Aonys® technology - Buccal administration for siRNA systemic delivery

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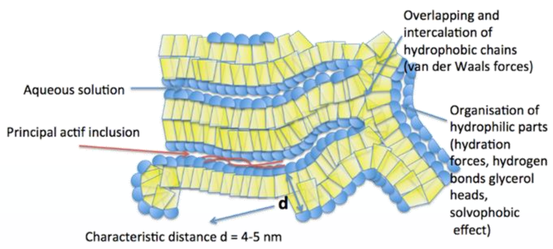
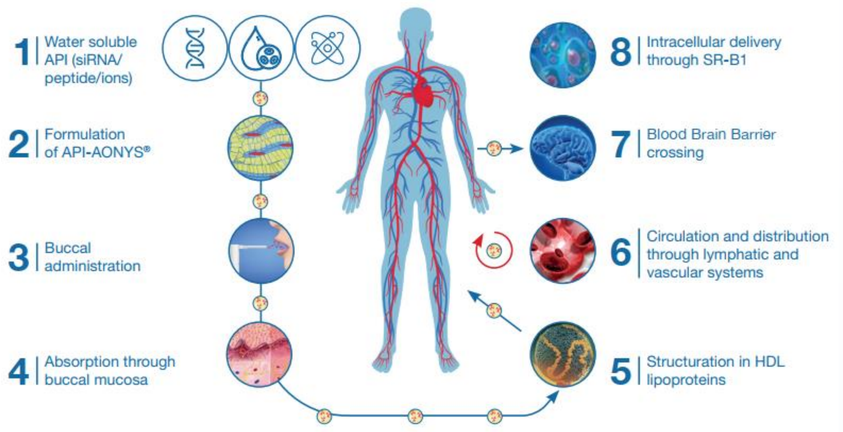
INTRODUCTION

Aonys® is a platform for water soluble therapeutic molecule delivery. It is an assembly of specific lipid components that spontaneously self-structure into a water-in-oil microemulsion. The active agent is solubilized in the aqueous phase of the microemulsion, in reverse micelles of 3 to 5 nanometers diameter.[1-3]





Aonys® in contact with mucosa self-structure in HDL-Lipoproteins, which then protect and transport the microemulsion. It is delivered inside the cytoplasm of all cells using lipoprotein receptor, SR-B1.

Biodistribution of the active ingredients in Aonys® technology has been characterized in various animal models (mice, rats, dogs, hamsters) and in healthy humans, demonstrating same profile regardless of the active ingredient: Cmax between 2 and 3 hours in animals and in humans; distribution homogenous and gradual in all tissues, including crossing of blood brain barrier, with concentrations balancing between organs (especially brain versus other organs) with repeated dose administration. [4-8]

siRNA-Aonys®: Biodistribution Study - Bioluminescence

Aim : Study the bio-distribution of a chemically unmodified siRNA when administered to the nude mouse via trans-mucosal administration in the Aonys® formulation or by intravenous (I.V) administration in saline solution.

Methods

siRNA against cyclin B1 annealed, labelled with Alexa 700 (sense strand). Imaging performed using a Hamamatsu OrcaBT bioluminescence imaging system. Images were taken at 0 and 15 minutes and at 1, 2-, 4-, 5- and 24-hours post-administration.

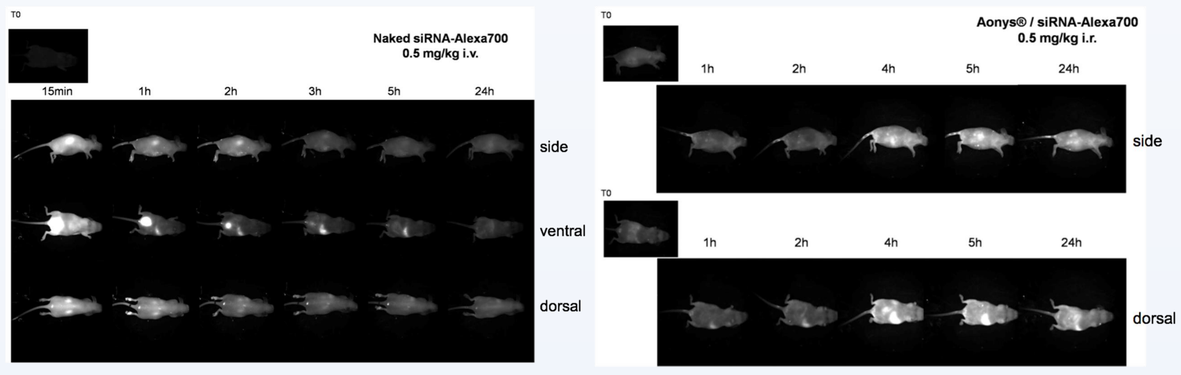
Group 1: Free siRNA: 10 µg siRNA / animal; volume = 200 µl /animal

Group 2: siRNA / Aonys® : 10 µg siRNA / animal; volume = 1 ml/kg (2x20 µl)

Results

Fluorescence of labelled siRNA in saline solution

Fluorescence of labelled siRNA in Aonys®



Simple intravenous administration of unmodified siRNA results in rapid renal clearance and limited tissue distribution. Mucosal administration of the same unmodified siRNA formulated in Aonys® shows complete absorption together with extensive and prolonged tissue distribution.

siRNA-Aonys®: Biodistribution Study - Radioactivity

Aim : Biodistribution in mice of a radiolabeled ^{32}P unmodified siRNA - Aonys®.

