vitro, the number of neurons responding to 200 nM capsaicin was significantly less in the DRG culture of naive Shp1-Tg mice compared to WTs (Fig. 2) (p=0.0044). Capsaicin injection led to significantly more time spent with paw licking compared to vehicle in both genotypes (Fig 3A) (p<0.0001vehicle vs capsaicin). WT mice showed significantly lower paw withdrawal threshold 4 hours after capsaicin footpad injection compared to vehicle (Fig 3B). Interestingly, in Shp1-Tg mice, capsaicin injection did not result in a significant drop in paw withdrawal threshold compared to vehicle (Fig 3B) (p=0.0167 WT vehicle vs WT capsaicin, p>0.9999 Shp1-Tg vehicle vs capsaicin), indicating these mice develop less mechanical allodynia in response to capsaicin. Capsaicin injection led to a significant increase in paw thickness in both genotypes, and no difference between genotypes was detected (Fig 3C) (p=0.0296 WT vehicle vs WT capsaicin, p=0.0022 Shp1-Tg vehicle vs Shp1-Tg capsaicin). Western blot and immunoprecipitation detected similar total TRPV1 protein expression in the DRG of WT and Shp1-Tg mice, however, less tyrosine phosphorylated TRPV1 appeared in the DRG of Shp1-Tg mice (data not shown).

DISCUSSION: Genetically enhanced SHP-1 expression resulted in reduced capsaicin-induced neuronal responses in the DRG. In a capsaicin-induced acute inflammatory pain model, SHP-1 overexpression led to reduced mechanical allodynia, without affecting early nocifensive pain behavior and paw thickness. Our results suggest that the observed difference is due to reduced tyrosine phosphorylation and activation of TRPV1 in the presence of enhanced SHP-1 phosphatase activity in the capsaicin-sensitive sensory neurons of the DRG.

SIGNIFICANCE/CLINICAL RELEVANCE: TRPV1 agonists and antagonists have been attractive drug targets for the treatment of MSK pain for several years. However, the potential of TRPV1 modulation by SHP-1 is underexplored and might represent a novel strategy for the future treatment of MSK pain, with potentially fewer adverse effects compared to TRPV1 agonist/antagonist drug candidates.

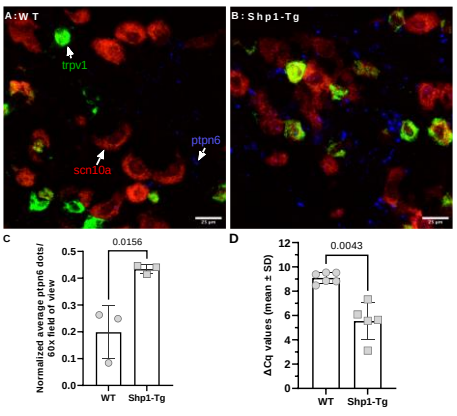

Figure 1. *Shp1* gene expression in the DRG. A-B: Representative RNAscope images of WT (A) and Shp1-Tg (B) DRG. Green-*Trpv1*, red-*Scn10a*, blue-*Ptpn6/shp1*. C: *Ptpn6/Shp1* expression based on the RNAscope (mean ± SD, n=3/group, unpaired t-test). D: *Shp1* gene expression in the DRG of WT and Shp1-Tg mice illustrated as ΔCq (unpaired t-test, n=5-6/genotype).

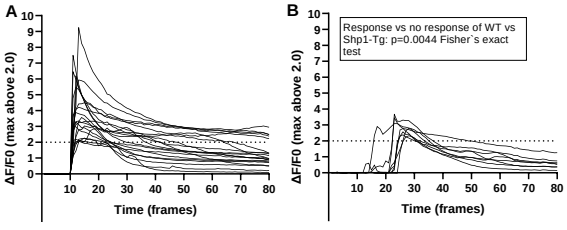

Figure 2. Capsaicin-induced neuronal responses. A: Responses of cultured WT DRG neurons. B: Responses of cultured Shp1-Tg DRG neurons. Response was considered as ΔF/F0>2.0. (Fischer`s exact test, n=1 culture/genotype).

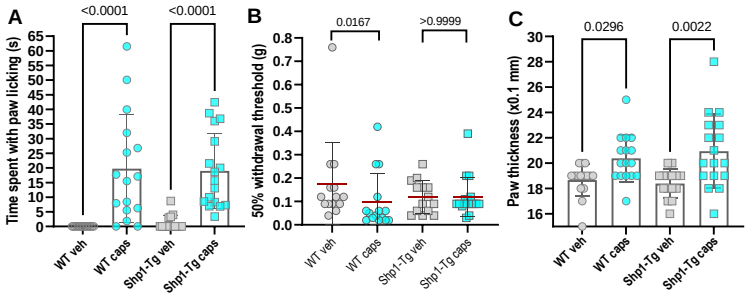

Figure 3. Capsaicin-induced pain behavior and paw oedema in WT and Shp1-Tg mice. A: Time spent with paw licking 0-5 minutes after capsaicin/vehicle injection. B: Mechanical allodynia 4 hours after capsaicin/vehicle injection. C: Paw thickness. (Mean ± SD, Kruskal-Wallis test and Dunn`s multiple comparison, n=14-17/group).