The Signaling Pathway of Sigma-1 Receptor Agonist PPBP Maleate (4-PPBP)

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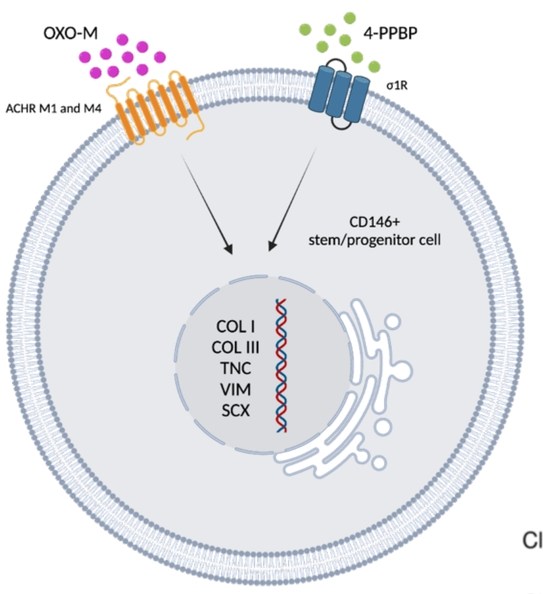
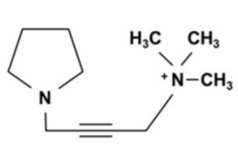
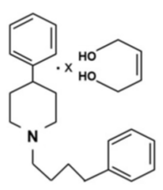
INTRODUCTION

- Pharmacokinetic small molecules Oxotremorine M (Oxo-M) and PPBP maleate (4-PPBP) induce differentiation of CD146⁺ stem/progenitor cells extracted from tendon and periodontal ligament (PDL) tissue.
- We have recently shown that Oxo-M acts via acetylcholine receptor signaling.
- 4-PPBP signaling mechanism remains unknown, despite the potential role of the sigma-1 receptor (σ1R) in other types of cells.
- This study sought to confirm 4-PPBP as a σ1R agonist and to identify the intracellular signaling molecules that elicit tendon and PDL regeneration following 4-PPBP treatment

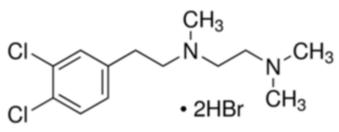




Oxotremorine methiodide (Oxo-M)
N,N,N,-Trimethyl-4-(2-oxo-1-pyrrolidinyl)-2-butyn-1-ammonium iodide



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



BD1047 dihydrobromide
N'-[2-(3,4-dichlorophenyl)ethyl]-*N*,*N*,*N*'-trimethylethane-1,2-diamine;dihydrobromide

Fig 1. Action of Oxo-M/4-PPBP.

