Intracellular disposition of the internalized CD36 receptor following its binding to azapeptides

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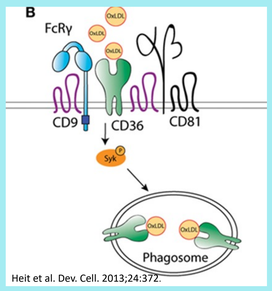
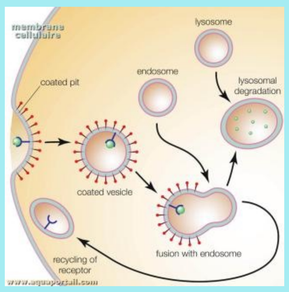
Introduction

The CD36 scavenger receptor, expressed in macrophages, is one of the main receptors involved in the internalization and metabolism of oxidized low-density lipoproteins (oxLDL). When stimulated, the CD36 receptor forms a complex with integrins, tetraspanins CD9/CD81 and the Fcγ receptor, which leads to an activation of the src kinases and tyrosine kinase Syk and to the internalization of the ligand-receptor complex. The internalized ligand-receptor complex will then be directed towards the lysosomes through the endosomal pathway or will be recycled back to the membrane.



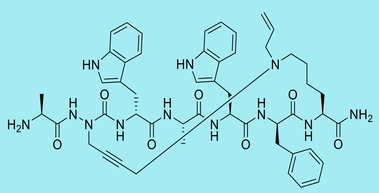
Heit et al. Dev. Cell. 2013;24:372.



MPE-298 is a selective synthetic azapeptide ligand of the CD36 receptor. Its binding site overlaps with the oxLDL binding site on the CD36 receptor.

Hypothesis: After its binding to the CD36 receptor, MPE-298 will promote the internalization of the receptor-azapeptide complex through the endosomal pathway in macrophages.

Objective: To demonstrate the internalization of the ligand-receptor complex (MPE-298-CD36) and the implication of the endosomal pathway in the intracellular disposition of the internalized receptor using a cell line of murine macrophages (J774).



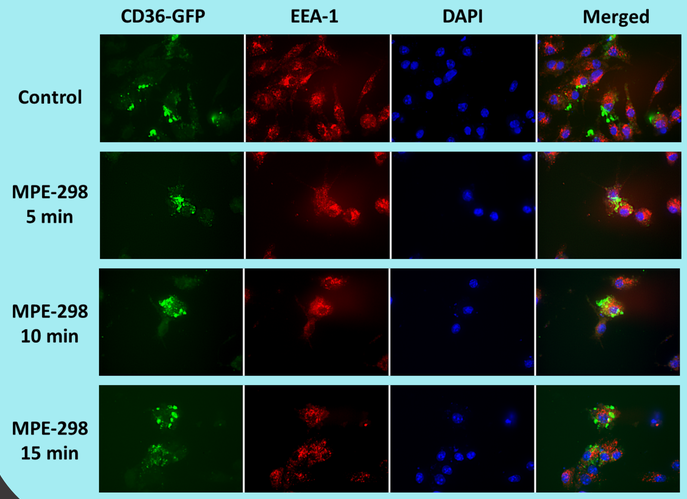
MPE-298

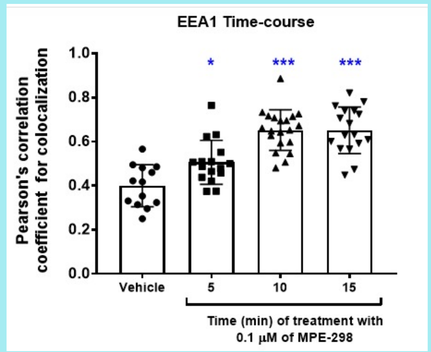
Methods

The J774 macrophages were transfected with an mCD36-GFP/pcADN plasmid x 24 h (TransitT-X2), weaned for 2 h (0.2% BSA), then incubated with 100 nM MPE-298 from 5 to 15 min. The cells (200,000/well) were fixed with 1.5% PFA. Markers for different endocytosis compartments were used: Early endosome antigen-1 (EEA-1), late endosome (Rab7) and recycling endosome (Rab11). The colocalization of the marked CD36 with the intracellular markers were analyzed with Image J plugin and the results are expressed as the mean of the correlation coefficient for colocalization.

Results

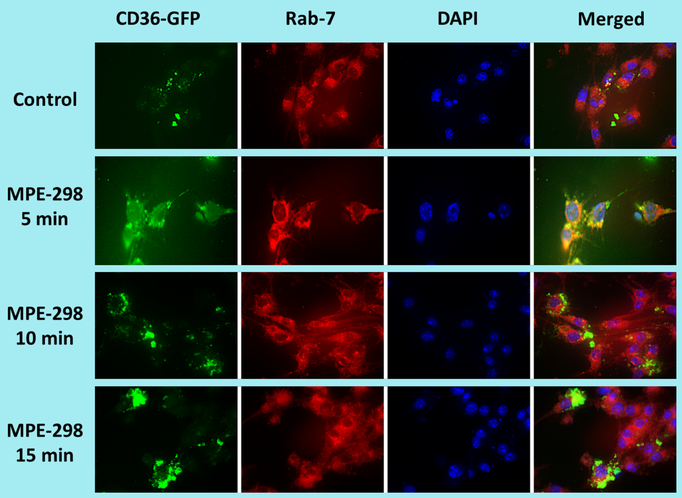
Colocalization of CD36 with early endosomes marker EEA-1 as a function of time

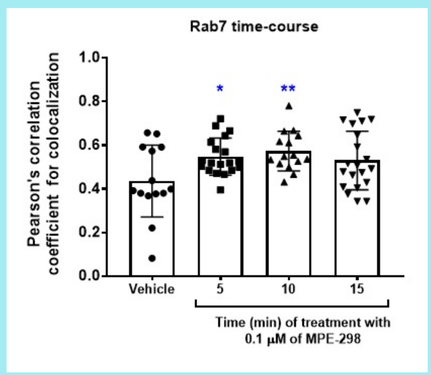




Results

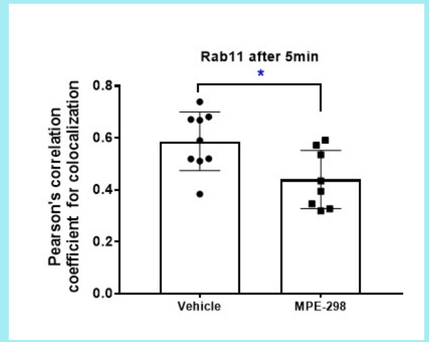
Colocalization of CD36 with late endosomes marker Rab-7 as a function of time





Results

CD36 receptor colocalization with the Rab11 marker (recycling endosomes) is reduced at t = 5 min



Conclusion

- Binding of azapeptide MPE-298 to the membrane CD36 receptor induces the rapid endocytosis of the MPE-298-CD36 complex in macrophages;
- The internalized GFP-tagged CD36 is rapidly accumulated in early and late endosomes following exposure of macrophages to azapeptide;
- The presence of GFP-tagged internalized CD36 in late endosomes suggests its potential transfer via multivesicular bodies to lysosomes;
- The results suggest the partial implication of the lysosomal pathway in the degradation of the internalized CD36 receptor following its binding to the azapeptide MPE-298

Acknowledgements

Cœur+AVC MC

Mperia