Universiteit
Leiden
The Netherlands

Cholesterol metabolism and hematopoiesis interaction in atherothrombosis

Ouweneel, A.B.

Citation
Ouweneel, A. B. (2019, March 21). *Cholesterol metabolism and hematopoiesis interaction in atherothrombosis*. Retrieved from https://hdl.handle.net/1887/70039

Version:	Not Applicable (or Unknown)
License:	Licence agreement concerning inclusion of doctoral thesis in the Institutional Repository of the University of Leiden
Downloaded from:	https://hdl.handle.net/1887/70039

Note: To cite this publication please use the final published version (if applicable).

Cover Page

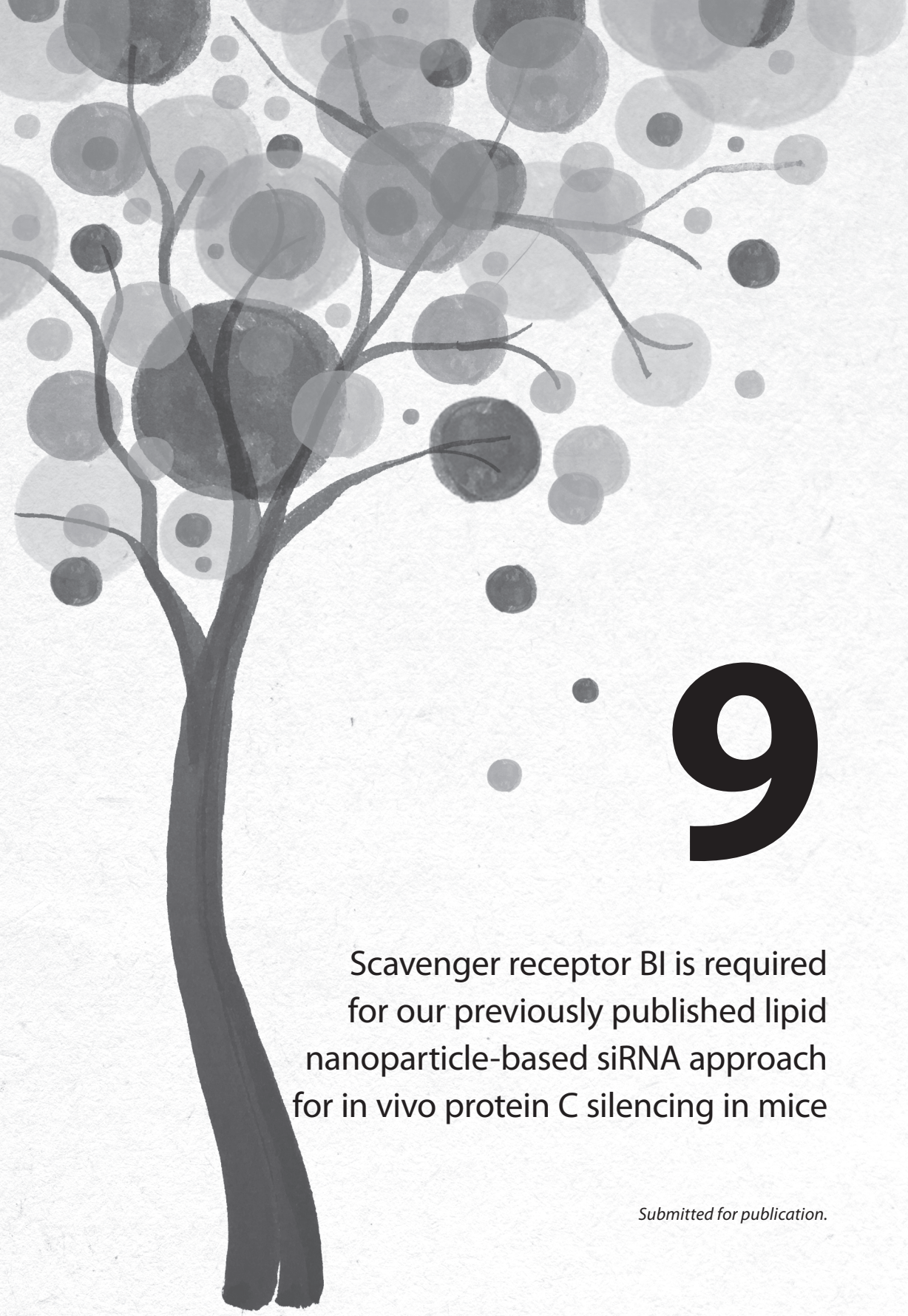
Universiteit Leiden

The handle http://hdl.handle.net/1887/70039 holds various files of this Leiden University dissertation.

Author: Ouweneel, A.B.
Title: Cholesterol metabolism and hematopoiesis interaction in atherothrombosis
Issue Date: 2019-03-21

Amber B. Ouweneel[1], Robin A.F. Verwilligen[1], Menno Hoekstra[1],
Marco Heestermans[2,3], Bart J.M. van Vlijmen[2,3]* & Miranda van Eck[1]*

[1] Division of BioTherapeutics, Leiden Academic Centre for Drug Research, Leiden University, Leiden, The Netherlands, [2] Einthoven Laboratory for Vascular and Regenerative Medicine, Leiden University Medical Center, Leiden, The Netherlands, [3] Department of Internal Medicine, Division of Thrombosis and Hemostasis, Leiden University Medical Center, Leiden, The Netherlands, * These authors contributed equally to the manuscript



9

Scavenger receptor BI is required for our previously published lipid nanoparticle-based siRNA approach for in vivo protein C silencing in mice

Submitted for publication.

To the editor,

