

Role of fibrinogen in the attenuation of ischemia reperfusion and remote ischemic preconditioning

El papel del fibrinógeno en la atenuación de la isquemia-reperfusión y el precondicionamiento isquémico remoto

Mohamed S. A. Mohamed*

Department of General Medicine, MSAM For Medical Consultations, Neumünster, Germany

Abstract

Background: Ischemic reperfusion injury (IRI) is a common hazard involved in many human diseases, such as cerebral stroke, myocardial infarction, solid organ transplant dysfunction or failure, and vascular diseases. Understanding the molecular bases of this injury is essential for the prevention and control of these life-threatening conditions. Ischemic and remote ischemic preconditioning techniques (IPC and RIPC, respectively) have gained increasing importance in the clinical practice to protect against the IRI; however, the exact mechanisms of these techniques are not fully understood, which renders their clinical application query. **Possible effectors:** Nitric oxide (NO) has been reported by multiple studies to be an important mediator of the protective effects of those techniques. While the physiological concentrations of NO and fibrinogen (FB) are known to antagonize each other, the circulating levels of both effectors increase in response to RIPC. **Hypothesis:** While NO has potential anti-inflammatory effects, non-soluble fibrinogen (sFB) shows pro-inflammatory effects. However, the sFB may have the potential to act synergistically rather than antagonistically with NO toward the attenuation of the IRI. **Conclusion:** While increased FB is considered a risk factor for cardiovascular and inflammatory conditions that is also able to decrease the efflux of NO, and increase the NO oxidative metabolites and S-nitroglutathione, the increased sFB during the acute phase reaction might have other protective aspects that should be carefully investigated.

Keywords: Ischemic reperfusion injury. Fibrinogen. Nitric oxide. Ischemic conditioning. Solid organ transplant. Inflammation.

Resumen

Antecedentes: La lesión por isquemia-reperfusión (LIR) es un riesgo común involucrado en muchas enfermedades humanas tales como derrame cerebral, infarto del miocardio, disfunción o falla de trasplante de órgano sólido, y enfermedades vasculares. Una comprensión de la base molecular de esta lesión es fundamental para la prevención y el control de estas enfermedades potencialmente mortales. Las técnicas de precondicionamiento isquémico y precondicionamiento isquémico remoto (PIR) han cobrado una creciente importancia en la práctica clínica para la protección contra la LIR, sin embargo, los mecanismos precisos de estas técnicas no se entienden plenamente, lo cual pone en duda su aplicación clínica. **Posibles efectores:** El óxido nítrico (ON) ha sido reportado por varios estudios como un importante mediador de los efectos

Correspondence:

*Mohamed S. A. Mohamed

E-mail: mohammed.shehatta1@gmail.com

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protectores de estas técnicas. Si bien se sabe que las concentraciones fisiológicas del ON y fibrinógeno son antagónicas, los niveles circulantes de ambos efectores aumentan en respuesta al PIR. **Hipótesis:** Aunque el ON tiene posibles efectos anti-inflamatorios, el fibrinógeno insoluble muestra efectos proinflamatorios. Sin embargo, el fibrinógeno soluble puede tener el potencial de actuar de manera sinérgica en lugar de antagónica con el ON hacia la atenuación de la LIR. **Conclusión:** Aunque el fibrinógeno elevado se considera un factor de riesgo para las enfermedades cardiovasculares e inflamatorias, que también puede disminuir la descarga de ON y aumentar los niveles de metabolitos oxidantes del ON y de S-nitrosoglutatión, el aumento de fibrinógeno soluble durante la reacción de fase aguda puede tener otros aspectos protectores que deben ser cuidadosamente investigados.

Palabras clave: Lesión por isquemia-reperusión. Fibrinógeno. Óxido nítrico. Acondicionamiento isquémico. Trasplante de órgano sólido. Inflamación.

Introduction

Fibrinogen (FB) is a glycoprotein, which is a hexamer, containing two sets of three different chains (α , β , and γ), linked to each other by disulfide bonds. FB plays an important role in coagulation cascade, where it can form bridges between platelets, by binding to their GpIIb/IIIa surface membrane proteins, in addition to the major role, where prothrombin is converted into thrombin, which then converts the soluble FB (sFB) into insoluble fibrin strands that are then cross-linked by factor XIII to form the blood clot¹.

Role of FB in the pathogenesis of diseases

FB plays a significant role in the pathogenesis of many diseases, mainly the diseases of cardiovascular and inflammatory backgrounds.

