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Role of the microbiota in the pharmacometabolomic response of statins and their therape

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Research Unit

Name : Research Unit on Cardiovascular and Metabolic Diseases

Code *(ex. UMR xxxx)* :

INSERM UMRS 1166

Doctorate School

Thesis supervisor's doctorate school (candidate's futur doctoral school) : ED394, École Doctorale Physiologie, Physiopathologie et Thérapeutique

PhD student currently supervised by the thesis supervisor (number, year of the first inscription) :

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Role of the microbiota in the pharmacometabolomic response of statins and their therapeutic efficacy.

General scientific context: Statins have a real efficacy in the prevention of cardiovascular (CV) events both in primary prevention (patients at high vascular risk: hypercholesterolemia, metabolic syndrome...) and in secondary prevention (after a first coronary or cerebrovascular event). The proven pharmacological effect of statins involves inhibition of cholesterol synthesis, and large-scale clinical studies show that low LDL cholesterol (LDL-C) levels are associated with a low risk of cardiovascular events.

However, at the individual level, patient response to statins in terms of both LDL-C lowering and side effects are variable, with good and poor responders to treatment and the existence of intolerant patients[1]. This heterogeneity in therapeutic response (pharmacokinetics or cytotoxicity) may result from genetic variants. However, this pharmacogenetic effect only contributes to about 5% of the LDL-C reduction[2]. This suggests other mechanisms that may play an essential role in statin therapy's overall response and benefit.

Pleiotropic effects of statins have been reported and include anti-inflammatory and immunomodulatory effects[3]. However, most of these studies have been performed in vitro or in vivo in mouse models using supra-physiological doses[4]. In humans, some statins reduce serum proinflammatory cytokine production, C-reactive protein, and monocyte and T-cell activation, some of which occur acutely[5] or after several weeks[6, 7] and sometimes in a manner independent of LDL-C reduction[8]. However, there is considerable heterogeneity among these clinical data, with some studies showing no immune effects[9, 10, 11]. In addition, some studies report the induction of proinflammatory cytokines by human leukocytes ex vivo[12, 13].

Therefore, given the potential public health implications, the effects of this widely used class of drugs deserve to be explored, beyond their traditional indications, in light of their impact on the gastrointestinal microbial ecosystem[14].

Our hypothesis: As the immune system is primarily regulated by the gut microbiota[15], with statins showing bactericidal and antibiotic properties[16, 17], statins likely act on the host immune system via modulation of the gut microbiota. These pleiotropic effects on immunity and inflammatory response are likely to impact the development of atherosclerosis lesions and their cardiovascular consequences. Thus, a quantitative understanding of the physiological, chemical and microbial factors that determine the microbiome's contribution to statin metabolism may help explain the interpersonal variability in cardiovascular response to statins and offer the opportunity for personalized treatment. In this context, defining markers of benefit or risk associated with statin use independently of LDL-C reduction may be helpful to optimize therapeutic choices.

In addition, several epidemiological, interventional, and preclinical studies demonstrate the link between microbiota composition and the development of atherosclerosis and cardiovascular risk in the host[18]. Of note, we have recently shown the influence of the gut microbiota on whole-body cholesterol flux and synthesis[19,20]. Indeed, by combining a series of in vivo experiments based on manipulation of the microbiota in dyslipidemic mouse models, we report, for the first time, how the gut microbiota regulates cholesterol synthesis, absorption, and trafficking. Importantly, we show that human dyslipidemia can be transferred from humans to mice by transplantation of the gut microbiota, demonstrating the causal role of the microbiota in the regulation of plasma cholesterol levels[20]. These data expand our knowledge of the potential mechanisms underlying the relative rates of cholesterol synthesis and absorption encountered in dyslipidemic patients at high cardiovascular

risk and challenge the current paradigm that elevated plasma cholesterol levels are solely related to genetic, dietary, age, and gender factors.

Preliminary data: Using axenization and fecal transfer approaches in experimental mouse models; our primary data show the direct effect of statins on the composition of the gut microbiota in vivo. Fecal and plasma metabolite analyses also show donor-dependent and statin-dependent signatures. Similarly, recent data suggest a contribution of the microbiota to the bioavailability and efficacy of other drugs[21, 22]. Interestingly, the intestinal microbiota produces many metabolites essential to the symbiotic relationship between the host and its microbiota. A disturbance of this balance (dysbiosis) is correlated to the development of many pathologies with chronic inflammatory components such as diabetes, obesity, steatosis, dyslipidemia, atherosclerosis[23].

