Protective cardiac conditioning by an atypical cytokine

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ABSTRACT

Ischemic heart disease (IHD) represents the leading cause of morbidity and mortality worldwide. Therapy options generally aim at restoring the blood flow to the heart muscle and relieve the ischemic insult. Paradoxically, coronary artery reperfusion itself, both during emergency intervention in STEMI patients or in the setting of elective cardiac surgery, damages the heart muscle, a phenomenon known as myocardial ischemia-reperfusion (I/R) injury (IRI). Ischemic preconditioning (IPC) is defined by episodes of 'sub-lethal' ischemia and reperfusion prior to prolonged coronary artery occlusion. It has been extensively studied as a promising approach to attenuate IRI, but two recent multicenter clinical trials of remote IPC (RIPC) on clinical outcomes have been disappointing. Macrophage migration-inhibitory factor (MIF) is a structurally unique chemokine-like inflammatory cytokine. MIF is pro-atherogenic, but has a complex function in the ischemic heart with a surprising potential as a local cardioprotective factor in early myocardial ischemia. A recent paper published in *Clinical Science* by Ruze et al. [*Clin. Sci (London)* (2019) **133**, 665-680], now suggests that MIF could be a key player mediating IPC in the ischemic heart. Employing a *Mif* gene knockout mouse model, the study indicates a role for endogenous MIF in IPC-mediated protection from myocardial IRI. It could assist in understanding how this atypical cytokine controls ischemic heart pathologies and may set the stage for novel MIF-based therapeutic strategies in IHD.

Non-standard abbreviations and acronyms

ACK	Atypical chemokine
ACS	Acute coronary syndrome
AIPC	Anesthetic-induced preconditioning
AMI	Acute myocardial infarction
AMPK	Adenosine-monophosphate kinase
CABG	Coronary artery bypass graft
CD74	Cluster of differentiation 74
CKR	Chemokine receptor
CXCL12	CXC motif chemokine 12
CXCR4	CXC motif chemokine receptor 4
D-DT	D-dopachrome tautomerase
IPC	Ischemic preconditioning
IPer	Ischemic perconditioning
IPost	Ischemic postconditioning
I/R	Ischemia/reperfusion
IRI	ischemia-reperfusion injury
MI	Myocardial infarction
MIF	Macrophage migration-inhibitory factor
NSTEMI	Non-ST elevation myocardial infarction
PPCI	Primary percutaneous coronary intervention
~~RIC~~	~~Remote ischemic (pre)conditioning~~
RIPC	Remote ischemic preconditioning
STEMI	ST elevation myocardial infarction

Ischemic heart disease (IHD), also termed coronary heart disease (CHD) or coronary artery disease (CAD), represents the leading cause of morbidity and mortality worldwide (1). It involves the reduction (blockage) of blood flow to the heart muscle due to atherosclerotic plaque build-up within the coronary arteries. Acute conditions caused by a reduced blood flow in the coronary arteries are often broadly called myocardial infarction (MI) or heart attack and are summarized under the umbrella term “acute coronary syndrome” (ACS). They are sub-classified into unstable angina, ST segment elevation myocardial infarction (STEMI), and non-ST segment elevation myocardial infarction (NSTEMI). Depending on the acuteness and extent of the event, age and condition of the patient, and comorbidities, primary percutaneous coronary intervention (PPCI), or coronary artery bypass grafting (CABG, referred to as "bypass surgery"), is the preferred therapy option (2) to restore blood flow to the heart muscle and relieve ischemic stress. Paradoxically, coronary artery “reperfusion”, achieved during emergency intervention by PPCI or in the setting of elective cardiac surgery by CABG, itself damages the heart muscle with cardiomyocyte death occurring during the reperfusion phase. This phenomenon is known as myocardial ischemia-reperfusion (I/R) injury (IRI) (3, 4). Of note, MI after CABG surgery is caused by acute IRI similarly to revascularization after STEMI, but an ageing population and the growing prevalence of comorbidities (e.g. diabetes mellitus), has led to an increased risk of developing perioperative IRI in CABG patients.