Methods

Wild-type mice received a single administration of Aonys® / [^{32}P] radiolabeled siRNA coding for GAPDH, at the dose of 800 µg/kg (equivalent to 500 µCi/kg). Rectal versus buccal routes were compared: (4 groups, n=16). 2 sampling time 1h and 24h.

Results

Figure 6: Biodistribution of ^{32}P-siRNA 24h post administration

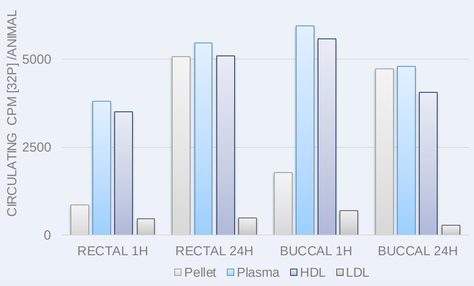
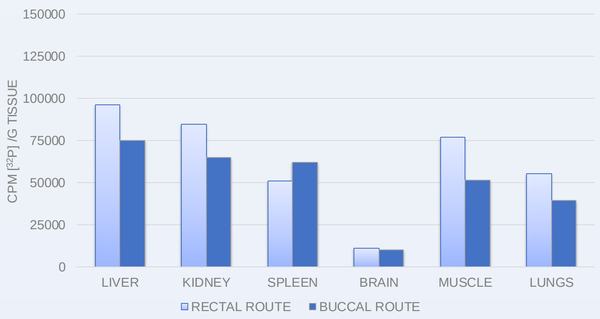


Figure 6: Biodistribution of ^{32}P-siRNA 24h post administration



Fractionation of the plasma fraction both at 1 hour and 24 hours showed that 98% of plasma radioactivity was associated with plasma lipoproteins. Majority of the plasma radioactivity was associated with pre-HDL/"VHDL" lipoproteins that have a high protein to lipid ratio.

Tissue distribution was extensive with approximately equal proportions of the radioactive dose contained in liver, kidney, spleen, muscle and lungs.

Oncology: Efficient targeted gene extinction in tumor (CCND1)

Aim: Evaluate the impact of cyclin D1 (CCND1) downregulation on tumor growth in mice following a permucosal treatment with specific siRNAs formulated in Aonys®.

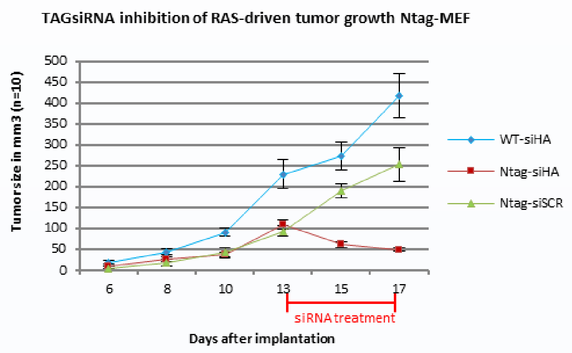
Method: Models used are nude mice transplanted

- with RAS/DNP53-transformed mouse mammary fibroblasts isolated from N- or C-terminally HA-tagged-CCND1 knock in C57BL/6J mice (expressing CCND1 at physiological levels)
- Or with mammary gland tumor cells isolated from animals obtained by crossbreeding of N- or C-terminally HA-tagged-CCND1 knock in C57BL/6J mice and MMTV/ErbB2 mice expressing the oncogene under control of the MMTV promoter

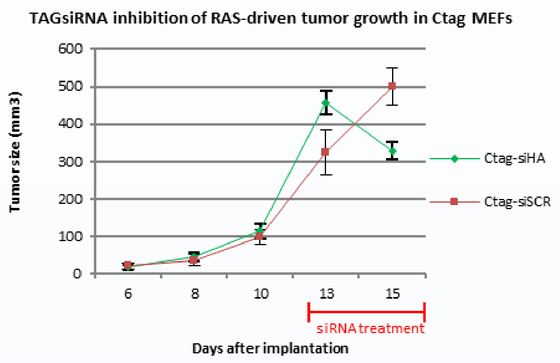
On separate flank of the same nude mouse, control CCND1 wild type was implanted as negative control. After significant growth on both flanks, we targeted Tagged-CCND1 using specific siRNA against the HA mRNA sequence formulated in Aonys.

