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Extracellular ATP modulates immunomodulatory property of periodontal ligament cells

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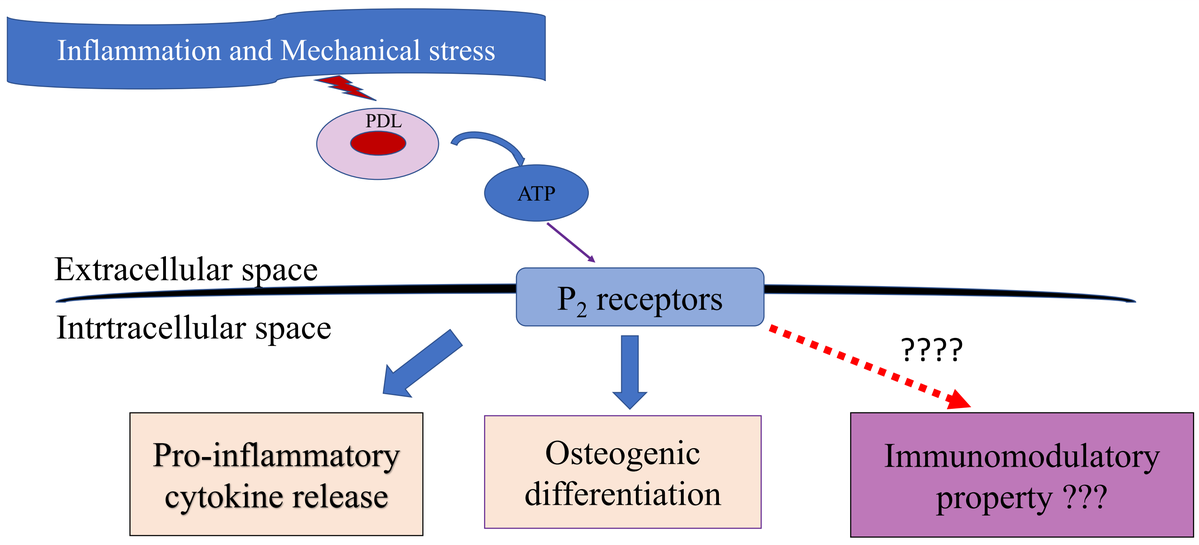
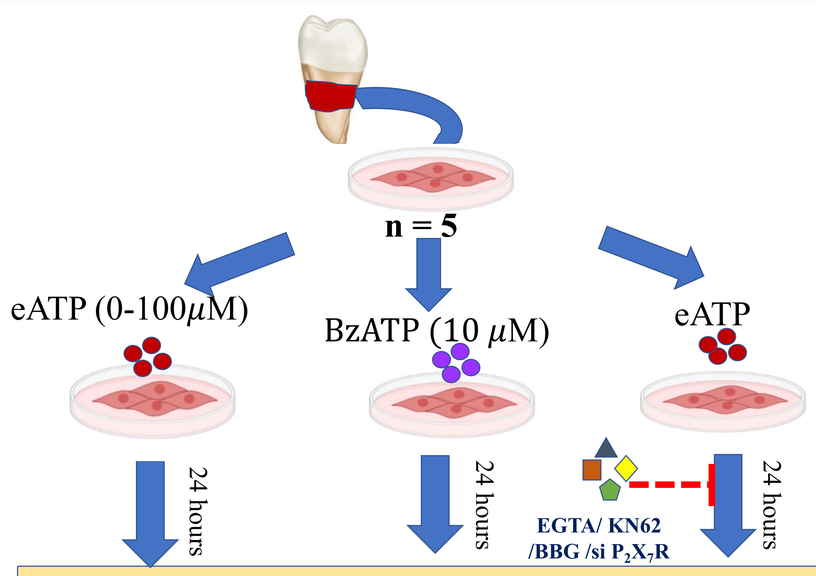
Background and Aims



Aims

1. To investigate the role of eATP on the immunomodulatory function of periodontal ligament cells
2. To identify the involvement of specific purinergic P_2 receptor on that function

Material and Method



- Real time polymerase chain reaction (RT-PCR) (Indoleamine 2,3 dioxygenase (IDO) and interferon-gamma (IFNγ)
- IDO enzymatic activity assay
- ELISA (IFNγ)

Statistical analysis
- Mean and S.D
- Significance analysis by One-Way Anova (p value≤ 0.05)

