

Objective Cancer-induced bone pain (CIBP) is a major clinical problem for which current treatments lack full efficacy. One of the major reasons is increased osteoclastogenesis activity within cancer bone microenvironment. Calpain inhibitor, a previously demonstrated osteoclastogenesis regulator, was applied both in murine RAW264.7 cells in vitro and in rat CIBP model in vivo to determine whether it could suppress CIBP.

Methods In vitro, Calpain inhibitor Calpastatin Peptide (50nM) was applied in Receptor Activator of NFκB Ligand (RANKL, 50ng/ml)-induced murine RAW264.7 cells for 6 days to investigate its inhibitory effect on osteoclastogenesis activity, through Tartrate-resistant acid phosphatase (TRAP) stain and pit formation assay. In the in vivo study in rat CIBP model using Walker 256 cell line injected into the tibia, the efficacy of Calpain inhibitor III (MDL28170, 1mg/kg) on pain-related behavior test on post-tumor day (PTD) 2, 5, 8, 11, 14, as well as on TRAP stain of the tumor bone on PTD14 were examined.

Results Calpastatin Peptide significantly inhibited TRAP positive cell count ($p < 0.05$) and pit formation area ($p < 0.05$) in murine RAW264.7 cells in vitro, compared with RANKL induction alone group. Moreover, the behavioral study showed that Calpain inhibitor III can effectively attenuate the operative side pain measured by mechanical withdrawal threshold (MWT) on PTD 5,8,11 in rat CIBP model in vivo ($p < 0.05$), but not normalize to the baseline degree ($p < 0.05$). Interestingly, the same attenuation effect was seen on the contralateral side of the operation ($p < 0.05$). TRAP staining of the tumor injected bone indicated that such analgesic effect was accomplished through inhibition of TRAP positive multinucleated osteoclast cell count ($p < 0.05$).

Conclusions Calpain inhibitor can effectively reduce CIBP originated from both ipsilateral and contralateral side of tumor injection in rat model through inhibition of RANKL-induced osteoclastogenesis both in vitro and in vivo. Calpain inhibitor could be a novel therapeutic target to treat CIBP and might involve in neuropathic pain mechanism. Future study is needed to clarify its role in treatment of CIBP.

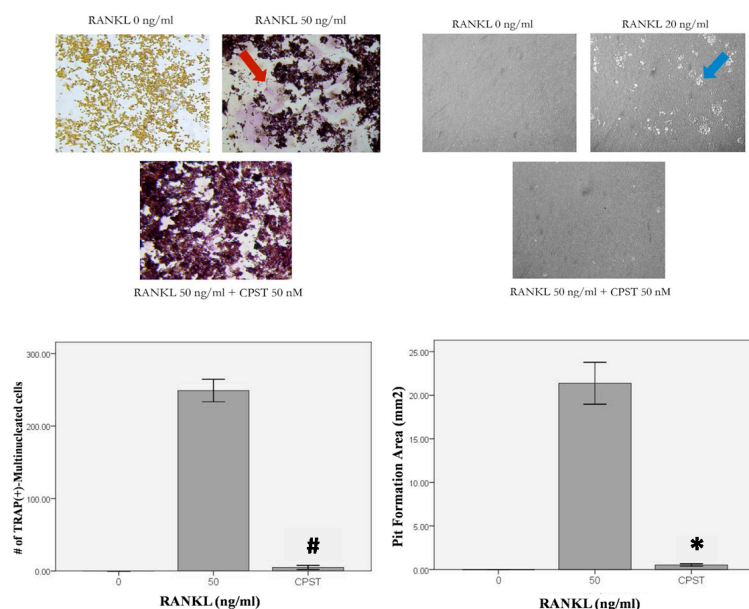


Fig 1 Calpain inhibitor suppresses RANKL-induced osteoclastogenesis in RAW264.7 cells. CPST=Calpastatin. Red arrow indicates TRAP(+) multinucleated cells, blue arrow indicates pit formation. #, * $P < 0.05$ for CPST inhibition vs. RANKL induction only in both TRAP(+) cell count and pit formation area.

