Non-Resolving Inflammation Post-Myocardial Infarction

803 ICA3 Literature Review

Introduction

Inflammation is an essential body's defence mechanism against infection and injury in higher organisms. During acute inflammation, pro-inflammatory compounds are released to eliminate infectious agents or damaged cells at the sites of injury, which eventually restores homeostasis. Inflammation will resolve as soon as the threats have been addressed – generally no longer than 6 weeks – through the release of anti-inflammatory and pro-resolving mediators. However, inflammation can persist indefinitely in some occasions, giving rise to chronic inflammatory conditions, such as atherosclerosis, Alzheimer's disease, cancer, and cardiovascular disease. In this review, I will focus on myocardial infarction (MI) as the recurrence of further cardiovascular complications as a result of unrestrained inflammation, is common and often with fatal outcomes.

Non-Resolving Inflammation

Myocardial infarction is the leading cause of heart failure worldwide. Approximately 13% of patients are diagnosed with heart failure at 30 days after hospital discharge for myocardial infarction, and another 20-30% at 1 year (Jenča et al., 2020). The prognosis is poor for post-MI heart failure patients: total mortality risk increases by 300% and cardiovascular mortality by 400% (Jenča et al., 2020). Post-MI heart failure is often of subtype heart failure with preserved ejection fraction (HFpEF), characterized by a stiff and hypertrophied left ventricular (LV) resulting from maladaptive LV remodeling, which reduces cardiac diastolic ability. The extent of adverse LV remodeling correlates positively with the efficacy of infarct wound healing (Nahrendorf, 2011). It is thus without a doubt that non-resolving inflammation is considered the primary determinant in the pathology of post-MI heart failure (Tourki and Halade, 2017).

Immediately after myocardial infarction, leukocytes infiltrate the infarct areas in response to damaged-associated molecular patterns released by apoptotic cardiomyocytes. The infiltration is aggravated should reperfusion injury occurs after the restoration of normal blood flow to the heart through reperfusion therapy – the most effective treatment against ischemic damage following a heart attack. In this case, the injury is mainly promoted by infiltrating neutrophils (Zhang et al., 2020). Disturbance to CXCL12-CXCR4 signaling post-myocardial infarction mediates neutrophilia in peripheral blood (Ma, 2021). Neutrophils die via spontaneous apoptosis and are mainly phagocytosed by macrophages in a process termed efferocytosis. This process precedes the resolution of inflammation and tissue repair, thus defective efferocytosis is deleterious. For instance, delayed removal of apoptotic neutrophils leads to secondary necrosis at which the cells secrete cytotoxic and antigenic contents that exacerbate inflammation (Blume et al., 2009). The delay also impedes inflammation resolution since efferocytosis induces the production of specialized pro-resolving mediators (SPMs), including lipoxin A4 and resolvin E1 (Kebir and Filep, 2013). SPMs in turn can further simulate neutrophils apoptosis and their removal by macrophages, thereby potentiating the resolution of inflammation (Kebir and Filep, 2013). In addition, previous studies have demonstrated that defective efferocytosis is linked to non-resolving inflammation diseases, including atherosclerosis and post-MI heart failure (Li et al., 2009; Wan et al., 2013). As such, promoting macrophages efferocytosis of neutrophils is beneficial to reduce non-resolving inflammation and the onset of post-MI heart failure.

Macrophage Polarization

During later stages of wound healing, the plasticity of macrophages enables them to polarize from pro-inflammatory M1 to pro-reparative M2 phenotype. M2 macrophages secrete MMP-12 which dampens neutrophil infiltration, and anti-inflammatory factors, including IL-4, IL-10, and TGF-β (Kim

et al., 2021). Importantly, the alternatively activated macrophages exhibit increased capacity of efferocytosis of neutrophils (Tourki and Halade, 2017). This is supported by a study conducted by Zhang et al. (2020), which demonstrated that mesenchymal stem cells transplant accelerates cardiac inflammation resolution and prevents ventricular remodeling after myocardial infarction reperfusion injury by enhancing M2 macrophage-induced efferocytosis of dead neutrophils. Similarly, Annexin A1 promotes inflammation resolution by modulating macrophage switch from M1 to M2 phenotype, increasing the elimination of effete neutrophils (Sugimoto et al., 2016). Therefore, this implies that impaired efferocytosis is associated with an imbalance of M1/M2 macrophages ratio, and therapies modulating the phenotypic switch may alleviate the pro-inflammatory response.