Results

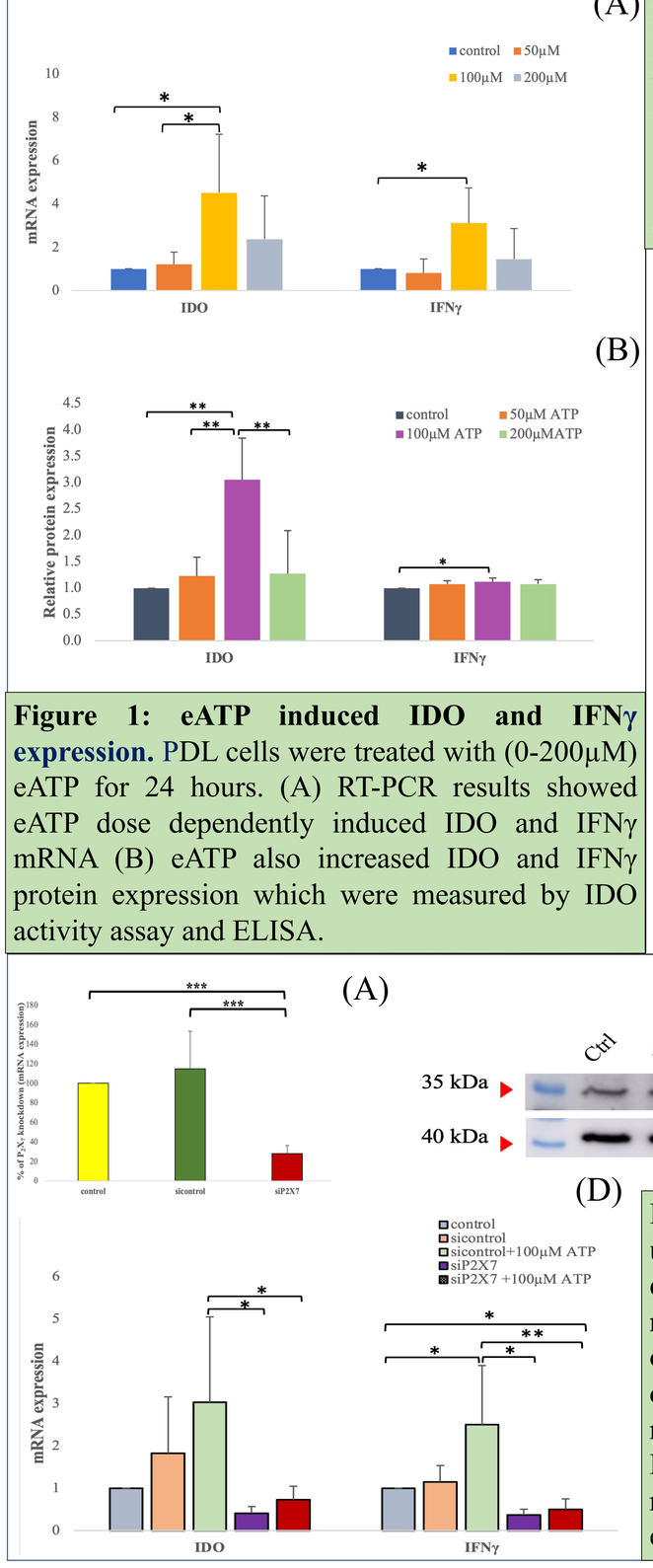
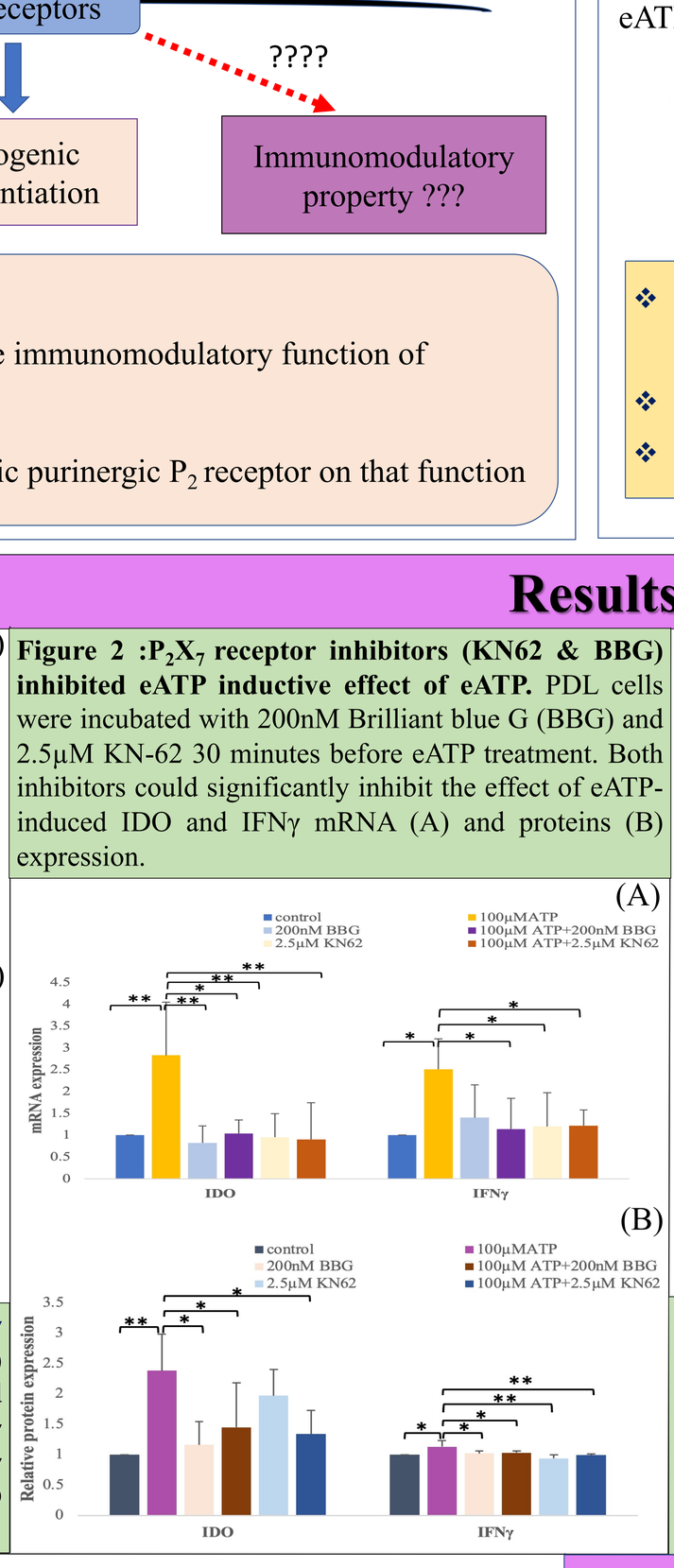


