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#01

Investigating Specificity of Oxo-M and 4-PPBP to CD146⁺ Stem/Progenitor Cells

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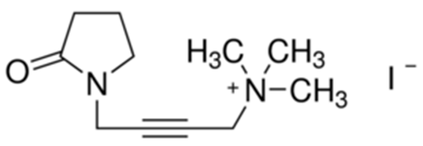
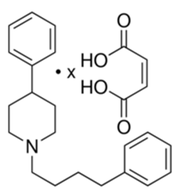
Introduction

- Connective Tissue Growth Factors (CTGF) have potential to guide regeneration of dense fibrous connective tissues by inducing endogenous CD146⁺ stem/progenitor cells via FAK and ERK1/2 signaling pathways, but entails several translational limitations.
- Recent data demonstrated that Oxotremorine M (Oxo-M) and PPBP maleate (4-PPBP) can replace functions of CTGF and induce fibrogenic differentiation of CD146⁺ stem/progenitor cells via non-neuronal muscarinic acetylcholine receptors (AChR) and σ1 receptor (σ1R) signaling.
- Accordingly, this study was designed to 1) determine the autocrine effect of ACh toward Oxo-M signaling and 2) investigate the intracellular link between Oxo-M and 4-PPBP and FAK and ERK1/2 signaling pathways by identifying secondary mediators.



Oxotremorine methiodide (Oxo-M)
N,N,N,-Trimethyl-4-(2-oxo-1-pyrrolidinyl)-2-butyn-1-ammonium iodide



