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Unpacking and understanding the impact of PCSK9 inhibitors on apolipoprotein B metabolism.

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Editorial

In the relatively short time from first identification of proprotein convertase subtilisin/kexin type 9 (PCSK9) as a potential therapeutic target to the imminent arrival of reports from large-scale outcome trials, there has been limited opportunity to explore the full impact of this regulatory protein on lipid and lipoprotein metabolism and to elucidate the detailed mechanism of action of PCSK9 inhibitors. On this occasion genomics did deliver, with the characterization of gain-of-function and loss-of-function variants leading to clinically understandable phenotypes associated with altered risk of cardiovascular disease [1,2]. When morbidity and mortality trials report in the next year or so, it will be important to understand which patients benefit most from this therapy and how PCSK9 inhibitors sit alongside other drugs such as statins and ezetimibe in their actions on the entire spectrum of atherogenic apolipoprotein B-containing lipoproteins.

Animal and cell based investigations have given signposts as to how these antibody-based drugs work but it is essential to consider the limitations of extrapolating from these to the clinical situation. With the production by the liver of particles across the entire VLDL-IDL-LDL spectrum and the generation of large quantities of circulating LDL via the action of intravascular lipases and transfer proteins, it is recognised that human lipoprotein metabolism is so distinctive that there is no good animal model that reflects reliably the intricacies of the metabolic pathways in man. Hence, there is no substitute for time-consuming and technically demanding in-vivo kinetic studies of the type reported in the current issue of the journal by Watts et al [3] and Reyes-Soffer et al [4]. Model systems have revealed that PCSK9 in addition to its key role in regulating cell surface LDL receptor abundance [1,2] potentially influences the behavior of other members of the LDL receptor family i.e. the VLDL receptor (VLDLr), CD36, apoER2 and CD81 due to shared binding motifs [5, 6]. The full effect of inhibiting this protein's action will therefore depend on the extent to which in man these receptors govern lipoprotein production, lipolysis and remodeling, and control tissue-specific lipoprotein uptake. Further, in experiments in cell culture and in investigations of animals with over-expression or knock-out of PCSK9 there is emerging recognition that the protein has a potentially important function in facilitating production of apoB100 lipoproteins in the liver, and apoB48 lipoproteins (chylomicrons) in the intestine [5 - 8]. The mechanism responsible for this effect is unclear but may involve increased lipogenesis, protection of newly synthesized apoB from intracellular degradation, or a reduction in the ability of LDL receptors to inhibit lipoprotein secretion (reviewed in [5, 6, 8]). An additional possible role in triglyceride metabolism appears to be in regulating adipose tissue lipid uptake. PCSK9-knock-out mice exhibit over-activity of VLDLr and CD36 and this has been suggested as the cause of the visceral obesity seen in this animal model [8]. With this multiplicity of effects being uncovered, it follows that there is the potential for

PCSK9 inhibition to have a broad ranging impact on the metabolism of cholesterol- and triglyceride-rich lipoproteins, and on apolipoprotein B kinetics.

Investigations of apoB metabolism in PCSK9 variants in man [9, 10] provide a background against which we can interpret the actions of PCSK9 inhibitors. For example, it is of interest to decipher to what extent monoclonal antibody-based inhibition of circulating PCSK9 recapitulates the impact of loss-of-function mutations [9], and if this therapeutic approach can correct fully the hyperlipidemia seen in gain-of-function carriers, some of which express a severe phenotype [1, 2, 10]. Comparison with the effects of statins on apoB metabolism [11] is also likely to be informative since these two therapeutic approaches share a common final pathway – enhanced LDL receptor activity.

The agreement between the two current investigations in their headline findings is striking. Watts et al [3] used a 2x2 factorial design to investigate apoB metabolism in (for a kinetic study) a large group of men. This provided helpful comparative data for the action of high dose atorvastatin versus evolocumab as monotherapies and in combination. In these normolipidemic subjects, the PCSK9 inhibitor increased the fractional catabolic rate (FCR) of VLDL, IDL and LDL apoB, consistent with stimulation of receptor-mediated removal of these particles from the circulation. Reyes-Soffer and colleagues [4] reported similar substantial increases in IDL- and LDL-apoB removal rates in a group of normal subjects (about half were women) on alirocumab. Given that at the doses administered virtually all circulating PCSK9 is sequestered in antibody complexes [3] these findings indicate that PCSK9 action in untreated normal subjects reduces cell surface LDL receptors by over half (since the rise in FCR was 80 to 100%, and about 25% of LDL clearance is by receptor-independent pathways). Both set of investigators report a decrease in LDL production during PCSK9 inhibition and Watts et al observed a fall in IDL apoB production as well. As pointed out [3, 4] this effect is attributable to the enhanced clearance of precursor IDL and VLDL particles leaving less to be converted to their respective products by delipidation.