Figure 1: eATP induced IDO and IFNγ expression. PDL cells were treated with (0-200μM) eATP for 24 hours. (A) RT-PCR results showed eATP dose dependently induced IDO and IFNγ mRNA (B) eATP also increased IDO and IFNγ protein expression which were measured by IDO activity assay and ELISA.

Figure 2 :P_2X_7 receptor inhibitors (KN62 & BBG) inhibited eATP inductive effect of eATP. PDL cells were incubated with 200nM Brilliant blue G (BBG) and 2.5μM KN-62 30 minutes before eATP treatment. Both inhibitors could significantly inhibit the effect of eATP-induced IDO and IFNγ mRNA (A) and proteins (B) expression.



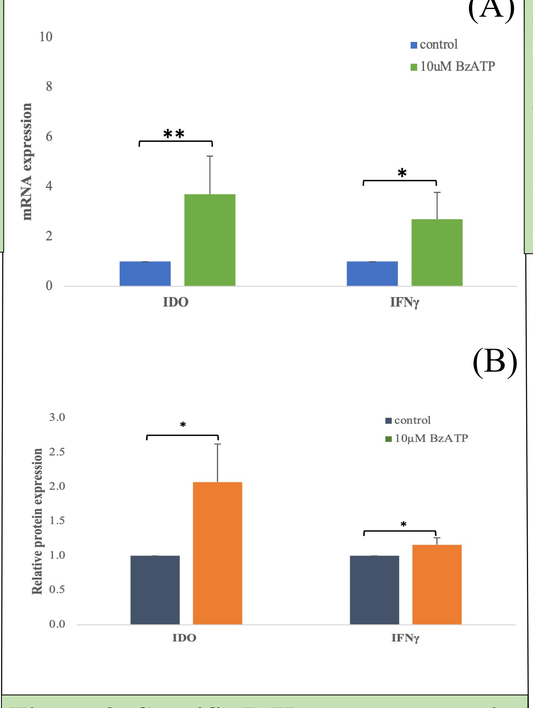


Figure 3: Specific P_2X_7 receptor agonist (BzATP) induced IDO and IFNγ expression. 10μM BzATP was use to activate PDL cells for 24 hours. BzATP markedly increased IDO and IFNγ expression at mRNA (A) and protein (B) levels.

Figure 4: Intracellular calcium participated in eATP induced IDO and IFNγ expression. PDL cells were pretreated with calcium chelator (10 μM EGTA) 30minutes before eATP treatment. Calcium chelator (EGTA) significantly downregulates eATP induced IDO and IFNγ mRNA (A) and proteins (B) expression.

