Doctoral School Health, Biological sciences and chemistry for the living

ED Santé, Sciences Biologiques et Chimie du Vivant (SSBCV) n°549

PhD Thesis offer (3-years funding)
EA4245 Transplantation, Immunology, Inflammation (T2I)
University of Tours, France

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Role of platelet factor 4 (PF4) and neutrophil extracellular traps (NETs) in cardiac ischemia-reperfusion injury

Key words: platelet factor 4, Ischemia-reperfusion, platelet, neutrophils, P2X7 receptor, antibody.

After myocardial infarction, ischemia-reperfusion (I/R) injury can induce tissue remodelling toward organ fibrosis affecting organ function and patient prognosis. During I/R, platelets, which are highly activated, release ATP and chemokines such as platelet factor 4 (PF4) that may play an important role in I/R injury. Moreover, ATP released during the I/R phase could promote, *via* the P2X7 purinergic receptor, the formation of neutrophil extracellular traps (NETs), generated by neutrophils infiltrating the tissue, amplifies the cardiac lesions associated with I/R.
In this context, the objectives of the thesis will be to study the effect of platelet factor 4 and NETs components (i.e. DNA, histones, proteases...) in fibrosis associated with cardiac I/R. The role of P2X7 in the formation of NETs will also be evaluated. Then, the effect on myocardial I/R lesions of anti-PF4 monoclonal antibodies, previously developed in the laboratory, as well as the effect of P2X7 antagonist will be studied in *in vitro* and *in vivo* models.
This thesis project should demonstrate that FP4 and/or P2X7 could be new therapeutic targets to limit cardiac I/R injury.

Context :
Ischemia-reperfusion (I/R) injuries occur when the blood supply to an organ is momentarily interrupted, then restored. These lesions induce the remodeling of the remaining tissue towards fibrosis of the organ which can affect its function and the prognosis of patients. The I/R situation associated with myocardial infarction induces massive platelet activation and

release of the contents of their intracytoplasmic granules into the bloodstream. These effects, with respect to the evolution of the I/R, are still little known (1). In addition, neutrophils infiltrate tissues after ischemic injury and exacerbate inflammation through various mechanisms, including the formation of NETs (neutrophil extracellular traps), which appear to have deleterious effects and generate I/R damage in various organs. (2).

Beyond their main role in haemostasis, platelets are also importantly involved in the inflammatory response associated with ischemia-reperfusion injury and in particular the development of tissue fibrosis. Platelet activation is associated with the secretion of intracytoplasmic granule content that contains many chemokines, including platelet factor 4 (PF4), and several studies suggest that it may promote the differentiation of fibroblasts into myofibroblasts with a pro- fibrosis in lung and skin models (3-4). On the other hand, there are very few data on the role of endogenous PF4 in the fibrotic remodeling of the myocardium in I/R condition, including on the underlying molecular mechanisms.

However, it is proposed that platelet-neutrophil interactions and the release of ATP during ischemia promote the formation of "neutrophil extracellular traps" (NETs) via the activation of the membrane purinergic receptor P2X7. NETs are composed of DNA, histones and many proteins such as neutrophil elastase (NE), myeloperoxidase (MPO) or PF4 and several studies have shown that an inhibition of the formation of NETs could be an interesting new therapeutic approach. At the same time, other studies have shown that the degradation products of NETs, such as circulating DNA, DNA/MPO complexes or histones, could also be deleterious via a cytotoxic effect in particular. In this regard, in a septic mouse model, the stabilization of NETs by an anti-PF4 antibody increases their resistance to endogenous nucleases, thus decreasing DNA release and improving mouse survival (6). Our laboratory has developed 4 anti-human PF4 chimeric antibodies with different specificities, three (1E12, 1C12 and 2E1) can bind to native PF4 while the 4th (5B9) only recognizes a complex formed of PF4 and glycosaminoglycans (7- 9). For this project, we will evaluate whether these Abs can constitute a new therapeutic approach in the myocardial I/R model.

Objectives of the PhD thesis :

- to study the role of platelet factor 4 in myocardial ischemia-reperfusion injury, and in particular in the mechanisms related to the differentiation of myofibroblasts.
- to evaluate the role of P2X7 in the formation of NETs and to evaluate the consequences of targeting this purinergic receptor in the myocardial I/R model
- to evaluate the effects of NET degradation products (DNA, histones, proteases, etc.) on the viability of cardiomyocytes and the differentiation of cardiac fibroblasts into myofibroblasts.
- to test the potential modulating effect of anti-PF4 monoclonal antibodies on myocardial I/R lesions.

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