Effects of Panobinostat, a Histone Deacetylase Inhibitor, on NETs Formation in Canine Neutrophils

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Introduction

Neutrophils play an important role in innate immunity by formation of neutrophil extracellular traps (NETosis).

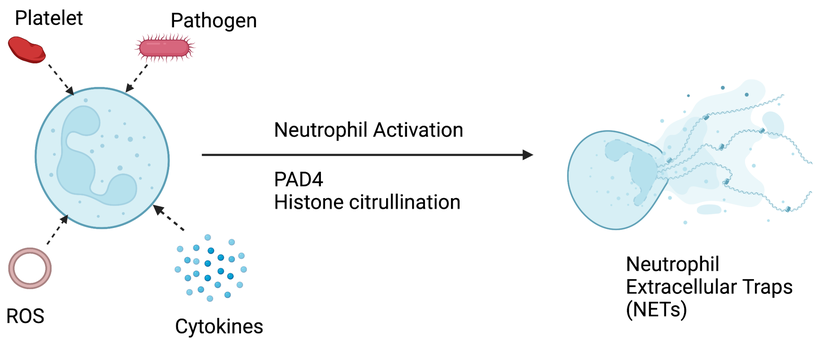


Fig. 1 Neutrophil extracellular traps formation in response to potential activators leading to histone citrullination via peptidyl arginine deiminase 4 (PAD4)

Overzealous inflammation and NET formation can also occur with **cancer**[1-3] → In people, NETs have been shown to contribute to tumor *progression* and *metastasis*[4-7]

Materials and Methods

Eligibility Criteria:

- Dogs deemed healthy, > 1 year of age, > 10kg, no vaccination within 30 days of enrollment, no comorbidities or medications
- Normal complete blood count within reference intervals

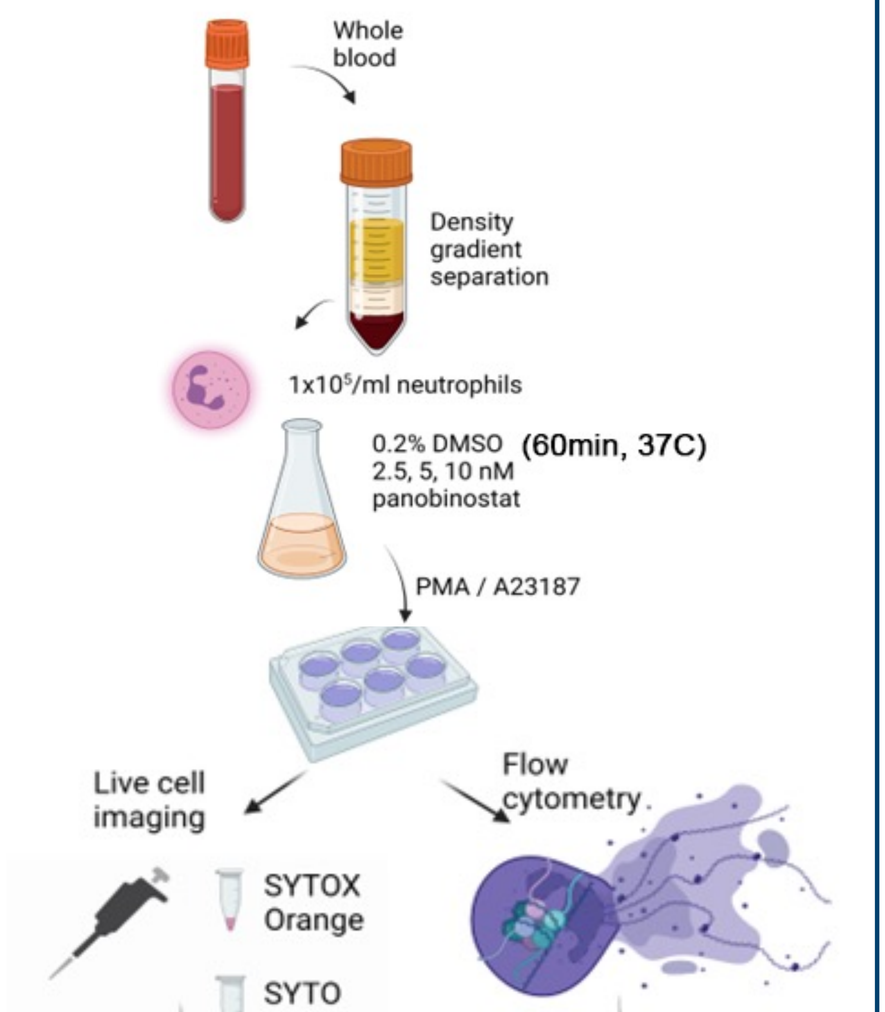


Fig. 3 Schematic illustration of the experimental procedures and assays. Isolated canine neutrophils from whole blood were pre-treated with increasing concentrations of panobionstat or its vehicle control (0.2% DMSO) before activation with 100 nM PMA or 16 uM A23187. NETs were evaluated by live cell imaging using immunofluorescence microscopy and intracellular histones were assessed by immunodetection and flow cytometry.

