MIDWESTERN UNIVERSITY

Development of Decoy CD47-Nanomedicine as Novel Therapeutic Strategy for Targeted Amelioration of Thrombospondin 1-Induced Vascular Dysfunction

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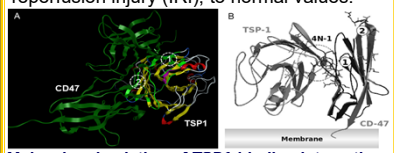
ABSTRACT

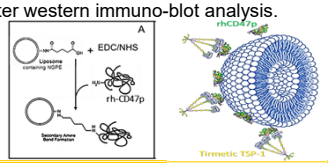
The trans-membrane receptor, integrin-associated protein CD47, is considered a central relay of thrombospondin 1 (TSP1)-mediated responses, involving impairment of endothelial vasoreactivity and vascular remodeling. Based on molecular modeling, combined with pre-clinical *in vitro* and *ex vivo* data, dosimetric TSP1 binding with soluble human CD47, serving as decoy recombinant protein (rh-CD47p), have been established. Starting at least 3:1 molar ratio of rhCD47p abolished all TSP1-vascular CD47 receptor communications and restored vascular tone. Our pharmaceutical prototype, where multiple rh-CD47p ligands cross-linked onto FDA-approved liposomes (NanoLip), can avoid mononuclear cells in blood (MNCs), while specifically binding many circulating TSP1 molecules. The resulting TSP1 scavenging CD47-NanoLip complexes are then eliminated by MNCs. Therefore, our newly developed rh-CD47p-nanomedicine can lower excessive plasma TSP1, often implicated in pulmonary arterial hypertension (PAH), and ischemia-reperfusion injury (IRI), to normal values.



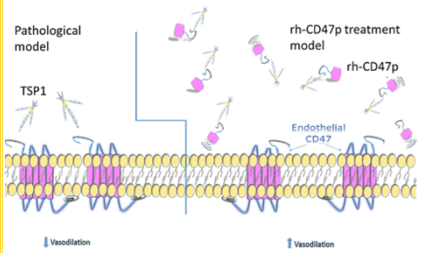
Molecular simulation of TSP1 binding interactions to CD47, *via slight opening of 4N-1 hydrophobic cleft.*

Methods

Direct bioconjugation of rh-CD47p, onto NanoLip surface was achieved using a sulfo-NHS-modified reaction between the rh-CD47p amino groups and a corbodiimide (EDC)-activated glutaryl-end of DOPE, already present in pre-formed NanoLip. Following column purification and physico-chemical characterization of prototype rh-CD47p-NanoLip, CD47 and modified TSP1-based ELISA were used to quantify specific binding of rh-CD47p-NanoLip to monomeric human TSP1 (mTSP1), at various ratios, after western immuno-blot analysis.



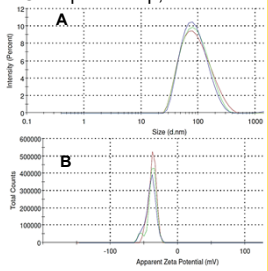
Pathological *ex-vivo* model of isolated mouse thoracic aorta (post-mTSP1 administration of rh-CD47p-NanoLip) was used to evaluate the efficiency of rh-CD47p-Nano Lip in mitigating TSP1-impaired vasodilation, at different dose levels.



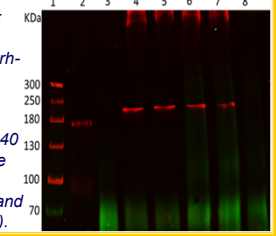
Schematics of putative mitigation mechanism of TSP1-impaired *vasodilation via recombinant human CD47 peptide (rh-CD47p), to be administered as novel targeted nanomedicine (CD47-NanoLip).*

Results

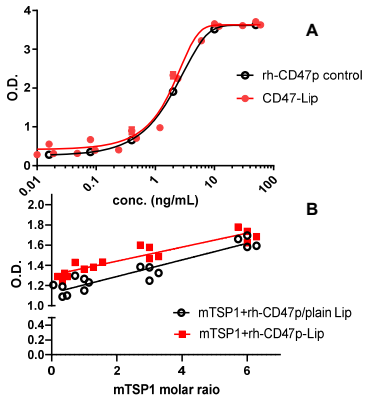
Prototype anti-TSP1 nano-formulation (av. particle size = 86nm) was pharmaceutically characterized, following successful bio-conjugation of rh-CD47p onto NanoLip, yielding 76±11µg/mL of protein (congruent with 8.5 mV reduction in NanoLip's negative surface potential, compared to plain/unconjugated NanoLip). Western blots and TSP1-ELISA revealed dosimetric binding of monomeric human mTSP1: rh-CD47p-NanoLip, at various levels.