Clinical treatment strategies against myocardial IRI are challenging and currently no effective treatment is available. A procedure called ischemic preconditioning (IPC) has been a promising approach. Over 30 years ago, Murry and colleagues discovered that brief episodes of ‘sub-lethal’ ischemia and reperfusion prior to prolonged coronary artery occlusion were able to markedly reduce myocardial infarct size (5). IPC research has considerably contributed to the field of cardioprotection and IPC-mediated cardioprotection has been successfully demonstrated in experimental studies across a variety of species. While the original IPC procedure was based on the principle of “intra”-myocardial protection, the IPC principle as appreciated today encompasses a variety of protocols including classical ischemic preconditioning (IPC), ischemic perconditioning (IPer), ischemic postconditioning (IPost), and remote ischemic

(pre)conditioning (RIPC); IPost and RIPC are of highest translational utility. However, while the preclinical studies and numerous smaller clinical trials have gathered convincing evidence for the translational value of IPC, two recent multicenter, randomized, controlled clinical trials of RIPC on clinical outcomes after cardiac surgery (the ERICCA and RIPHeart studies) have come out neutral. On the other hand, the failure of the cardioprotection field to deliver myocardial infarct size-reducing drugs that are effective in patients has been partially attributed to an insufficient reproducibility in the preclinical studies, with recommendations leveled for future protocols in various animal models (the “CAESAR protocols”) (6). Together, this has highlighted the challenges in translating IPC-based cardioprotection into clinical practice (7, 8).

A recent study published in last month’s issue of *Clinical Science (London)* applied an experimental IRI mouse model and the gene knockout technology and identified one of the factors that mediates RIPC (9). It is macrophage migration-inhibitory factor (MIF), a long-known chemokine-like inflammatory cytokine that has previously been implicated in inflammation, but for which a dichotomic role in cardiovascular disease has been suggested. Ruze and colleagues induced I/R *ex vivo* in a Langendorff-perfused heart and *in vivo* with or without preceding cycles of ischemia and reperfusion. Strikingly, they found that the protective effect of RIPC in wildtype hearts/mice, as evidenced by strongly reduced infarct size and cardiac dysfunction in combination with increased cardioprotective signaling, was completely lost in mice, in which the *Mif* gene had been globally deleted ($Mif^{-/-}$) (9).

MIF was already discovered over half a century ago as the first cytokine to be described (10), but its molecular characterization and the identification of its receptors and signaling pathways were only achieved in the past 25 years (11). Today, MIF is known as a pleiotropic inflammatory cytokine and upstream mediator of innate immunity that is characterized by several unique features. It is evolutionarily conserved and, structurally, does not belong to any of the known cytokine classes. Initially discovered as a T-cell and macrophage factor, it has been found to be widely expressed and can be secreted from intracellular stores by non-

conventional secretion. Important for the current work by Ruze et al., inflammation- or cell stress-triggered MIF secretion is not confined to immune cells but has been observed in endothelial and parenchymal cells, including cardiomyocytes. These features have led to the notion that MIF may have local, tissue-based, alarmin-like characteristics. MIF is viewed as an extracellular-acting cytokine and chemokine, but intracellular MIF may contribute to certain of its functions. Immune and inflammatory activities of MIF are mediated by high-affinity interactions with its cognate receptor CD74 or the chemokine receptors CXCR2 or CXCR4. These receptors are expressed on myeloid cells and lymphocytes, but can also be upregulated on endothelial and tissue cells upon inflammation and hypoxia. The MIF/CD74 axis primarily drives cell-proliferative and metabolic responses, while the MIF/chemokine receptor pathways are pivotal in controlling leukocyte recruitment. MIF has generally been considered a pro-inflammatory cytokine and disease-exacerbating factor in numerous inflammatory and autoimmune conditions as well as cancer. However, there are exceptions to this general rule and the role of MIF in cardiovascular disease has turned out to be complex. Owing to its potent CXCR2/4-based leukocyte recruitment and pro-inflammatory capacity, MIF markedly promotes atherosclerosis through enhancing atherogenic monocyte and T-cell infiltration and by sparking plaque inflammation and destabilization. This has been unanimously shown in numerous experimental atherosclerosis models and is supported by correlations of circulating MIF protein and MIF promoter polymorphism with clinical outcome in human atherosclerotic disease (as e.g. summarized in (12, 13)).

In contrast, the local role of MIF in the ischemic heart and its contribution to myocardial I/R is complex. Studies in mouse models of brief (15-30 min) myocardial ischemia followed by a brief (up to 4 h) period of reperfusion demonstrated a marked cardioprotective role for MIF in myocardial I/R. This effect is intriguing and initially appeared surprising given the overall pro-inflammatory capacity of MIF. However, it was unraveled to be driven by a specific cardioprotective metabolic effect of MIF on I/R-stressed cardiomyocytes that is mediated by the CD74/AMP kinase (AMPK) signaling pathway, and which is further amplified by a MIF redox mechanism (12, 14-17). In line with these findings, pharmacologic augmentation of MIF

activity enhances the cardioprotective capacity of MIF in a murine myocardial I/R model (18). Augmentation is based on the therapeutic administration of a small molecule compound termed MIF20 that binds to the conserved N-terminal cavity of MIF, invoking a conformational change. This enhances MIF binding activity to CD74, which in turn leads to an increase in cardioprotective CD74/AMPK signaling in cardiomyocytes (18). Subsequent studies then indicated a more complex role of MIF in cardiac ischemia. In settings of longer ischemic episodes and towards later time intervals after the onset of reperfusion, the CXC chemokine receptor-mediated inflammatory activity of MIF appears to prevail with cardioprotection seen in *Mif*$^{-/-}$ mice (19, 20). This latter notion is in line with clinical data suggesting a correlation between high admission MIF levels in STEMI patients and adverse outcomes (21) as well as high MIF, inflammation markers, and unstable IHD.

