UNIVERSITÀ DEGLI STUDI DI PADOVA

PROJECT

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1) Project title

Inclisiran, statins and monoclonal antibodies anti PCSK9: exploring the elements of differentiation in their pharmacological action

2)Abstract (max 500 words)

A recently discovered means of decreasing PCSK9 levels is represented by the development of small interfering RNA (siRNA) molecules (Fitzgerald, Frank-Kamenetsky et al. 2014). Inclisiran is a long-acting, subcutaneously delivered, synthetic siRNA directed against PCSK9 and conjugated to triantennary N-acetylgalactosamine carbohydrates (siRNA-GalNAc) (Fitzgerald, Frank-Kamenetsky et al. 2014). These carbohydrates bind to abundant liver-expressed asialoglycoprotein receptors, leading to inclisiran uptake specifically into hepatocytes. The siRNA was modified with a combination of phosphorothioate, 2'-O-methyl nucleotide, and 2'-fluoro nucleotide modifications to improve molecular stability (Nair, Willoughby et al. 2014). Single *i.v.* dosing of radiolabeled siRNA-GalNAc conjugate showed robust and fast (5÷20 minutes) liver-specific uptake of the conjugate in mice (Nair, Willoughby et al. 2014). In phase 1 study, a single injection of inclisiran at a dose of 300 mg or more was associated with 75% reductions from baseline in the PCSK9 levels, effect that started after 3-5 days post injection and reached a maximal inhibition in 10-15 days.

Although the earlier meta-analysis of Kinlay et al (Kinlay 2007) supported a strong correlation between LDL-C reduction and lowering of hs-CRP, the case is certainly different for the newly developed PCSK9 antagonists (evolocumab and alirocumab) where the dramatic reduction of LDL-C, in the range of 50-60%, is only marginally associated with changes in hs-CRP (Sahebkar, Di Giosia et al. 2016). Surprisingly, patients receiving a single dose of inclisiran 300 or 500 mg had non-significant reductions of 16.2% and 19.8%, respectively of hs-CRP, with a wide distribution (Ray, Landmesser et al. 2017). Conversely, patients at a two-dose regimen of inclisiran (300 mg) showed a 16.7% significant decrement in hs-CRP (Ray, Landmesser et al. 2017). Thus, the effect of inclisiran on hs-CRP represents a very interesting element of differentiation with monoclonal antibodies anti PCSK9.

Evidence suggests that pro-inflammatory responses to PCSK9 in macrophages and arterial atherosclerotic lesions may primarily depend on LDLR. PCSK9 enhances the release of pro-inflammatory cytokines IL-6 and TNF-α, the latter being directly correlates with PCSK9 plasma levels in humans (Ricci, Ruscica et al. 2018). Recombinant PCSK9 induced TNFα mRNA in bone marrow-derived macrophages mainly in an LDLR-dependent fashion (Ricci, Ruscica et al. 2018). Furthermore, overexpression of human PCSK9 in macrophages promoted atherosclerotic lesions (Giunzioni, Tavori et al. 2016). Deletion of the PCSK9 gene in the liver reduced atherosclerotic lesions, primarily via mechanisms dependent on LDLR (Denis, Marcinkiewicz et al. 2012). More recent study demonstrated that circulating PCSK9 induces

macrophage activation and vein graft lesion development in LDLR-independent mechanisms (Katsuki, Kumar Jha et al. 2022). The relative contributions of the LDLR-dependent and independent mechanisms, however, remain unknown.

In the present research project, we propose to define the kinetic of PCSK9 inhibition by inclisiran and to determine the effect of this inhibitor on ApoB secretion and expression of hs-CRP in human hepatocarcinoma cell line Huh7. The anti-inflammatory effect of inclisiran will be further investigated in a co-culture system where PCSK9 derived from HepG2 cells will act as pro-inflammatory factor on THP-1 derived macrophages.

In addition, we will determine the efficacy of inclisiran to modulate the LDL receptor expression and intracellular lipids in human hepatocarcinoma cell line Huh7 with high and low levels of LDL receptor.

Finally, we will characterize the alterations in the lipidome of lipoprotein particles released by Huh7 cell line in response to PCSK9 inhibition by inclisiran.

All these analyses will be compared to the effect of simvastatin and evolocumab/alirocumab in order to identifying the element of differentiation of inclisiran compared to the other hypocholesterolemic drugs.

References

Denis, M., et al. (2012). "Gene inactivation of proprotein convertase subtilisin/kexin type 9 reduces atherosclerosis in mice." Circulation **125**(7): 894-901.

Fitzgerald, K., et al. (2014). "Effect of an RNA interference drug on the synthesis of proprotein convertase subtilisin/kexin type 9 (PCSK9) and the concentration of serum LDL cholesterol in healthy volunteers: a randomised, single-blind, placebo-controlled, phase 1 trial." Lancet **383**(9911): 60-68.

Giunzioni, I., et al. (2016). "Local effects of human PCSK9 on the atherosclerotic lesion." J Pathol **238**(1): 52-62.

Katsuki, S., et al. (2022). "Proprotein Convertase Subtilisin/Kexin 9 Promotes Macrophage Activation via LDL Receptor-Independent Mechanisms." Circ Res: e320056.

Kinlay, S. (2007). "Low-density lipoprotein-dependent and -independent effects of cholesterol-lowering therapies on C-reactive protein: a meta-analysis." J Am Coll Cardiol **49**(20): 2003-2009.

Nair, J. K., et al. (2014). "Multivalent N-acetylgalactosamine-conjugated siRNA localizes in hepatocytes and elicits robust RNAi-mediated gene silencing." J Am Chem Soc **136**(49): 16958-16961.

Ray, K. K., et al. (2017). "Inclisiran in Patients at High Cardiovascular Risk with Elevated LDL Cholesterol." N Engl J Med **376**(15): 1430-1440.

Ricci, C., et al. (2018). "PCSK9 induces a pro-inflammatory response in macrophages." Sci Rep **8**(1): 2267.

Sahebkar, A., et al. (2016). "Effect of monoclonal antibodies to PCSK9 on high-sensitivity C-reactive protein levels: a meta-analysis of 16 randomized controlled treatment arms." Br J Clin Pharmacol **81**(6): 1175-1190.