An integral player in macrophage's functional plasticity is cellular metabolism. Macrophages adapt their metabolic state according to their surrounding microenvironment (e.g., nutrient and oxygen availability), thus metabolic imbalance can dysregulate the cardiac macrophage's functionality. For instance, hypoxia reduces mitochondrial oxidative phosphorylation and increases reliance on glycolysis, thereby supporting the biosynthesis of proinflammatory cytokines and M1 polarization. In contrast, reoxygenation tends to restore mitochondrial metabolism and fatty acid oxidation, facilitating macrophage transition to M2 phenotype. The metabolic switch from OXPHOS to glycolysis is dependent upon the activation of hypoxia-inducible factor 1-alpha (HIF-1α). Deletion of this gene in obese mice has been reported to diminish adipose tissue inflammation and improve insulin sensitivity (Takikawa et al., 2016). It is important to note that myocardial infarction patients usually already have a background of various health problems that increase their risk of chronic inflammation and subsequent development of heart failure. For instance, myocardial infarction patients with diabetes are 30-42% more likely to develop heart failure than non-diabetic patients (Table 1). Impaired healing process and unrestrained inflammation reaction in diabetic wounds are associated with dysfunctional efferocytosis and deficiency in macrophage phenotypic switch to M2 (Huang et al., 2020). However, to the best of my knowledge, diabetes has not been shown to induce similar macrophage dysregulation and impaired efferocytosis of neutrophils in inflamed heart post-myocardial infarction. In addition, little is known about the mechanisms by which diabetes affects cellular metabolism to favor pro-inflammatory phenotype in this disease-specific profile. Given that hyperglycaemia has been implied to induce microenvironmental hypoxia in cancer (Li et al., 2018), I hypothesize that diabetes promotes M1 macrophage polarization post-myocardial infarction through the activation of HIF-1α.

Conclusion

Myocardial infarction patients are at a higher risk of developing heart failure, and this is particularly true in the context of diabetic patients. Non-resolving inflammation underlies the pathogenesis of post-MI heart failure as it promotes maladaptive LV remodeling. The prolonged inflammation may occur when macrophage efferocytosis of apoptotic neutrophils is deficient. Neutrophil infiltration following myocardial infarction is enhanced, particularly if reperfusion injury occurs after reoxygenation therapy. Failure to remove those neutrophils may lead to secondary necrosis and ongoing inflammatory reaction. A core player in the efferocytosis of effete neutrophils is M2 macrophages. During non-resolving inflammation, macrophage polarization from pro-inflammatory M1 to pro-reparative M2 phenotype is likely to be dysregulated because therapies targeting the phenotypic switch have been shown to enhance efferocytosis and inflammation resolution post-myocardial infarction. Given that both M1 and M2 macrophages have their preferential metabolic pathway, the microenvironment in a prolonged inflamed heart is likely to favor reliance on glycolysis. Therefore, the significantly higher incidence of heart failure among post-MI patients with diabetes is possibly due to hyperglycemia-induced cellular hypoxia which activates HIF-1α signaling, an important regulator of the metabolic switch to glycolytic metabolism.

Table 1. Clinical risk factors for post-MI heart failure (Jenča et al., 2020)

Clinical risk factors	**Increase in risk of post-MI HF**
Age, increase by 10 years	20–50%
Female sex	15–34%
History of previous MI	21–89%
Hypertension	7–70%
Diabetes	30–42%
Glomerular filtration, decrease by 10 mL/min/1.73 m^2	10%
Heart rate, increase by 10 b.p.m.	7–23%
Atrial fibrillation	20–51%

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Experimental Plan

This plan will outline a series of experiments to test my hypothesis that: 1) diabetes promotes M1 macrophage polarization and hence defective efferocytosis of neutrophils in the heart post-myocardial infarction; 2) the dysregulation is mediated via the activation of HIF-1α as a consequence of hyperglycaemia-induced cellular hypoxia. The expected timeline for this project is 7 months as detailed in the Gantt chart (Table 2).

