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Reducing Steatosis with Safe-by-Design Autophagy-Inducing i-Particles

Stavroula Zagkou[1,2], Valentine Marais[1], Narimane Zeghoudi[1], Capucine Phelip[2], Charlotte Primard[1], Bernard Verrier[1,2]

[1] Adjuvatis, 69007 Lyon, France

[2] Laboratory of Tissue Biology and Therapeutic Engineering, UMR5305, CNRS, 69007, Lyon, France

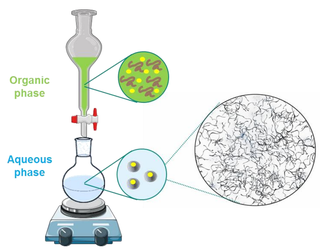
Correspondence: charlotte.primard@adjuvatis.com, stavroula.zagkou@adjuvatis.com

Background

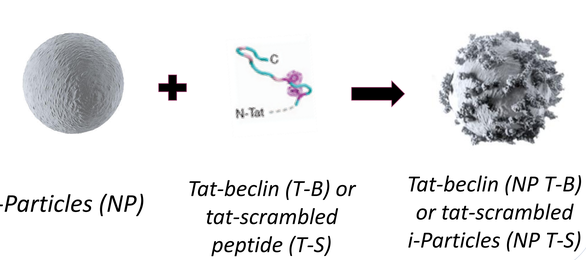
Non-alcoholic fatty liver disease (NAFLD) is the most common liver disease of the Western world and is characterized by the efflux of free fatty acids in the liver, where steatosis and lipotoxicity contribute to disease progression (Fotbolcu H., 2016). Autophagy is a cellular recycling process that has been shown to be deregulated in states of nutrient oversupply, such as high-fat diet (Koga H., 2010). Recently, a novel role of autophagy in degrading lipid droplets has been identified (Singh R., 2009). Here, we attempted to develop and characterize autophagy inducing i-Particles, made from poly-L-lactic acid, for targeted autophagy induction in the liver as a therapeutic strategy for NAFLD.

A. Formulation Development & Characterization

i-Particles (NP) prepared from poly-L-lactic acid by nanoprecipitation



Tat-beclin (NP T-B) and tat-scrambled i-Particles (NP T-S) prepared by passive adsorption

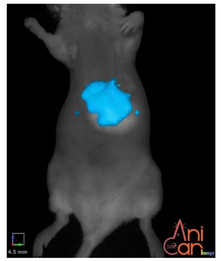


i-Particles (NP) — *Tat-beclin (T-B) or tat-scrambled peptide (T-S)* — *Tat-beclin (NP T-B) or tat-scrambled i-Particles (NP T-S)*

Formulation	Dh (nm)	PDI	Zeta potential (mV)	Ads. Efficiency (%)
NP	177,8 ± 8,0	0,07 ± 0.01	-55,3 ± 0,3	NA
NP T-B	201,8 ± 4,0	0,13 ± 0.02	32,9 ± 3,7	81,24 ± 3,2
NP T-S	195,1 ± 3,68	0,11 ± 0,03	19,8 ± 3,4	36,07 ± 5,4

B. *In vivo* biodistribution

NIR fluorescent i-Particles target the liver



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C. Autophagy induction in HeLA

Autophagy modulation is more potent and longer-lasting with NP T-B compared to the free peptide

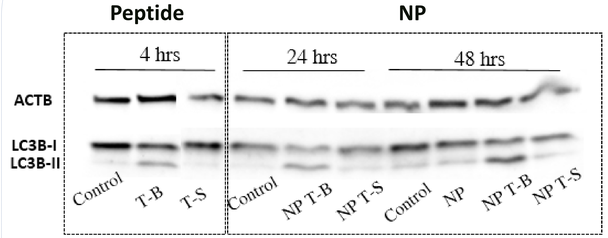


Figure 1: Comparison of autophagy modulation by tat-beclin or tat-scrambled at its soluble or NP form at their peak of autophagic activity (4hrs and 24 or 48hrs, respectively). An increase in LC3B-II band intensity indicates autophagy modulation.

NP T-B induces autophagy

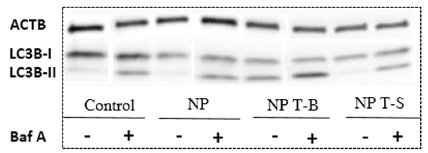
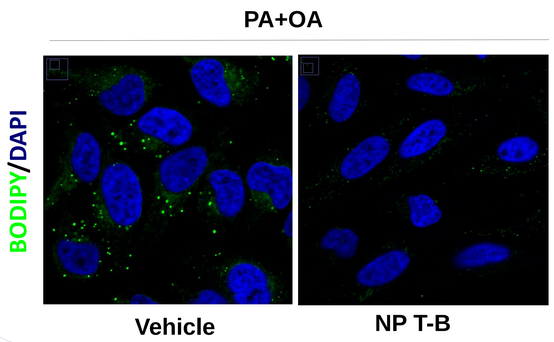
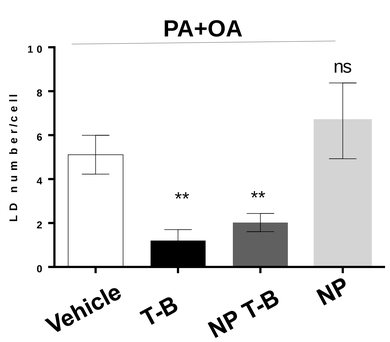


Figure 2: An increase in LC3B-II band intensity in the presence of bafilomycin A, which blocks autophagosome degradation, indicates induction of autophagy. Here, NP T-B strongly induce autophagy while NP T-S and NP slightly induce autophagy.

D. Lipid droplet breakdown in a model of steatosis





NP T-B induces LD breakdown

Figure 3: NP T-B (1µM) induces lipid droplet breakdown at a lower dose compared to the free peptide (10µM) in an *in vitro* model of steatosis (HeLA cells supplemented with palmitic and oleic acid). ** P<0.01 by one-way ANOVA with Dunnett's multiple comparison test (compared to Vehicle)

Conclusion & Perspectives

- ✓ **A stable and reproducible formulation of tat-beclin i-Particles was developed and characterized**
- ✓ **Tat-beclin i-Particles induce autophagy more efficiently and for a longer time compared to the free peptide**
- ✓ **Tat-beclin i-Particles induce lipid droplet breakdown at a lower dose compared to the free peptide in an *in vitro* model of steatosis**

Autophagy inducing i-Particles may be a promising tool for treating NAFLD.

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