In conclusion, the data in the literature show that statins modify the bacterial ecosystem, and we demonstrate in our primary data that this impacts the production of metabolites involved in the host-microbiota dialogue. The overall effect of statins thus results from the addition of beneficial effects via inhibition of endogenous cholesterol synthesis and uncharacterized effects on the eubiosis/dysbiosis balance and repercussions on host clinical parameters and consequently on the development of atherosclerotic lesions.

Strategy and rationale: To go beyond correlations between pathology and the spectrum of microbial species composing the microbiota or the repertoire of microbial genes collectively present in the microbiota, we propose approaches based on the relative or absolute quantification of metabolites produced by the microbiota in response to statins on hypercholesterolemic mouse experimental models and their consequences on lipid metabolism and atherosclerosis development.

Among the metabolites studied, we will pay specific attention to sterols and bile acids (BAs) which are particularly attractive metabolites for the following reasons: - BAs represent a vast pool of host-derived metabolites co-metabolized by the microbiota allowing a permanent molecular dialogue between the host and its microbiome[24] - The BAs are produced in the liver (primary BAs) from hepatic cholesterol, then during enterohepatic recycling, they will undergo numerous enzymatic modifications (deconjugation, dehydrogenation, dehydroxylation, epimerization, oxidation) in particular by the intestinal microbiota forming a large number of secondary BA molecular species - Sterols are at the heart of HMGCoA-Reductase metabolism and, therefore, likely to influence the response to statins.

We will analyze hepatic, plasma, and fecal sterols. The molecular modifications of BAs are likely to impact their biological properties:
- Bactericide. The BAs are powerful detergents; they contribute to regulating the composition of the microbiota.
- Hydrophobic BAs are involved in the absorption of lipids.
- Agonist/antagonist. Through their activities on their nuclear (FXR, VDR) and membrane (TGR5) receptors, BAs will regulate lipid and carbohydrate metabolism, energy homeostasis, and host immune responses.

Main objective: The main objective of the study is to measure the direct effect of HMG-CoA reductase inhibitors (Atorvastatin and Rosuvastatin) on the production of metabolites derived from the intestinal microbiota of several bacterial communities from hypercholesterolemic patients pre-selected for their quality of good or poor responders in terms of lowering cholesterol levels and possibly in terms of reducing inflammation.

Secondary objectives: To correlate metabolic signatures favoring eubiosis or dysbiosis and clinical benefits according to the statin. To propose mechanisms underlying the responses of good responders, poor responders, and statin intolerants.

Our objectives will take into account:
- The evolution of all metabolomic/lipidomic profiles at the plasma and fecal level related to statin treatments.
- The evolution of all sterol, primary & secondary hepatic, plasma and fecal BA profiles - The evolution of genes related to the metabolism of BAs and cholesterol in the organs of synthesis and absorption (liver, jejunum, ileum).
- The progression of atherosclerosis lesions in response to treatment. - The evolution of clinical phenotypes related to carbohydrate metabolism (weight gain and OGTT and ITT analyses if glycemia altered).
- The evolution of inflammatory parameters related to intestinal permeability (endotoxemia, lipocalin 2, calprotectin and fecal albumin, inflammatory cytokines, transit time).
- The evolution of bacterial phylogeny by 16S sequencing and metagenome analysis (these analyses will be performed in independent funding applications).

Experimental approach: the project is based on a prospective, randomized, double-blind, placebo-controlled interventional trial (EudraCT Number: 2017-002048-34). We accurately detect a large number of metabolites, including microbial derivatives, in various matrices (plasma, tissue, feces) by mass spectrometry methods on our dedicated metabolomics and lipidomics platforms. During two weeks, the mice receive human microbiota five times at each gavage. Rosuvastatin or Atorvastatin are administered to the mice once a day for three weeks. Mice are then euthanized to analyze metabolites in feces and blood, gene expression in tissues, and atherosclerotic lesions in the aortic sinus[26]. Cecal, duodenal/jejunal, and ileum contents are also sampled for further metabolic analyses[20].

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