Results:

TAGsiRNA inhibition of RAS-driven tumor growth Ntag-MEF

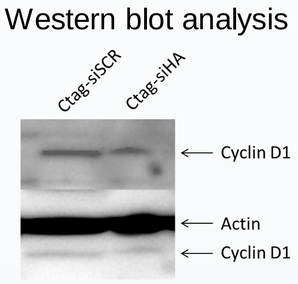


TAGsiRNA inhibition of RAS-driven tumor growth in Ctag MEFs



- Tumor regression was observed within hours only on the flanks expressing HA-CCND1, but not with scramble siRNA..
- Western blot analysis confirmed that tagged CCND1 expression decreased in response to HA-siRNA
- Tumor size re-increased after treatment stopped; Restarting treatment reduced tumor growth.

Western blot analysis



siRNA-Aonys®: Efficient targeted gene extinction in brain

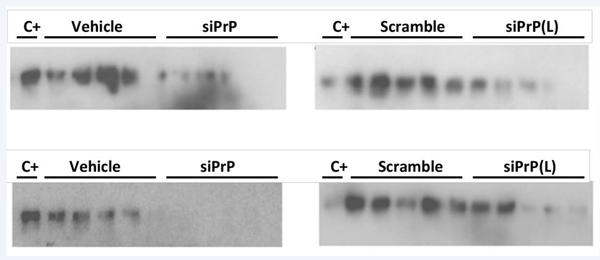
Aim : Evaluate the inhibition of the PrP(C) gene (Prion Protein) in several tissues after a 12-Day rectal mucosa administration of Aonys®-siRNA in wild-type mice (non infected mice model).[9]

Methods

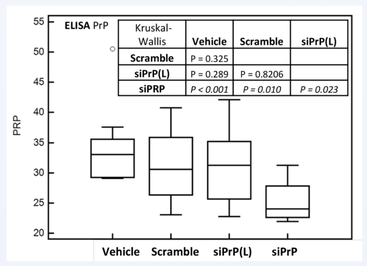
C57Bl/6J mice received repeated daily rectal administration of Aonys® PrP(C) siRNA (300 or 600 µg siRNA/mL) or scramble siRNA or not treated for 12 days (1 ml/kg). Brain, muscle (Tibialis) and spleen were collected on Day 13, after sacrifice. PrP(C) protein levels were evaluated in tissues by ELISA and Western-Blot techniques.

Results

PrP levels evaluated by western-blot

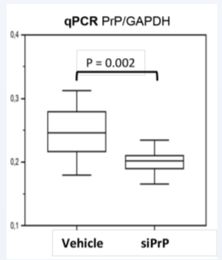


PrP levels (ELISA)



Kruskal-Wallis	Vehicle	Scramble	siPrP(L)
Scramble	P = 0.325		
siPrP(L)	P = 0.289	P = 0.8206	
siPRP	*P < 0.001*	*P = 0.010*	*P = 0.023*

PrP mRNA levels (ELISA)



siPrP (L): 300 µg; siPrP: 600 µg versus vehicle or scramble. 12 days treatment.

PrP levels were lower in the groups treated with Aonys/PrP-siRNA. These results which were most important with the highest dose level of PrP-siRNA for which a significant 28% decrease (p = 0.01 vs scramble) was observed. These results were correlated with a 17.6% decreased level of PrP mRNA determined by RT-QPCR.

CONCLUSIONS AND OUTLOOK

These studies evidenced Aonys® value as drug delivery technology for the mucosal administration of siRNA. Aonys® through HDL transport and intracellular delivery acts as a Trojan horse for siRNA. The technology overcomes most of the hurdles encountered in siRNA delivery :

- ✓ Buccal administration
- ✓ No first pass metabolism
- ✓ No toxicity due to circulating active ingredient
- ✓ No immune system activation
- ✓ CNS and Intratumoral Active Ingredient Delivery
- ✓ Optimized Intracellular delivery by lipoprotein receptor

REFERENCES

1. Mouri A, et al. *Int J Pharm. 2014 Jul 15;475(1-2):324-334.* ; 2. Mouri A, et al. Int J Pharm. 2016 Apr 11;502(1-2):117-24.; 3. Wilson EN, et al. *J. of Alzheimer's Disease, 73 (2020) 723-739*.; 4 Wilson EN, et al. *Current Alzheimer Research, 2018, 15, 1220-1230*.; 5. Wilson EN, et al. *Translational Psychiatry (2017) 7, e1190*.; 5. Mahmoud A. et al. *Neurobiology of Disease 48 (2012) 282–289*. ; 6. Marelli C and Maschat F. *Orphanet Journal of Rare Diseases (2016) 11:24*. 7. Y. Arribat, et al. *Acta Neuropathologica Communications 2014, 2:86*. ; 9. Lehmann S, et al. *PLoS One. 2014 Feb 14;9(2):e88797*.