Animals and Diets

Three groups of mice will be enrolled in the project: diabetic mice with HIF-1α-deficient macrophages (diabetic HIF-1α$^{flox/flox}$/LysM-cre KO), diabetic mice with intact HIF-1α (diabetic HIF-1α$^{flox/flox}$), and non-diabetic mice with intact HIF-1α (non-diabetic HIF-1α$^{flox/flox}$). The minimum number of mice to be included in each group will be determined by power analysis. The diabetic HIF-1α$^{flox/flox}$ and non-diabetic HIF-1α$^{flox/flox}$ mice are particularly needed to test the first hypothesis, whereas investigating the role of HIF-1α requires both diabetic HIF-1α$^{flox/flox}$/LysM-cre KO and diabetic HIF-1α$^{flox/flox}$ mice. To delete the HIF-1α gene in myeloid cell lineage, I will cross HIF1α^{flox} mice (JAXMICE: 007561) with LysMcre mice (JAXMICE: 004781) to yield HIF-1α$^{flox/flox}$/LysM-cre KO mice. For the control group, I will pick mice with floxed HIF-1α allele that lack Cre from the same cross. Floxing could cause altered gene expression, thus it must be accounted as a possible confounding factor. All mice will be housed in standard polypropylene cages containing 3 animals per cage, under a 12:12 light-dark cycle and an ambient temperature of 22-25°C. Ad libitum access to water and regular chow will be provided. For the subsequent experiments, only male mice will be included because female mice tend to be resistant to streptozotocin (STZ)-induced diabetes, and both sexes differ in their immune responses (Wu and Huan, 2008).

Induction of Diabetes

Ten-week-old mice in diabetic groups will each receive a single dose of STZ intraperitoneally for 5 consecutive days at 40 mg/kg body weight (Wu and Huan, 2008). Besides being cost-effective and time-efficient, STZ works in most strains of rodents, thus it is expected to successfully induce diabetes in both groups. STZ is toxic to pancreatic beta cells, leading to decreased insulin production and elicitation of type 1 diabetes (T1D). Induction of T1D is preferred over T2D because T2D is highly interlinked to obesity. In fact, induction of T2D using STZ will require a high-fat diet throughout the experiment. Obesity could contribute to cellular hypoxia and activation of HIF-1α, thus it might distort the measure of association between hyperglycaemia and the dysregulation. In this experiment, the non-diabetic group will serve as vehicle control. They will be subjected to the same dose, duration, and mean of injection as the diabetic groups, but with vehicle citrate buffer (used to dissolve STZ). The body weight and blood glucose level of every mouse will be measure immediately before the injection and after every week for one month by tail snip and diabetes test strips for comparisons. At week 3, around 50% of mice in diabetic groups should have developed severe diabetes (Wu and Huan, 2008). If more than half of the mice still do not exhibit mild hyperglycaemia (>150 mg/dl) by week 4, the mice will receive STZ injection for another 5 days. Only mice with severe hyperglycaemia (>300 mg/dl) will be considered for the next experiment to make similar initial severity of diabetes.

Induction of Myocardial Infarction

Myocardial infarction is commonly induced by permanent or temporary surgical ligation of the left anterior descending artery (LAD). Although permanent ligation can induce a larger and more consistent infarct, ischaemia-reperfusion (IR) or the temporary approach will be selected. The reason is reperfusion therapy is the first-line treatment for myocardial infarction patients, thus IR approach is more representative of human myocardial infarction and reperfusion injury. I will follow an IR approach described by Zhang et al. (2020). In brief, all mice from the three groups will undergo

anaesthesia and left-sided thoracotomy, exposing the heart. Then, using a small piece of tubing, their LAD will be ligated for 45 minutes. The occlusion duration of 30 minutes is commonly used in mice, however, with this setting, the mice could end up with infarct sizes as small as 4% (Villiers and Riley, 2020). Therefore, a longer duration as performed by Zhang et al. (2020) is preferred.