The main difference between the two studies is in the reported effect of PCSK9 inhibition on VLDL apoB clearance. This may be due to small subject numbers since there was a trend for VLDL apoB and triglyceride FCR to rise in the Reyes-Soffer study [4] to an extent comparable to that seen by Watts et al [3]. Also, while the starting triglyceride levels were virtually identical in the two trials it may be significant that there was a difference in sex distribution and body mass index (and thus in the underlying metabolic properties of VLDL). It is noteworthy that the results of these studies are in line with the hypercatabolism of apoB in VLDL, IDL and LDL seen in the two loss-of-function variants studied by Cariou et al [9]. The lack of an effect of PCSK9 inhibition on VLDL apoB production rates in both investigations (and direct IDL and LDL apoB production in [3]) indicates that at least in normolipidemic subjects treatment with antibodies does not impact apoB100

lipoprotein assembly and secretion. This may be explained by an inability of these agents to act on intracellular PCSK9 or that the facilitatory action of PCSK9 on lipoprotein production is not quantitatively important in these individuals (in apparent distinction to those possessing a gain-of-function variant [10]). Likewise with the same caveat, the lack of an effect of arilocumab on post-prandial lipemia reported by Reyes-Soffer et al [4] did not offer support for a major role of the PCSK9/LDL receptor axis in chylomicron production.

At about 70mg/dl the basal plasma triglyceride in the healthy volunteers examined was in the low normal range [3, 4]. It is known from statin turnover studies that subjects with higher starting triglycerides exhibit an even more marked (about a doubling) acceleration of VLDL catabolism on treatment [11], an action that explains the well-established observation that the ability of statins to lower plasma triglyceride is a function of the initial level of this lipid. Preliminary data from a meta-analysis of evolocumab trials indicated that the same phenomenon pertains to PCSK9 inhibition i.e. triglyceride lowering is greater when the starting level is high, but overall the degree of VLDL lowering is less than that seen on statins [12].

An initially unexpected feature of PCSK9 inhibitor treatment is the substantial lowering of circulating levels of Lp(a) [1, 2, 13], a particle that is not reduced by statin therapy. Conceptually, there are four possible ways for PCSK9 inhibition to influence Lp(a) metabolism, namely blocking PCSK9 mediated stimulation of apo(a) synthesis and secretion, reducing the direct secretion of LDL (which may be promoted by PCSK9), enhanced receptor-mediated degradation of LDL particles in the space of Disse before they can combine with apo(a), or promotion of catabolism of circulating Lp(a) by LDL receptors. Reyes-Soffer et al [4] observed a trend to increased clearance of apo(a) on alirocumab suggesting that particle removal is enhanced, and the lack of an effect on direct LDL production [3] suggests that the second mechanism is unlikely. The finding of accelerated clearance is consistent with the observations that Lp(a) binds albeit with low affinity to LDL receptors and reductions in this lipoprotein are seen even in homozygous FH unless there is a 'double-negative' mutation present [13].

These metabolic studies [3, 4] although conducted in normal subjects provide important insight into the general mechanism of action of antibody-based PCSK9 inhibitors. They help establish the conceptual framework that enhanced LDL receptor activity with attendant accelerated removal of VLDL, IDL and LDL particles is the primary cause of the profound cholesterol lowering seen with these agents. As noted above, no effect was seen on VLDL apoB production either due to the choice of subjects or because the agents did not access the appropriate intracellular pathways. In this regard, it will be of interest to know if alternative approaches such as small, interfering RNA to PCSK9 lead to an incremental reduction in apoB levels via an action on lipoprotein production (see ORION trial NCT02597127; www.clintrials.gov). If, as appears to be the case [3, 8, 12] current PCSK9 inhibitors have less impact on

VLDL kinetics and plasma triglyceride levels compared to statins then cognizance has to be taken of this in strategies for treating hypertriglyceridemic states such as type 2 diabetes. There is clearly a case now to proceed to determine how PCSK9 inhibitors influence VLDL kinetics in subjects with elevated plasma triglyceride levels and how the combination of these agents with statins may be best deployed. The apparent additive effects of the two classes of agents observed by Watts et al [3] on VLDL catabolic processes is important when addressing the need to reduce remnant particles as well as LDL to new lows.

Conflict of interest disclosures

Dr Packard has received grants for MSD relating to lipid lowering research studies, and honoraria/ consulting fees from MSD, Sanofi/ Regeneron, Pfizer, and Amgen related to PCSK9 inhibitors and lipid lowering.

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