Current knowledge on the complex role of MIF in atherosclerotic heart disease including clinical myocardial I/R is probably best reconciled by the following mechanistic model: i) cardiac MIF released in the ischemic or early reperfusion phase exerts a potent but temporal cardioprotective effect that is mediated by cardiomyocyte CD74/AMPK signaling and MIF redox effects ("1st wave cardioprotective MIF"); ii) as ischemia becomes more profound and inflammatory infiltration into the heart occurs in later reperfusion phases, inflammatory MIF, produced both locally in the heart and by infiltrating monocytes takes over with a prevalence of MIF-triggered CXCR2/4 pathways ("2nd wave inflammatory MIF"). How this could translate into specific clinical strategies for IHD patients has remained unanswered.

This is where the current paper by Ruze et al. may come into play. In demonstrating that *Mif* deficiency abrogated the protective effect of RIPC on myocardial injury and dysfunction, while blunting the increase in signaling through the cardioprotective "reperfusion injury salvage kinase" (RISK) and AMPK pathways and improving cardiomyocyte glucose uptake, they identified a possibility of how the cardioprotective potential of MIF could be made clinically accessible. Although currently limited to a preclinical mouse model, their data might imply that MIF mimics RIPC-based cardioprotection. In fact, specific molecular strategies that

mimic RIPC protection might bear a potential to “replace” the rather crude I/R conditioning cycles that may lead to a broad activation of numerous factors. This has been associated with unfavorable side effects due to the ischemia *per se* or the repetitive clamping, ranging from embolic risks to complications during open heart surgery in aged patients, respectively. The extensive margin of RIPC-based reduction in infarct size of >30% that is fully abolished in the absence of MIF, suggests that MIF could be a critical cardioprotective factor released in RIPC. However, MIF is not the first molecular entity that has been associated with RIPC. Several blood-borne factor(s) as well as neural pathways have been suggested to constitute the “remote signal” conveying cardioprotection in RIPC. Vicencio and colleagues demonstrated that exosomes deliver protective signals to the myocardium via TLR4 (22) and Davidson et al. showed that RIPC stimulates the release of SDF-1α/CXCL12, a chemokine that shares with MIF the receptor CXCR4. They found that CXCL12 at least partially accounts for the cardioprotective effect of RIPC in a rat model. Moreover, the concept of MIF as a RIPC mediator is not entirely new. An *in vitro* study by Goetzenich and colleagues suggested a role for MIF in anesthetic-induced myocardial preconditioning (AIPC) (23), a phenomenon following a comparable mechanistic principle. Moreover, a previous RIPC study has raised doubts whether MIF-based strategies may have translational potential, as exogenously administered recombinant MIF did not elicit a cardioprotective effect in a Langendorff-perfused heart, when administered before the ischemic insult or at reperfusion (24).

In contrast, using a *Mif* gene knockout approach, the current study by Ruze et al. has directly addressed the role of “endogenous” MIF and found a clear-cut role of MIF in cardiac RIPC (9). In combination, these two studies show a role for MIF in cardiac RIPC, while emphasizing the inherent complexities of using recombinant protein technology. To this end, the MIF protein has been suggested to be prone to redox-modulation, an effect that may make it more difficult to control protein activity in hypoxia/hyperoxia-based experimental set-ups.

Several questions have remained open. How and from which cell sources is MIF released? Do MIF-based approaches represent viable strategies to achieve molecule-specific

cardioprotective conditioning? This and questions related to the mechanism(s) of how MIF may control the critical RISK and SAFE pathways will have to be addressed to eventually translate the findings by Ruze et al. into clinical settings. The above-mentioned pharmacologic augmentation strategy for MIF employing MIF20 (18) or similar compounds and approaches may offer an intriguing solution to at least some of these issues. While clinical applications of MIF20 are subject to future studies, it is appealing to predict that a small molecule-based MIF augmentation strategy could serve to amplify or improve the cardioprotective potential of endogenous MIF in RIPC.

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