Atherothrombosis, characterized by the development of a superimposed thrombus upon rupture or erosion of an atherosclerotic plaque, is the underlying cause of common acute cardiovascular events, such as myocardial infarction or ischemic stroke [1]. Although atherosclerosis development is widely studied in (genetically modified) mouse models, these atherosclerotic plaques do not spontaneously progress towards the atherothrombosis stage. For this purpose, we have recently developed the first spontaneous atherothrombosis mouse model. As previously reported in *Arteriosclerosis, Thrombosis and Vascular Biology* [2], siRNA-mediated lowering of the natural anticoagulant protein C (si*Proc*) induced the occurrence of atherothrombosis in the aortic root of hypercholesterolemic apolipoprotein E (APOE) knockout mice at one week after siRNA administration. However, spontaneous thrombus formation only occurred at a low incidence (12-25%) [2,3], which highlights that further optimization of the model is necessary before we can identify conditions that affect the atherothrombosis incidence.

Platelets play an important role in the pathogenesis of atherothrombosis [4]. In this context, mice lacking a functional high-density lipoprotein receptor, i.e. scavenger receptor BI (SR-BI) knockout mice, exhibit an interesting platelet phenotype. More specifically, SR-BI deficiency-associated hypercholesterolemia in plasma results in cholesterol accumulation in platelets, causing them to circulate in an activated state [5–7]. Importantly, this activated platelet phenotype is associated with a higher susceptibility for $FeCl_3$ damage-induced arterial thrombosis in the carotid artery [5]. Hence, it is of clear interest to study whether modifying SR-BI functionality can also increase the incidence of atherothrombosis in si*Proc*-treated mice.