4-PPBP maleate
4-phenyl-1-(4-phenylbutyl)-piperidine maleate

Mechanism for specificity of Oxo-M and 4-PPBP

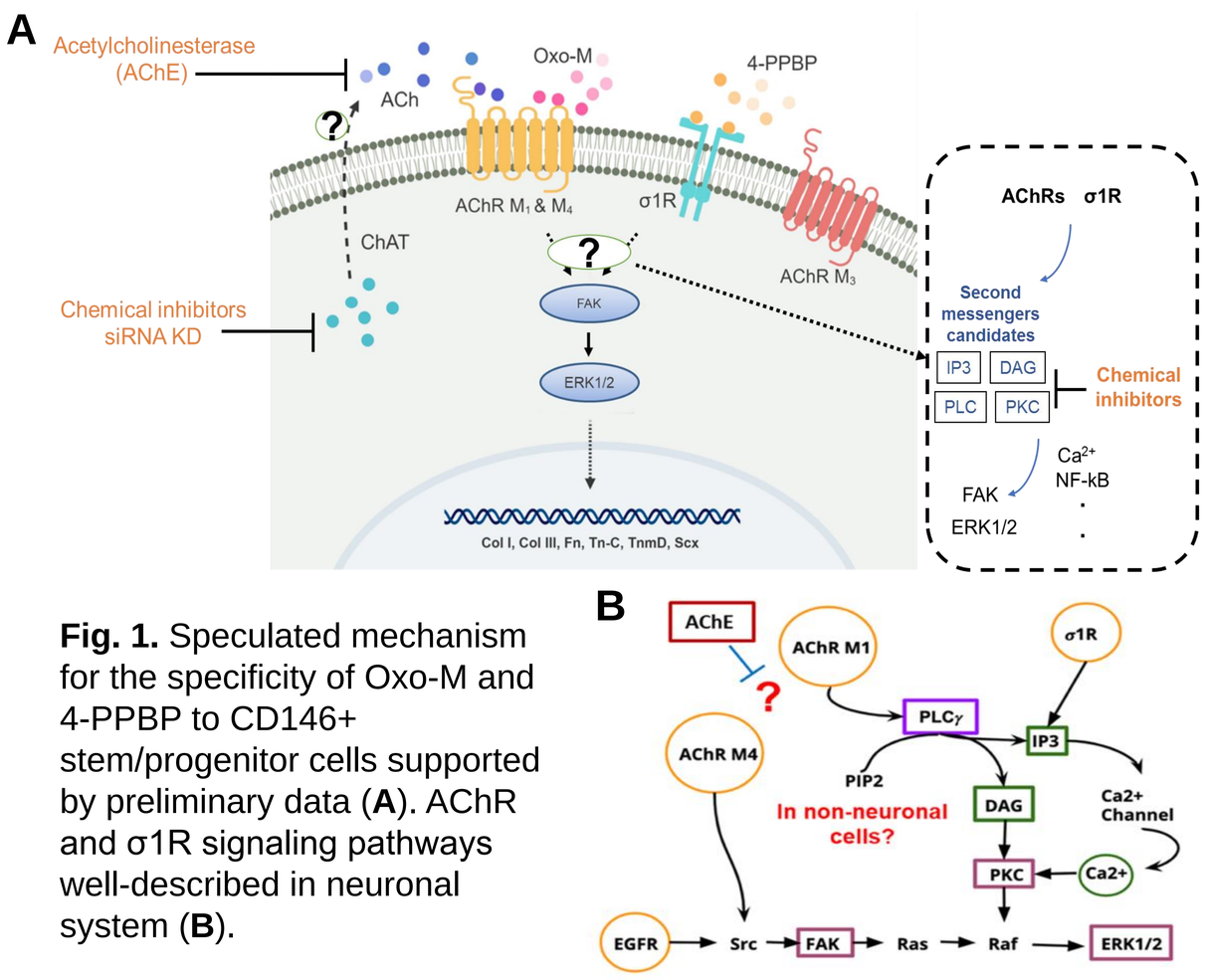
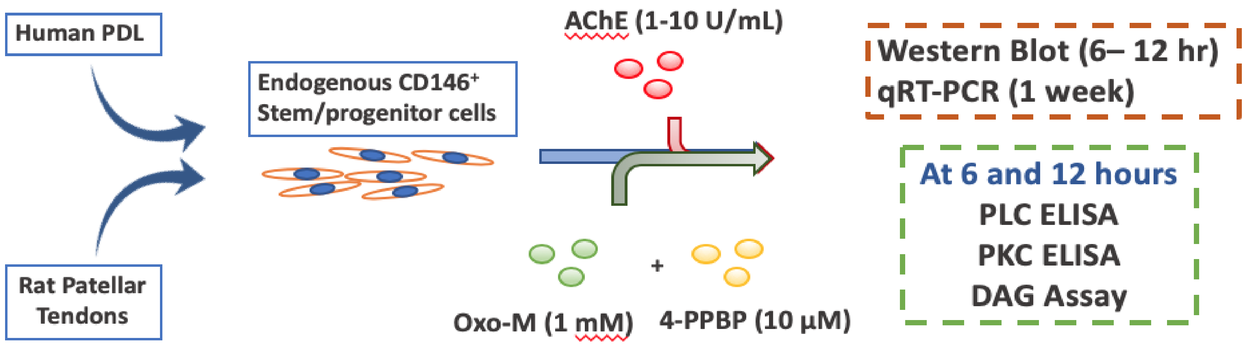


Fig. 1. Speculated mechanism for the specificity of Oxo-M and 4-PPBP to CD146+ stem/progenitor cells supported by preliminary data (**A**). AChR and σ1R signaling pathways well-described in neuronal system (**B**).

Methods



- CD146⁺ stem/progenitor cells were isolated from human PDL from adult patients and patellar tendons of skeletally mature Sprague-Dawley rats by enzyme digestion and magnetic-activated cell sorting

Role of ACh Signaling in Oxo-M and 4-PPBP

- ELISA assay was performed to confirm secretion of ACh from CD146⁺ stem/progenitor cells (**Fig. 2A).**
- Acetylcholinesterase (AChE) (1 – 10 U/ml) was applied to degrade secreted ACh from the cells treated with 1mM Oxo-M and 10 μM 4-PPBP. Western Blot was performed at 6 – 12 hrs after treatment to check phosphorylation of ERK1/2 (**Fig. 2B**) and qRT-PCR was performed at 1 week after treatment to check mRNA expressions (**Fig. 2C**).

Potential Secondary Mediators for AChR and σ1R Signaling

- PLC ELISA, PKC ELISA, DAG assay kits were use as per established methods to measure expressions of PLC, PKC, and DAG at 6 – 12 hours after Oxo-M and 4-PPBP treatment (**Fig. 3 A-C**).