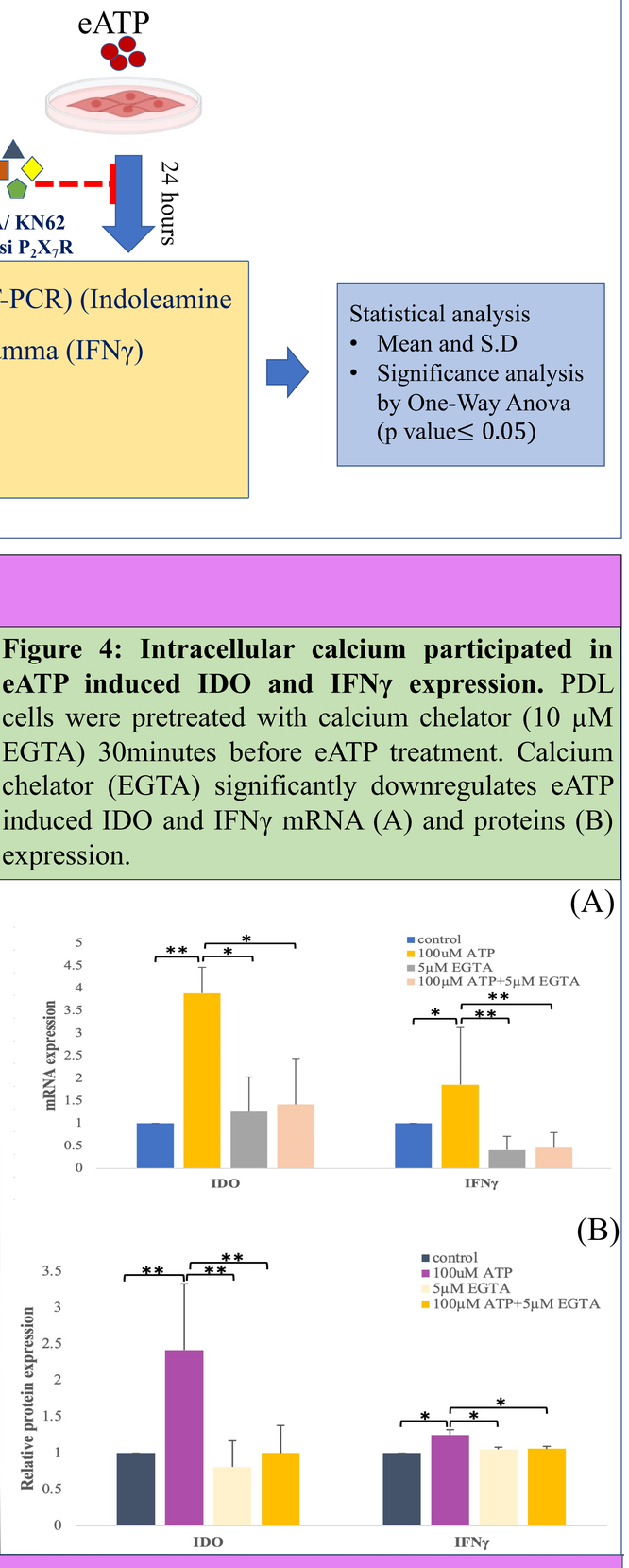


Figure 5: Knockdown of P_2X_7 receptor using siRNA attentuated inductive effect of eATP. PDL cells were transfected with P_2X_7 receptor siRNA for 24-72 hours. Knockdown of P_2X_7 receptor by siRNA showed dramatically decrease in expression of P_2X_7 receptor as shown in RT-PCR (A) and Western Blot analyses (B and C). siRNA targeting P_2X_7 receptor significantly decreased mRNA expression of IDO and IFNγ induced by eATP.

Results

- Extracellular ATP promotes immunosuppressive molecules (IDO and IFNγ) expression by periodontal ligament cells.
- Significant inhibition of eATP effect by chemical inhibitors (KN62 and BBG) and siRNA, stimulation by BzATP showed that P_2X_7 receptor participated in eATP –induced IDO and IFNγ expression.
- As P_2X_7 receptor is cationic channel receptor that is highly permeable to calcium, downregulation of eATP-induced IDO and IFNγ expression by calcium chelator (EGTA) confirmed the involvement of P_2X_7 receptor and intracellular calcium signaling in this mechanism.

Conclusion

- Our findings showed that eATP induced immunosuppressive property of periodontal ligament cells through P_2X_7 receptor signaling.
- Since immunosuppressive molecules triggers tissue healing process, eATP may serve as a regulatory molecule in tissue healing and regeneration.
- eATP may become one of the important promising molecule used for future periodontal regeneration therapy.

Acknowledgement

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Selected References

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