In order to determine if SR-BI deficiency increases the susceptibility for si*Proc*-induced atherothrombosis, we challenged age- and sex-matched wild-type and SR-BI knockout mice with an atherogenic diet containing 15% fat, 1% cholesterol, and 0.5% cholic acid for 8 weeks before injection with *siProc* or a non-targeting control siRNA (siNEG). After 8 weeks dietary challenge, wild-type mice developed significant hypercholesterolemia (plasma total cholesterol level: 205±8 mg/dL; n=26) and early lesions in the aortic root ($14\pm2x10^3$ μm^2; n=24). In line with the notion that early lesions are not susceptible for atherothrombois, no atherothrombosis was observed in the aortic root of si*Proc*-treated wild-type mice despite a 50% reduction in hepatic mRNA levels of *Proc* (siNEG: 0.064±0.009 (n=7), si*Proc*: 0.032±0.0005 (n=20); $p<0.0001$). We previously consistently observed an atherothombosis incidence of 12-25% in APOE knockout mice [2,3]. However, the previously used APOE knockout mice had advanced aortic root atherosclerotic plaques (plaque size ~$400\text{-}600x10^3$ μm^2). Moreover, the extent of hypercholesterolemia in APOE knockout mice (~1000 mg/dL) was much larger than in the wild-type mice of the current study, possibly resulting in increased inflammatory and plaque destabilizing conditions. Dedicated studies on the effect of plaque size and hypercholesterolemia on atherothrombosis susceptibility are warranted.

Atherogenic diet-fed SR-BI knockout mice developed exacerbated hypercholesterolemia (plasma total cholesterol levels: 1549±85 mg/dL; n=29). As a result, SR-BI knockout mice did develop advanced plaques in the aortic root with an average size of 425±21x10^3 μm^2 (n=26). Surprisingly, despite the advanced stage of the lesions, no aortic root atherothrombosis was observed in these animals. Analysis of *Proc* mRNA expression in livers of si*Proc* treated SR-BI knockout mice revealed that this was probably due to an impaired hepatic targeting of the siRNA complex. Instead of a decrease, the relative expression of *Proc* actually tended to increase (0.064±0.005 for siNEG (n=6), 0.083±0.005 for si*Proc* (n=19); p=0.058). Therefore, unfortunately no conclusions regarding the possible prothrombotic effects of SR-BI deficiency under conditions of *Proc* silencing can be drawn from this study.

In order to inhibit *Proc* production, we complexed our siRNA with commercially available Invivofectamine™ 3.0 Reagent (Invitrogen). This reagent is specifically designed to target hepatocytes and has previously been shown to be highly effective and non-toxic when used for liver-targeted RNA interference [8]. Although the exact composition of this compound is not publicly known for competitive reasons, the supplier has indicate that it is an animal-origin free lipid nanoparticle [9]. Previous studies have described a role for APOE and the LDL receptor in the uptake of neutral, cationic and ionizable nanoparticles (liposomes) in mice [10,11]. However, in our earlier studies Invivofectamine™ 2.0 Reagent and Invivofectamine™ 3.0 Reagent-complexed si*Proc* silencing was successful [2,3]. Moreover, transfection was also efficient in APOE*3Leiden.CETP mice (unpublished data). Our current study suggests that SR-BI is rather involved in the hepatic uptake of Invivofectamine™ 3.0 Reagent-complexed siRNA. SR-BI is widely recognized as a multi-ligand scavenger protein, and it clears, among other things, native and modified lipoproteins [12], and anionic phospholipids [13]. Therefore, it is not unlikely that SR-BI is also directly mediating the uptake of Invivofectamine™ complexed siRNA.

In conclusion, our studies show that SR-BI is required for an Invivofectamine™ 3.0-based siRNA approach to silence Protein C in hepatocytes in vivo. Our studies highlight that caution is warranted in the design and interpretation of experiments involving liver-targeted siRNA in mouse models of disturbed scavenger receptor functioning.

ACKNOWLEDGEMENTS

This work was funded by Leiden University, and in part supported by a VICI grant 91813603 from the Netherlands Organization for Scientific Research (M.V.E). M.V.E is an Established Investigator of the Dutch Heart Foundation (grant number 2007T056).

The authors thank Ronald J. van der Sluis, Lidewij R. de Leeuw, Mara J. Kröner, Joya E. Nahon, Ezra J. van der Wel, Robbert Wever for excellent technical assistance.