Results

Role of ACh Signaling in Oxo-M and 4-PPBP

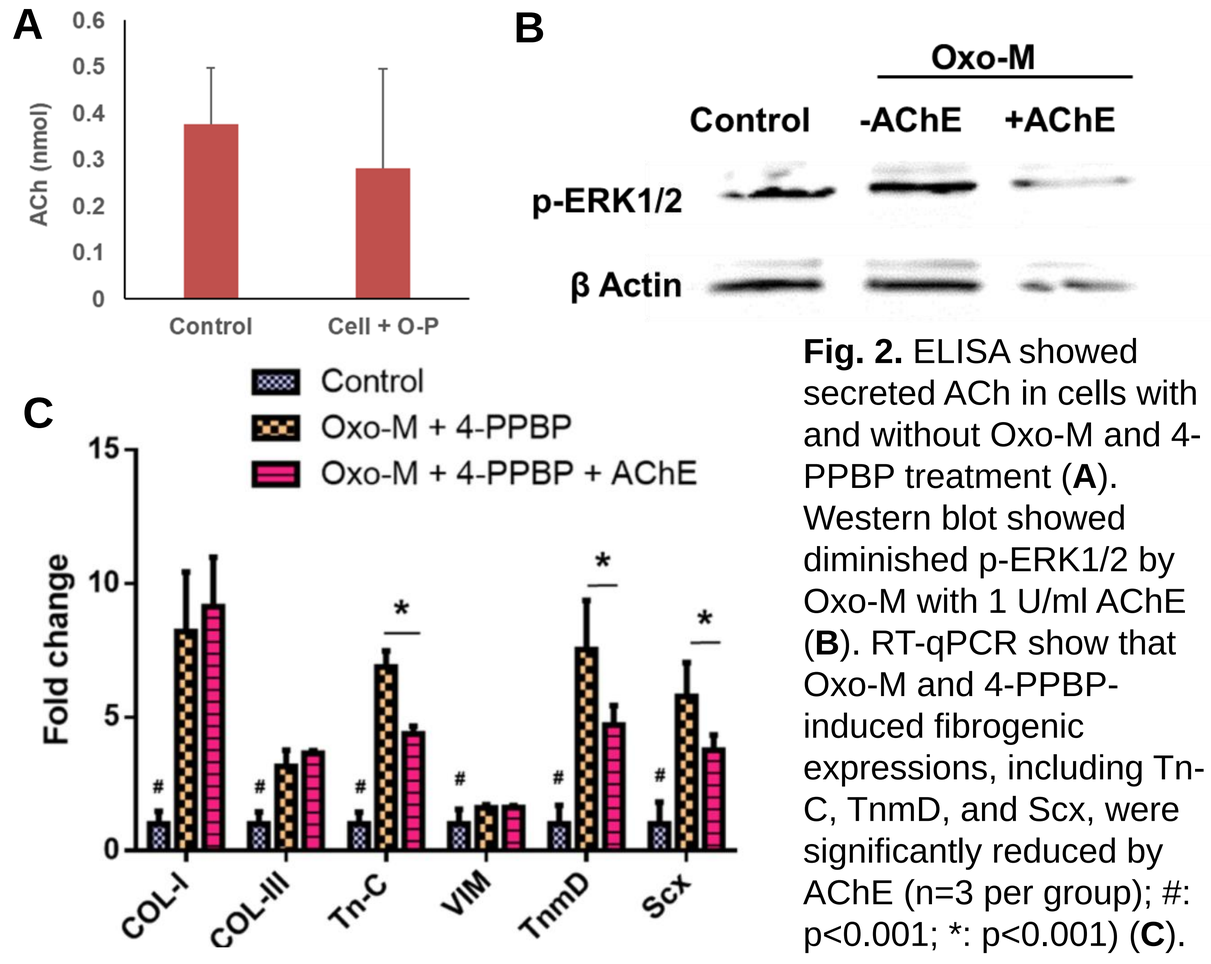


Fig. 2. ELISA showed secreted ACh in cells with and without Oxo-M and 4-PPBP treatment (**A**). Western blot showed diminished p-ERK1/2 by Oxo-M with 1 U/ml AChE (**B**). RT-qPCR show that Oxo-M and 4-PPBP-induced fibrogenic expressions, including Tn-C, TnmD, and Scx, were significantly reduced by AChE (n=3 per group); #: p<0.001; *: p<0.001) (**C**).

