Thesis proposal 2022:

Interactions between omega-3 and prostacyclin pathways in the management of pulmonary hypertension of group 3

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Abstract and background:
This thesis project concerns the omega-3 polyunsaturated fatty acids (n-**3** PUFA) and their association with prostacyclin (prostaglandin (PG) I_2) analogues as a possible treatment targeting the development of pulmonary hypertension (PH). PH is characterised by pulmonary vascular vasoconstriction, smooth muscle cell proliferation, and inflammation (thrombosis). PH remains a major health problem despite current therapies, like PGI_2 an omega-6 (n-6 PUFA) and its analogues[1]. These PGI_2 analogues (iloprost, treprostinil…) play an important role in the reduction of the pulmonary vascular tone and inhibition of cell proliferation[2]. On the other hand, n-3 PUFA, like (Docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA)) mainly found in fish oils and their metabolites (resolvin (Rv), protectin (PD), maresin (MaR)), are involved in the resolution of inflammation[3,4]. These specialized pro-resolving lipid mediators (SPM) have been recently reported to be protective against cardiovascular events[3-5] and pulmonary inflammation[6]. Many studies have shown that DHA and EPA dietaries are protective against cardiovascular events[3,5] while only two studies showed that RvD1 and RvE1 are able to normalize arterial hyper-reactivity induced by pro-inflammatory mediators in a PH model[5]. To our knowledge, the role of n-3 PUFA in APH of group 3 PH and their interaction with the prostacyclin pathway have not yet been investigated.

PH is a disabling chronic disorder of the pulmonary vasculature, which is characterised by increased pulmonary pressure (0.3% of prevalence in the population). It is classified according to its etiology into five groups. Group 3 PH is the most common and lethal form, it is secondary to lung disease frequently associated with inflammation (COPD, emphysema or fibrosis). These changes are considered as a consequence of endothelial dysfunction with an imbalance between several molecular families[2,7]: vasodilators (PGI_2, nitric oxide) and vasoconstrictors (thromboxane (Tx) A_2, endothelin)... Our group has shown that prostacyclin analogues (iloprost, treprostinil) commonly used to treat group 1 PH are also active in pulmonary bronchial and arterial preparations obtained from group 3 PH patients[8,9]. This *ex vivo* results, along with other clinical studies, led to US FDA approval of inhaled treprostinil (Tyvaso) as the first treatment for patients with group 3 PH interstitial lung disease[10].

Aims of the project: 1/In this project we would like to extend our pharmacological researches by comparing the roles and effects of n-3 PUFA on human pulmonary arteries (HPA) derived from group 3 PH and non PH patients. 2/In addition, the effects of some n-3 PUFA of interest used in combination with PGI_2 analogues (a classical PH-treatment) will be tested on vasoconstriction, remodelling and inflammation in these HPA.

Scientific objectives
We aim at first to compare the presence and role of omega-3 between pulmonary vascular samples derived from group 3 PH and non-PH patients. We will measure (RvD1, RvD2, RvD3, RvD4, RvD5,

MaR1, 7(S)-MaR1, MaR2, PDX, PD1, 17(R)-HDOHE, 17(S)-HDOHE, 14(R)-HDOHE, 14(S)-HDOHE, RvE1, RvE2, 18(R)-HEPE, 18(S)-HEPE) after stimulation of fresh HPA with DHA, EPA or nothing.

For all the experiments of the project we will focus and use the 2-3 SPM produced at the highest level and/or displaying the greatest different levels between PH and non-PH samples. The HPA (+/-PH) will be incubated with our n-3 PUFA (the 2-3 SPM of interest selected, DHA or EPA) and their effects will be studied on the three major physiopathological axes of pulmonary hypertension which are pulmonary vasoconstriction, remodelling and inflammation:

-The vasoconstriction, few *ex-vivo* studies on human vascular tone using DHA or EPA have been performed; we have recently shown in human saphenous vein that the vasoconstriction induced by noradrenaline is reduced after their pre-treatment with DHA[11]. In isolated non-PH HPA only one study describes the inhibitory effect of RvE_1 on the contractions induced by an analogue of TxA_2[12]. This last work will be extended with our HPA samples and n-3 PUFA.

-The remodelling will be analysed by measuring the proliferation and migration of smooth muscle cell derived from HPA after treatments with the 2-3 SPM of interest.

-The inflammatory state will be detected by measuring cyclooxygenase (-1 and -2) expressions, endogenous PGE_2 and C reactive protein levels in HPA.

At second aim, we will investigate the effect of the 2-3 SPM of interest, EPA or DHA on the prostacyclin pathway and PGI_2.analogues effects in HPA.

-In our previous publication, we showed that the activity of the PGI_2 pathway is significantly reduced in APH derived from group 3 PH patients[7]. Now, the PGI_2 synthesis, the expression of the PGI_2 receptor (IP) and metabolic enzyme (PGIS) will be measured in APH +/-PH after incubations the 2-3 SPM of interest, EPA or DHA.

-The HPA relaxations induced by PGI_2 analogues will be tested and compared after incubation with or without our selected n-3 PUFA.

-The proliferation and migration of smooth muscle cell derived from HPA after treatments with a combination of PGI_2 analogues and our selected n-3 PUFA will be tested.

-Furthermore, in human, EPA can produce *via* cyclooxygenase activity, (n-3) prostacyclin (PGI_3). PGI_3 has similar antiaggregatory and vasorelaxing effects than PGI_2[13]. The level and vasorelaxant properties of PGI_3 will be measured in HPA derived from group 3 PH and non-PH patients.

Methods

The experiment will be done by using HPA samples obtained at Bichat hospital (with informed consent of the patient and ethic advisory board "GHU Nord" agreement), an array of complementary *ex-vivo* pharmacological studies (organ bath system), *in-vitro* experiments (Western blot, ELISA, Boyden chamber, Real-time PCR, mass spectrometry LC-MS, Immunohistochemistry) and cell culture will be applied.

References

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