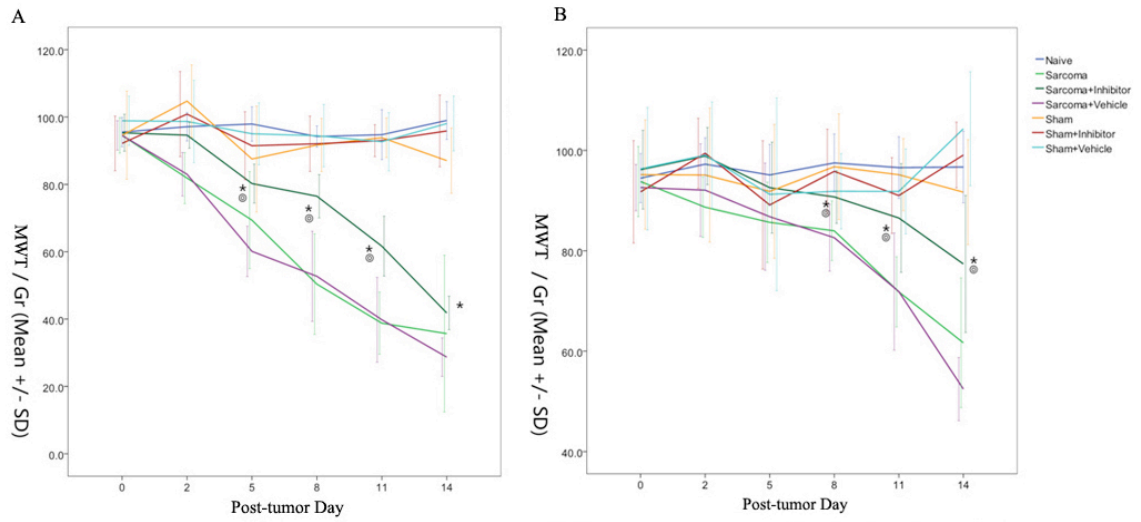


Fig 2 Mechanical Withdrawal Threshold (MWT) of operative side (A) and non-operative side (B) limbs of each group. *, P<0.05 for Sarcoma+Inhibitor vs. Naive; ◐, P<0.05 for Sarcoma+Inhibitor vs. Sarcoma+Vehicle.

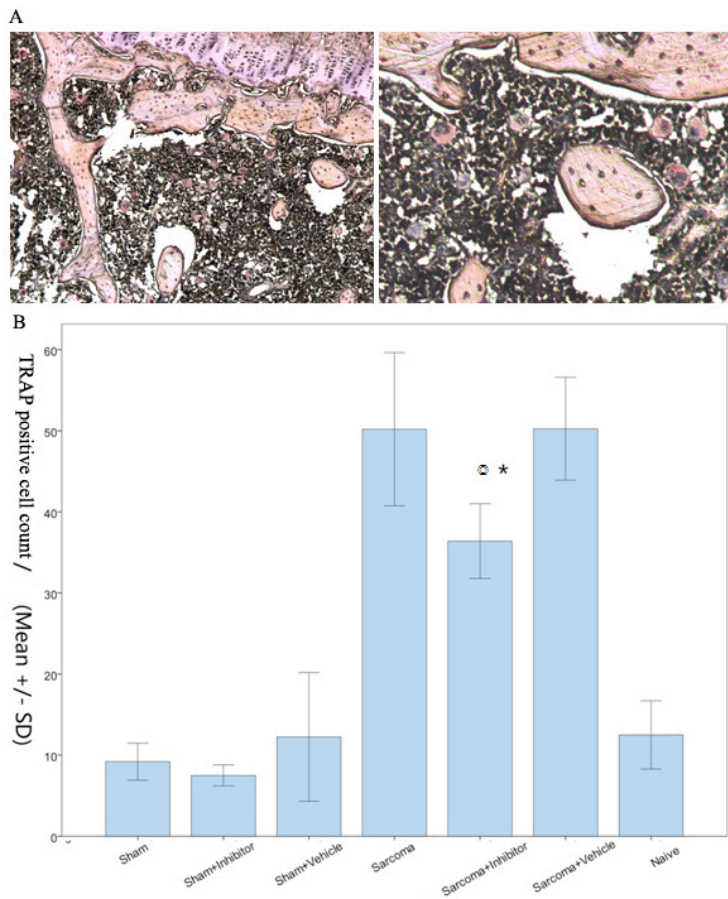


Fig 3 A, TRAP stain of matured osteoclasts in tumor bone site (left 10×10, right 10×20). Arrow indicates matured multinucleated osteoclasts. B, TRAP positive cell count in tumor injected tibia bone of each group. *, P<0.05 for Sarcoma+Inhibitor vs. Naive; ◐, P<0.05 for Sarcoma+Inhibitor vs. Sarcoma+Vehicle.