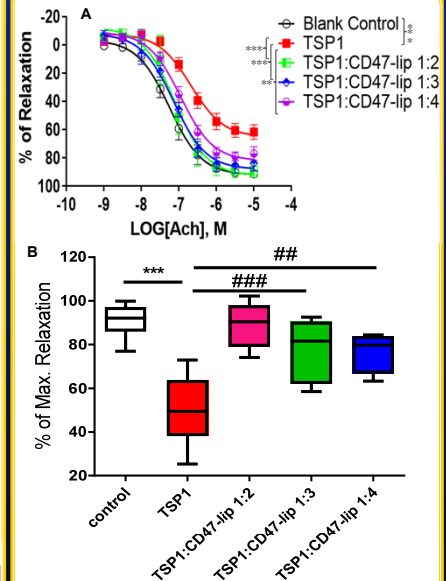
Physico-chemical characterization of rh-CD47p-NanoLip. *Average particle size (A); and surface potential (B), (n=5).*



Western blot of combination of TSP1 and rh-CD47p-NanoLip *incubated at 37 °C for 60 min. Lane 1: molecular weight marker; 2: mTSP1 control; 3: rh-CD47p control; 4-5: mTSP1 and rh-CD47p; 6-7: mTSP1 and rh-CD47p-Lip; and 8: rh-CD47p-Lip control. Mixture of mTSP1 and rh-CD47p-Lip showed bands at 240 KDa (Lane 6-7), the same molecular weight as mTSP1 and rh-CD4p (Lane 4-5).*



ELISA of titrated mTSP1 and rh-CD47p-NanoLip doses. *(A) Bound CD47 ELISA activity assay of control/native rh-CD47p vs. CD47-NanoLip (n=3); (B) Remaining mTSP1 ELISA assay of mTSP1 and rh-CD47p-NanoLips, co-incubated at 37 °C for 30 min., at 0.33:1 – 6:1 molar ratios of mTSP1:rh-CD47p, shown here vs. same ratios for control extemporaneous mixtures of rhCD47p and plain NanoLip (n=4).*



Ex-vivo treatment with rh-CD47p-NanoLip re-established vasodilation, reduced by pre-existing TSP1, in therapeutic isolated mouse thoracic aorta model*. tTSP1 alone vs. tTSP1+CD47-Lip (1:2, 1:3 and 1:4 molar ratios) treatments, p<0.0001 (n=4); blank control vs. tTSP1+CD47-Lip (1:2, 1:3 and 1:4 molar ratios) treatment, p<0.01 (n=4), shown as concentration-response curves (**A**) and Percentage of maximal relaxation in response to 10 µM acetylcholine, presented as Box and Whisker plots (**B**).*

Conclusions

This is the first report about CD47-based therapy specifically targeting TSP1-induced tissue and organ damage as well as complicated cardio/cerebro-vascular disorders. The extracellular domains of native CD47 receptors of TSP1 were successfully "nano-modified" into a candidate systemic decoy pharmaceutical, rh-CD47p-NanoLip, to scavenge and eliminate pathologically elevated TSP1 plasma levels. Encouraging characterization and stability data of rh-CD47p-NanoLip, combined with qualitative and quantitative evidence of its TSP1-specific activity, confirmed the functionality of our novel design. Phagocytosis studies further demonstrated effective CD47 self-marker efficacy, capable of ultimately binding and eliminating excess TSP1 as nano-immune complex, owing to prolonged circulation of rh-CD47p-NanoLip formulation in blood.

Taken altogether, our pre-clinical data represent successful basic early-stage development of a unique TSP1-specific pharmaceutical prototype, rh-CD47p-liposomes to abrogate TSP1-associated cardiovascular pathologies.

References

1. Yao M, and Elbayoumi T. et al., Biomedicines. 2021; 9(6):642.
2. Yao M, and Elbayoumi T. et al., Methods Protoc, 2021; 4(4):74.
3. Yao M, J Am Soc Nephrol. 2014 ;25(6):1171-86
4. Csanyi G, Yao M., et al., Arterioscler Thromb Vasc Biol. 2012;32(12):2966-73.
5. Oldenborg, P., et al., Science, 2000. 288(5473): p. 2051-4.
6. Isenberg, J., et al., Cell Mol Life Sci, 2008. 65(5): p. 728-42
7. Elbayoumi, T. et al., Mol Pharm, 2009. 6(1): p. 246-54.

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