REFERENCES

1. Lippi G, Franchini M, Targher G. Arterial thrombus formation in cardiovascular disease. *Nat Rev Cardiol*. 2011;8(9):502-512. doi:10.1038/nrcardio.2011.91.
2. Ouweneel AB, Heestermans M, Verwilligen RAF, Gijbels MJJ, Reitsma PH, Van Eck M, van Vlijmen BJM. Silencing of Anticoagulant Protein C Evokes Low-Incident but Spontaneous Atherothrombosis in Apolipoprotein E-Deficient Mice-Brief Report. *Arterioscler Thromb Vasc Biol*. 2017;37(5):782-785. doi:10.1161/ATVBAHA.117.309188.
3. Heestermans M, Ouweneel AB, Hassan J, Kloosterman M, Reitsma PH, Gijbels MJJ, van Vlijmen BJM, van Eck M. Predilection of Low Protein C-induced Spontaneous Atherothrombosis for the Right Coronary Sinus in Apolipoprotein E deficient mice. *Sci Rep*. 2018;8(1):15106. doi:10.1038/s41598-018-32584-y.
4. Davì G, Patrono C. Mechanisms of disease: Platelet activation and atherothrombosis. *N Engl J Med*. 2007;357(24):2482-2494. doi:10.1056/NEJMra071014.
5. Korporaal SJA, Meurs I, Hauer AD, Hildebrand RB, Hoekstra M, Cate HT, Pratico D, Akkerman J-WN, Van Berkel TJC, Kuiper J, Van Eck M. Deletion of the High-Density Lipoprotein Receptor Scavenger Receptor BI in Mice Modulates Thrombosis Susceptibility and Indirectly Affects Platelet Function by Elevation of Plasma Free Cholesterol. *Arterioscler Thromb Vasc Biol*. 2011;31(1):34-42. doi:10.1161/ATVBAHA.110.210252.
6. Dole VS, Matuskova J, Vasile E, Yesilaltay A, Bergmeier W, Bernimoulin M, Wagner DD, Krieger M. Thrombocytopenia and Platelet Abnormalities in High-Density Lipoprotein Receptor – Deficient Mice. 2008. doi:10.1161/ATVBAHA.108.162347.
7. Ma Y, Ashraf MZ, Podrez EA. Scavenger receptor BI modulates platelet reactivity and thrombosis in dyslipidemia. 2017;116(11):1932-1942. doi:10.1182/blood-2010-02-268508.An.
8. Invivofectamine® 3.0 Reagent. https://www.thermofisher.com/nl/en/home/life-science/rnai/introduction-to-in-vivo-rnai/invivofectamine-reagent.html. Accessed October 3, 2018.
9. Invivofectamine™ 3.0 Reagent. https://www.thermofisher.com/order/catalog/product/IVF3001. Accessed October 23, 2018.
10. Yan X, Kuipers F, Havekes LM, Havinga R, Dontje B, Poelstra K, Scherphof GL, Kamps JAAM. The role of apolipoprotein E in the elimination of liposomes from blood by hepatocytes in the mouse. *Biochem Biophys Res Commun*. 2005;328(1):57-62. doi:10.1016/j.bbrc.2004.12.137.
11. Akinc A, Querbes W, De S, Qin J, Frank-Kamenetsky M, Jayaprakash KN, Jayaraman M, Rajeev KG, Cantley WL, Dorkin JR, Butler JS, Qin L, Racie T, Sprague A, Fava E, Zeigerer A, Hope MJ, Zerial M, Sah DWY, Fitzgerald K, Tracy MA, Manoharan M, Koteliansky V, Fougerolles A de, Maier MA. Targeted delivery of RNAi therapeutics with endogenous and exogenous ligand-based mechanisms. *Mol Ther*. 2010;18(7):1357-1364. doi:10.1038/mt.2010.85.
12. Hoekstra M, Berkel TJC Van, Eck M Van. Scavenger receptor BI : A multi-purpose player in cholesterol and steroid metabolism. *World J Gastroenterol*. 2010;16(47):5916-5924. doi:10.3748/wjg.v16.i47.5916.
13. Rigotti A, Acton SL, Krieger M. The class B scavenger receptors SR-BI and CD36 are receptors for anionic phospholipids. *J Biol Chem*. 1995;270(27):16221-16224.