

The unexpected roles of extracellular ADP and P2Y₁₃ receptor in reverse cholesterol transport

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Received: 7 December 2010 / Accepted: 7 December 2010 / Published online: 14 December 2010
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Fabre AC, Malaval C, Ben Addi A, Verdier C, Pons V, Serhan N, Lichtenstein L, Combes G, Nijstad N, Tietge U, Briand F, Collet X, Robaye B, Perret B, Boeynaems JM and Martinez LO (2010) P2Y₁₃ receptor is critical for reverse cholesterol transport. *Hepatology* 52: 1477–1483

Summary of the article

In this article, the authors show that P2Y₁₃-deficient mice exhibit a decrease in hepatic HDL cholesterol uptake, hepatic cholesterol content and biliary cholesterol output, while plasma HDL and other lipids are unchanged. Furthermore, cangrelor, a partial agonist of P2Y₁₃, stimulates hepatic HDL uptake and biliary secretion of cholesterol and bile acids. This effect is abolished in P2Y₁₃^{−/−} mice, but maintained in mice with hepatic deficiency of the scavenger receptor SR-BI. This article leads thus to three important conclusions:

- The P2Y₁₃ receptor regulates in the liver a pathway of HDL uptake independent from the classical pathway mediated by SR-BI.

- P2Y₁₃ deficiency decreases reverse cholesterol transport without a change in plasma HDL cholesterol.
- Pharmacological activation of P2Y₁₃ increases reverse cholesterol transport, supporting its potential role as a new target for treatment of dyslipidemia and atherosclerosis.

Background: HDL and reverse cholesterol transport

The role of HDL in the protection against atherosclerosis is now well established [1]. It involves two distinct mechanisms:

- One is reverse cholesterol transport, a process in which excess cholesterol from peripheral tissues and particularly macrophages/foam cells is taken up by HDL particles that deliver it to the liver for excretion into the bile as free cholesterol or after transformation into bile acids.
- In addition, HDL have a direct atheroprotective effect on the vascular wall through anti-inflammatory, antioxidant and antithrombotic actions involving inter alia the production of NO.

Hepatic uptake of HDL particles is a key step in reverse cholesterol transport [1]. Two distinct mechanisms of HDL cholesterol uptake coexist in hepatocytes:

- Selective cholesterol uptake at the level of the plasma membrane, a mechanism by which cholesterol (free or esterified) is taken up while the protein components of the HDL particle are not;
- HDL endocytosis resulting in the uptake and degradation of HDL holoparticle.

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The role of the scavenger receptor SR-BI (also called CLA-1 in human) in selective cholesterol uptake is now well established [1]. Deletion of the SR-BI gene in mice slows hepatic HDL cholesterol uptake and increases plasma HDL cholesterol level. This is associated with an accelerated onset of atherosclerosis in mice deficient in both SR-BI and apolipoprotein E. Interestingly, these data support the concept that the flux of HDL cholesterol is more important than steady-state concentrations for protection against atherosclerosis [1].

In contrast with the well-established role of SR-BI in selective cholesterol uptake by hepatocytes, the molecular mechanisms involved in HDL holoparticle endocytosis have not been characterized, although the potential role of SR-BII, a SR-BI splice variant, has been proposed [2].

Reverse cholesterol transport: the connexion with purinergic signalling

The current paper by the team of Laurent Martinez from INSERM U563 in Toulouse represents the end of a long quest starting in 2003. At that time, they published a seminal paper in *Nature* that linked for the first time purinergic signalling to reverse cholesterol transport [3]. They demonstrated indeed that the β -chain of mitochondrial ATP synthase was expressed on the plasma membrane of hepatocytes. Apolipoprotein A-I (apoA-I), the major apolipoprotein in HDL particles, bound with high affinity to this ecto-ATPase. This resulted in an increased hydrolysis of extracellular ATP into ADP. Finally, ADP stimulated the internalization of HDL by hepatocytes. Later on the same group showed that siRNA targeting the P2Y₁₃ receptor abolished the stimulatory effect of apoA-I and ADP on the uptake of HDL by the HepG2 human hepatocyte cell line [4]. In the same paper, they reported that the P2Y₁₂ antagonist cangrelor behaved as a partial agonist of P2Y₁₃ and increased the uptake of HDL in HepG2 cells. In addition they showed that HDL particle endocytosis in HepG2 cells might result from RhoA stimulation [5]. Indeed, ADP stimulated RhoA and siRNA targeting the Rho kinase ROCK1 prevented ADP-stimulated HDL endocytosis. The new paper by the group of Martinez validates these concepts in intact animals. Furthermore, it reports two important new findings. First, thanks to the use of SR-BI-deficient mice, it shows conclusively that the P2Y₁₃-regulated pathway is totally independent from the classical pathway of HDL uptake involving SR-BI, or from SR-BII which is encoded by the same gene. On the other hand, it demonstrates that changes in P2Y₁₃ activity have an impact on the flux of HDL cholesterol, but not on its steady-state concentration in plasma. The reason for that discrepancy is not entirely clear,

but could be related to a compensatory decrease of HDL formation in the liver of P2Y₁₃-deficient mice. Indeed, the mRNA level of ABCA1 and ABCG1, two factors involved in cholesterol efflux and lipidation of nascent apoA-I, was decreased in the liver of P2Y₁₃^{−/−} mice.

P2Y₁₃: a therapeutic target to activate reverse cholesterol transport?

Various strategies are being pursued to increase reverse cholesterol transport [1]. Inhibitors of cholesteryl ester transfer protein (CETP), that increase the plasma level of HDL cholesterol, might also accelerate reverse cholesterol transport, but this remains unproven [6]. One such drug candidate, torcetrapib, was a failure, possibly due to an off-target related increase in blood pressure. Other projects (dalcetrapib, anacetrapib) are still going on. Another strategy involves the infusion of recombinant apoA-I Milano, a variant which is associated with low rates of vascular diseases despite reduced HDL level. ApoA-I mimetic peptides constitute an alternative approach which is actively pursued. The paper of Fabre et al. validates the P2Y₁₃ receptor as a target to accelerate reverse cholesterol transport through an increased HDL uptake by the liver, and possibly upregulation of ABCA1 and ABCG1. However the extrapolation to man of these findings in mice must be very cautious in view of major species differences in the distribution of cholesterol between lipoproteins. Indeed mice lack CETP and most of their plasma cholesterol is in HDL. Cangrelor, a P2Y₁₂ antagonist in clinical development as antithrombotic agent [7], is also a partial agonist of the P2Y₁₃ receptor, and it increased the uptake of HDL and biliary excretion of cholesterol and bile acids. However, the lack of oral bioavailability and very short half-life preclude its clinical use for this indication. The development of an uncharged (partial) agonist of P2Y₁₃ suitable for oral administration will be a tremendous challenge [7].

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