Rationale/Hypothesis/Objectives

Rationale:

- Cancer is the leading cause of death in older dogs and new therapeutic options are needed[2]
- Given the potential role NETs may play in cancer progression, treatment strategies targeting their formation should be pursued
- This study is the first step to determine if HDACis may reduce *in vitro* NETosis in dogs and provides the basis for future *in vivo* studies

Hypothesis:

The HDACi, panobinostat, will dose-dependently modulate NET formation in canine neutrophils induced by phorbal myristate acetate (PMA) or A23187, by inhibiting histone citrullination.

Objectives:

1. Evaluate if increasing concentrations of panobinostat would modulate in vitro NETs formation by PMA or A23187
2. Evaluate if panobinostat inhibits PMA or A23187-induced NETosis by inhibiting histone citrullination

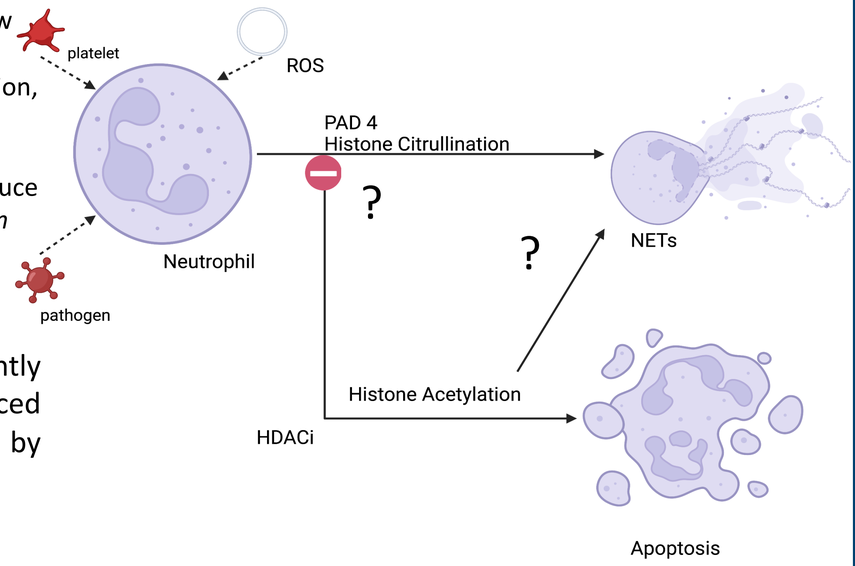
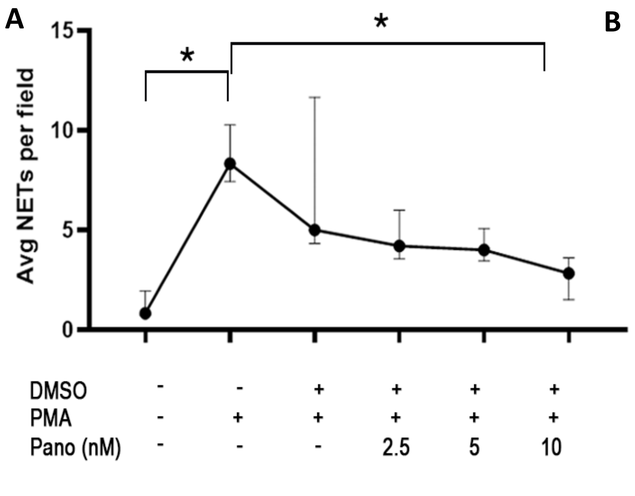


Fig. 2. Proposed mechanisms of NETosis inhibition by panobinostat (HDACi)

Results



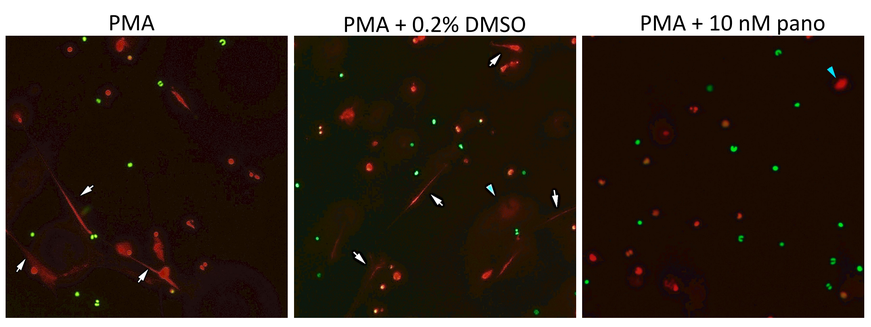
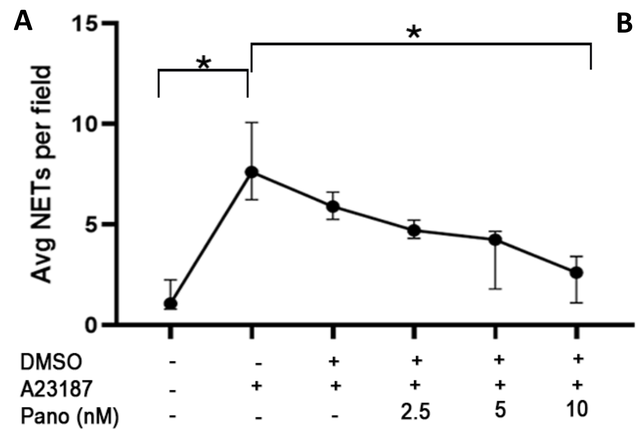