Hypothesized mechanism of action for 4-PPBP and BD1047

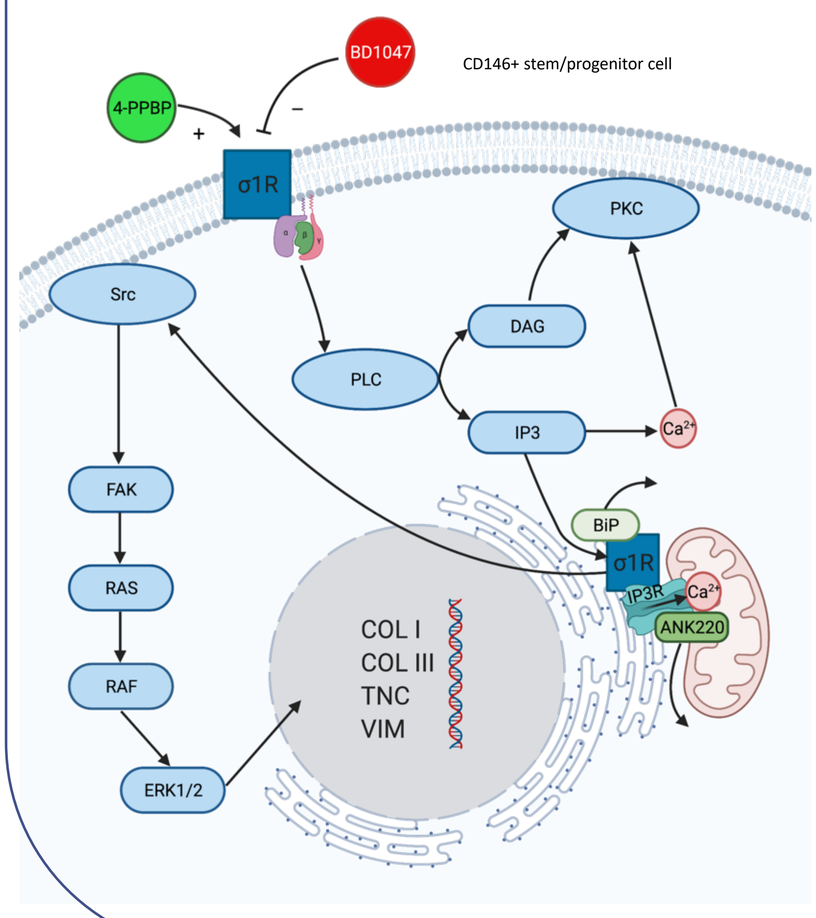
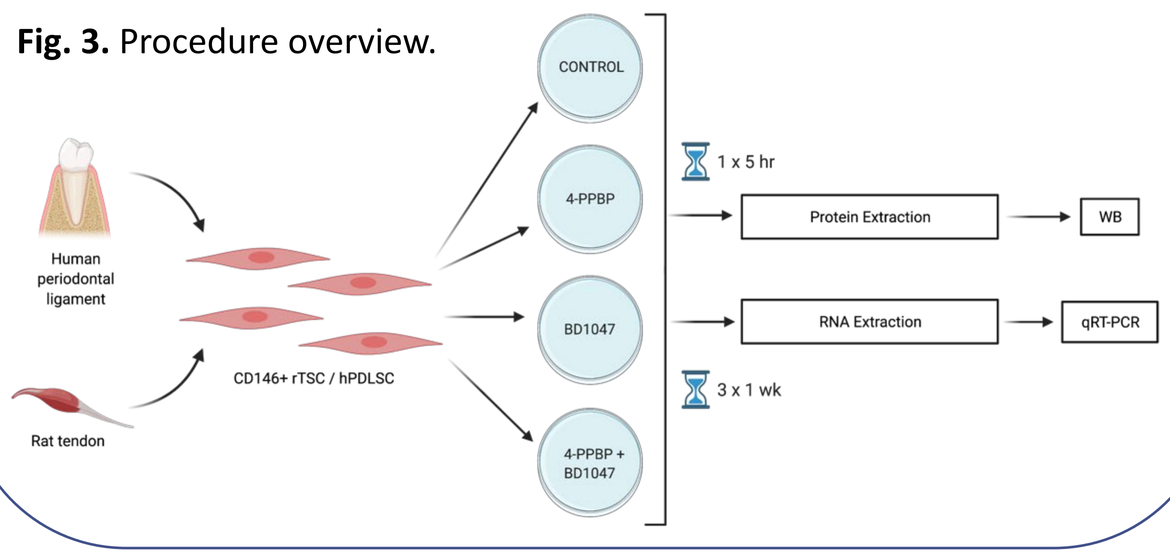


Fig 2. Intracellular σ1R colocalizes with IP3 receptor at the at the mitochondrion-associated endoplasmic reticulum membrane (MAM). We propose that a plasma membrane-localized form of σ1R initiates second messenger signaling to activate the intracellular σ1R and induce its translocation from the MAM to the plasma membrane. Subsequent interaction between σ1R and Src results in ERK1/2 activation and transcription of genes associated with fibrogenic differentiation.

MATERIALS & METHODS

- CD146⁺ rat tendon stem cells (rTSC) and CD146⁺ human PDL stem cells (hPDLSC), were treated with σ1R agonist (50 μM 4-PPBP), selective σ1R antagonist (200 μM BD1047), and their combinations (**Fig. 3**).
- Following one treatment with 5-hour incubation, activity of signaling proteins p-ERK1/2 and Src were assessed using western blot.
- Following three treatments with 1 week incubation each, Expression of COLI, COLIII, TNC, and VIM genes were assessed using qRT-PCR

Fig. 3. Procedure overview.