Potential Secondary Mediators for AChR and σ1R Signaling

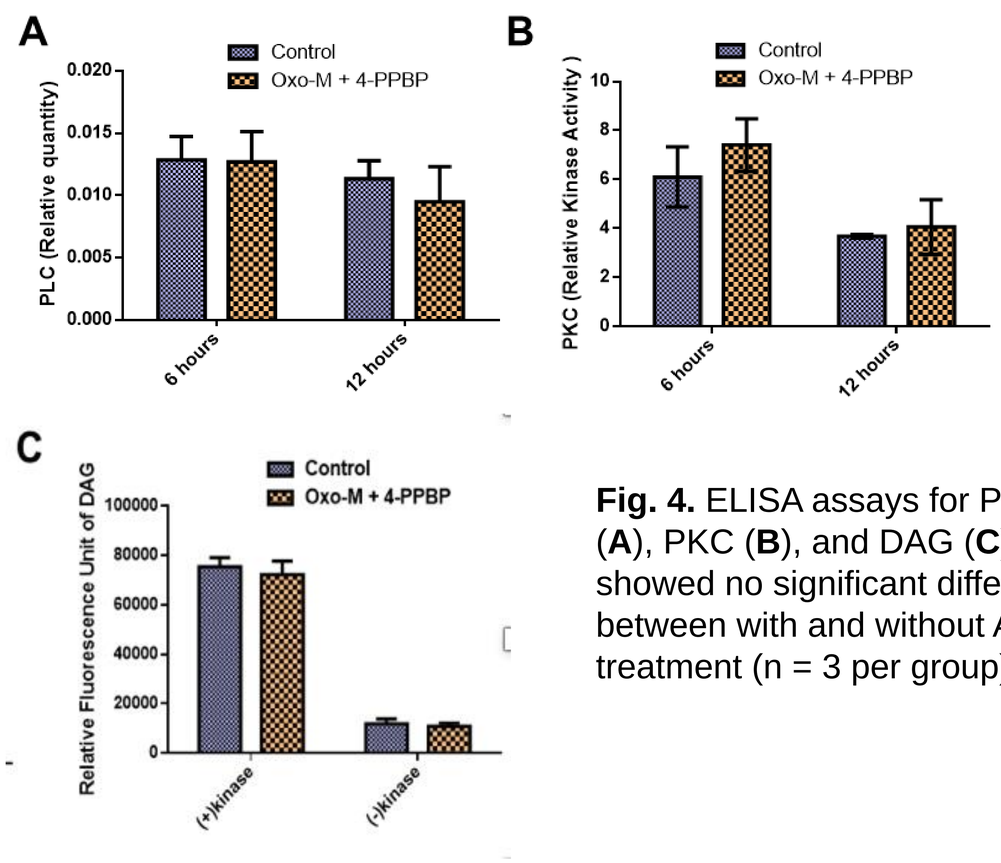


Fig. 4. ELISA assays for PLC (**A**), PKC (**B**), and DAG (**C**) showed no significant difference between with and without AChE treatment (n = 3 per group).

Discussion & Conclusion

- The present findings support our hypothesis that Ach-to-AChR signaling plays essential roles in Oxo-M and 4-PPBP-induced fibrogenic differentiation of CD146⁺ stem/progenitor cells from PDL and tendons.
- Degrading Ach with AChE diminished fibrogenic gene expressions induced by Oxo-M, which is an AChR agonist.
- Key secondary messengers involved in AChR M_4 and σ1R signaling in neuronal system are likely not involved in fibrogenic differentiation of Oxo-M and 4-PPBP induced CD146⁺ stem/progenitor cells.
- Potential translocation of σ1R from mitochondrion-associated endoplasmic reticulum membrane (MAM) to plasma membrane suggests the activation of Src and neurotrophic tyrosine kinase receptor type 2 (TrkB) signaling, which necessitates follow-up investigation.
- In conclusion, the present study extends our knowledge in mechanism of the specificity of Oxo-M and 4-PPBP to CD146⁺ stem/progenitor cells in tendon and PDL via AChR and σ1R signaling pathways.

Acknowledgment

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