Measurement of Hypoxia, Glycolysis, Efferocytosis, M1/M2 Ratio, and LVEDP

According to a previous RNA-sequencing analysis, macrophages from normal mice already exhibit increased expression of genes related to OXPHOS 3 days post-myocardial infarction, indicative of metabolic reprogramming (Mouton et al., 2018). Therefore, I expect lower glycolysis activity at 3 days post-surgery in non-diabetic HIF-1α$^{flox/flox}$ and knock-out mice (Table 1). Although hyperglycaemia is supposed to induce cellular hypoxia, the knock-out group should exhibit less sensitivity to the microenvironmental cue due to lack of oxygen-sensing HIF-1α. I will sacrifice one-third of the mice from each group to perform immunohistochemical staining on glycolysis-related proteins: Glut1 and hexokinase II. The expression levels of Glut1 and hexokinase II are expected to be higher in diabetic HIF-1α$^{flox/flox}$ because hypoxia should activate HIF-1α to promote glycolysis in that group. I will also assess the level of hypoxia in their heart tissue using Hypoxyprobe™ (pimonidazole hydrochloride) immunohistochemical analysis (Aguilera and Brekken, 2014). This step will confirm that both diabetic groups are indeed hypoxic, but only one group is responsive.

The macrophage phenotype shifts to a predominantly M2 by day 7 post-myocardial infarction as reported by Mouton et al. (2018). I will sacrifice another one-third of mice from all groups one week after the surgery to obtain their cardiac tissue sections. The sections will be double immunostained against M1 macrophages (CD68+/CD80+) and M2 macrophages (CD68+/CD163+). Then the M1/M2 ratio will be calculated from the total number of M1 and M2 as inferred from the staining. The result will be validated using quantitative real-time PCR targeting pro-inflammatory IL-1β, IL-6, and TNF-α. The expression of those genes should be highest in diabetic HIF-1α$^{flox/flox}$ due to the expected predominant M1 polarization. The level of efferocytosis will be determined by labelling the slides with anti-MPO (to detect neutrophils) and anti-CD163 as described by Zhang et al. (2020). Lastly, the remaining one-third of mice will be kept for another 3 weeks for cardiac function analysis. At the end of the final week, the left ventricular filling pressure of each mouse will be measured using a catheter. High left ventricular end-diastolic pressure (LVEDP) may reflect heart failure with preserved ejection fraction, which is likely to occur in some mice in the diabetic HIF-1α$^{flox/flox}$ group as a result of dysregulated macrophage polarization and efferocytosis, mediated by increased activation of HIF-1α in hyperglycaemia-induced hypoxic microenvironment.

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Table 1. Expected outcomes from each experiment

Experiment	Diabetic HIF-1α$^{flox/flox}$/LysM-cre KO	Diabetic HIF-1α$^{flox/flox}$	Non-Diabetic HIF-1α$^{flox/flox}$ (taken as baseline)
Induction of diabetes with STZ	Severe diabetes in ~50% of mice at 3rd week	Severe diabetes in ~50% of mice at 3rd week	Normal blood glucose
Induction of MI with IR approach	Infarct formed and potential reperfusion injury	Infarct formed and potential reperfusion injury	Infarct formed and potential reperfusion injury
Measurement of hypoxia 3 days post-surgery	Hypoxia	Hypoxia	Normoxia
Measurement of glycolysis 3 days post-surgery	Lower expressions of Glut1 and hexokinase II than diabetic HIF-1α$^{flox/flox}$, possibly higher than non-diabetic group	High expressions of Glut1 and hexokinase II	Low expressions of Glut1 and hexokinase II
Measurement of efferocytosis 7 days post-surgery	Higher than diabetic HIF-1α$^{flox/flox}$, possibly lower than non-diabetic group	Low	High
Measurement of pro-inflammatory IL-1β, IL-6, and TNF-α	Lower expressions than diabetic HIF-1α$^{flox/flox}$, possibly higher than non-diabetic group	High	Low
Measurement of M1/M2 ratio 7 days post-surgery	Less M1 and more M2	More M1 and less M2	Less M1 and more M2
Cardiac Function Analysis	Lower LV end-diastolic pressure than diabetic HIF-1α$^{flox/flox}$, possibly higher than non-diabetic group	High LV end-diastolic pressure	Normal or slightly increased LV end-diastolic pressure

Table 2. Research project timeline

Action	1	2	3	4	5	6	7
Mice breeding and growing until 10 weeks old							
Induction of diabetes							
Induction of MI							
Measurement of hypoxia							
Measurement of glycolysis							
Measurement of efferocytosis							
Measurement of pro-inflammatory genes							
Measurement of M1/M2 ratio							
Measurement of LVEDP							