Fig. 3 A. Dose-response to panobinostat of neutrophils isolated from 8 dogs. Dose-dependent inhibition was noted with neutrophil extracellular traps (NETs) inhibition in PMA-activated neutrophils. DMSO-treated neutrophils served as vehicle control. **B.** Representative fluorescence microscopy images of PMA activated neutrophils in the presence of DMSO or 10 nM panobinostat (pano). Note that NETs (white arrows) in PMA-activated neutrophils were absent when treated with 10nM pano. Blue arrow represents necrotic cells. Original magnification 10x.



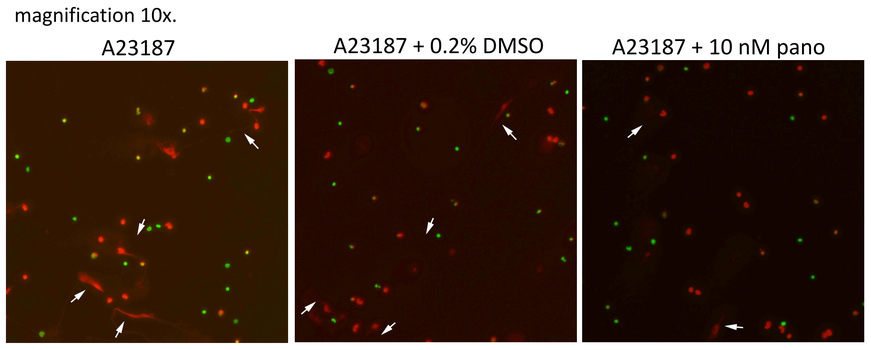


Fig. 4 A. Dose-response to panobinostat of neutrophils isolated from 8 dogs. Dose-dependent inhibition was noted with neutrophil extracellular traps (NETs) inhibition in A23187-activated neutrophils. DMSO-treated neutrophils served as vehicle control. **B.** Representative fluorescence microscopy images of A23187 activated neutrophils in the presence of DMSO or 10 nM panobinostat (pano). Note that NETs formation (white arrows) in A23187-activated neutrophils were reduced when treated with 10nM pano. Original magnification 10x.

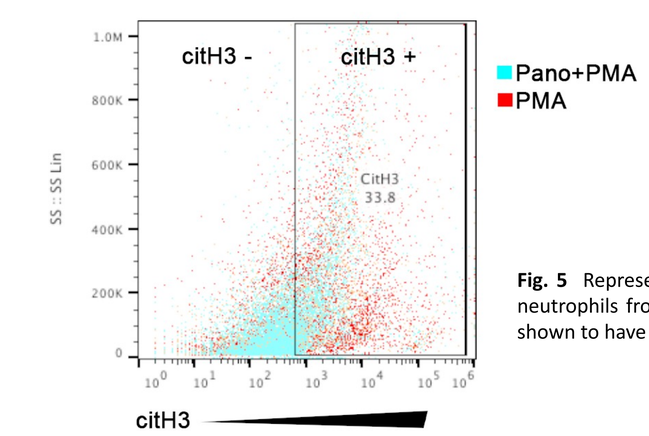


Fig. 5 Representative scatter dot plot of intracellular citrullinated histone H3 (citH3) expression in isolated neutrophils from 1 dog. PMA-activated neutrophils pre-treated with 10 nM panobionstat (blue dots) were shown to have modulated expression of citH3 compared to PMA-activated neutrophils (red dots).

Conclusion/Future Directions

Conclusions:

- Panobinostat modulated NETosis in canine neutrophils in a dose-dependent manner
- Unlike human neutrophils, panobinostat did not further stimulate NETosis
- Inhibition of NETosis by panobinostat may be secondary to a reduction in histone citrullination

Future Directions:

- Assessment of apoptosis in panobinostat-treated canine neutrophils
- Western blot analysis to evaluate histone citrullination and acetylation
- Future pharmacodynamic studies to assess if panobinostat decreases NETosis in dogs with cancer

References

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