RESULTS

σ1R antagonist BD1047 attenuates 4-PPBP-induced p-ERK1/2

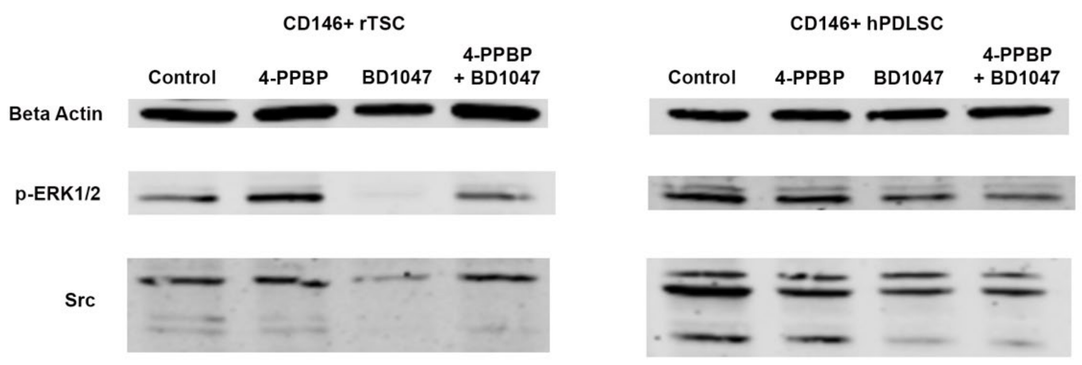


Fig 4. Western blot for p-ERK1/2 and Src in CD146+ rTSC and hPDLSC at 5 hours.

σ1R antagonist inhibits 4-PPBP-induced gene expressions

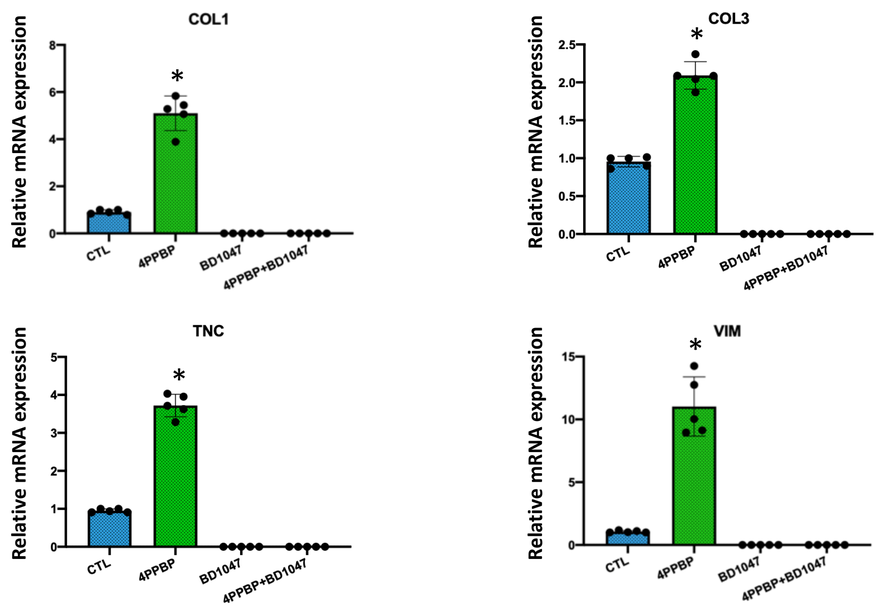


Fig 5. qRT-PCR revealed that expressions of COL-I, COL-III, TNC, and VIM induced by 4-PPBP were significantly diminished by BD1047 (*:p<0.0001 compared to control; n=5 each group).

DISCUSSION & CONCLUSION

- Western blot data suggested that ERK1/2 and Src signaling are involved in the σ1R-activated intracellular cascade and potentially linked with improved regeneration of tendon and PDL tissue by Oxo-M and 4-PPBP.
- hPDLSC displayed notable difference in sensitivity to antagonist.
- Increased expression of COLI, COLIII, TNC, and VIM genes indicated tenogenic differentiation of CD146+ rTSC following treatment with 4-PPBP.
- Silenced gene expression in antagonist and combination groups suggested that lower BD1047 concentration and shortened incubation times are necessary in order to accurately assess gene expression following inhibitor treatment.
- In contrast to uniform rat tissue, human tissue donors' genetic variation, age, and medical history may affect cellular activity and protein expression. COVID-19 limited hPDLSC collection; therefore, future studies will include hPDLSC from a variety of donors in order to establish universal patterns.
- Follow-up studies will investigate how Oxo-M and 4-PPBP apply to an additional intracellular signaling pathway. Using inhibitors of signaling molecules, the chronological order of the cascade will be determined.
- Clinical use of Oxo-M/4-PPBP treatment has potential to accelerate healing following tendon injury, dental trauma, orthodontic tooth movement, and advanced periodontal disease.

ACKNOWLEDGEMENTS

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