Role of FB in cardiovascular diseases

There is a confirmed agreement that FB is an important contributor to the cardiovascular events, including myocardial infarction and cerebral stroke. In addition to the traditional cardiovascular risk factors, FB has been identified as an additional risk factor that could predict new events within 10 years². This may refer to the traditional role of FB in blood clotting and platelet aggregates formation.

Role of FB in inflammatory diseases

Many studies have confirmed FB as a pro-inflammatory effector. In addition to, its ability to stimulate the proliferation of B-lymphocytes, T-lymphocytes, and monocytes³, immobilized FB and fibrin have high affinity to macrophage antigen 1 (MAC-1) and can activate neutrophils and monocytes⁴⁻⁶. In neutrophils,

FB/MAC-1 interaction activates the NF- κ B pathway, which is an anti-apoptotic and inflammatory cytokine-inducing pathway⁴.

Ischemic and remote ischemic preconditioning (IPC and RIPC)

IPC is a technique, where the prior application of repeated short cycles of ischemia and reperfusion could attenuate the severity of the subsequent ischemic reperfusion injury (IRI).

RIPC describes the ability of the technique to function through distance. For example, the application of short, repetitive ischemia-reperfusion cycles of the limb would protect distant organs such as heart, kidney, brain, and liver during subsequent IRI. Both phenomena indicate the involvement of local, paracrine as well as remote, circulating mediators⁷. IPC showed ability to significantly reduce the DNA fragmentation and the apoptotic death of the myocytes, usually associated with IRI⁷.

During limb ischemia, the diminished flow and shearing stress are associated with inhibition of Na⁺/K⁺ ATPase and the inward driving K⁺ channels. This leads to increased Na⁺ influx and persistent membrane depolarization. The increased intracellular Na⁺ activates Na⁺/Ca⁺⁺ exchanger to let Na⁺ out and Ca⁺⁺ in. In addition, the inhibition of K⁺ channels results in the activation of T type Ca⁺⁺ channels, leading to increased Ca⁺⁺ influx into the endothelial cells. Increased intracellular Ca⁺⁺ activates Ca⁺⁺ - dependent endothelial nitric oxide synthase (eNOS), which results in increased Nitric oxide (NO) production^{8,9}.

Xanthine oxidoreductase (XOR) is a complex molybdoflavin protein, which catalyzes the terminal two reactions in purine degradation (hypoxanthine \rightarrow xanthine \rightarrow uric acid) in primates. In humans, hypoxia and the inflammatory cytokines TNF- α , interleukin 1-beta (IL-1 β), and IFN- γ induce XOR expression in vascular

endothelium, where it can be released into the circulation^{10,11}. XOR is transcribed as a single gene product in the xanthine dehydrogenase (XDH) form, in which the enzyme exists intracellular, where substrate-derived electrons reduce nicotine amide adenine dinucleotide (NAD⁺) to NADH. However, during ischemia and inflammation, reversible oxidation of critical cysteine residues (535 and 992) and/or limited proteolysis converts XDH to xanthine oxidase¹².

In the oxidase form, the affinity for oxygen is significantly enhanced, resulting in univalent and divalent electron transfer to O₂ generating O₂⁻ and hydrogen peroxide, respectively¹³. Moreover, the inhibition of ATP-sensitive potassium channels, and the persistence of cell membrane depolarization result in increased activity of NADPH oxidase, and dysfunction of mitochondrial respiration, leading to more increase in the production of the reactive oxygen species (ROS)^{7,9}. Increased production of both NO and ROS results in NO oxidation to produce nitrite (NO₂⁻).

Role of NO to protect the vascular endothelium during IRI

NO contributes to vascular homeostasis by inhibiting the smooth muscle contraction and growth, platelet aggregation, and leukocyte adhesion to the endothelium. NO acts through the stimulation of the soluble guanylate cyclase, which is a heterodimeric enzyme with subsequent formation of cyclic-GMP. Cyclic-GMP activates protein kinase G, which causes reuptake of Ca⁺⁺ and the opening of calcium activated potassium channels that play an important role in the protection against IRI^{14,15}.

Several studies documented the important role of NO in mediating the protective effect of IPC and RIPC. While the locally produced NO can exert its action in case of IPC, it can't be accused for RIPC protective effect because of its short blood half-life (≤ 2 milliseconds)¹⁶. However, it was observed that NO inhalation in human provides protection against IRIs, while being associated with a significant increase in the circulating levels of nitrite. In addition, NO₂⁻ showed the ability to protect against IRI, to exert cytoprotective effects, and to decrease the infarction size similar to NO¹⁷⁻²⁴. Moreover, it has recently been confirmed that the application of brachial artery RIPC results in the activation of eNOS and increased plasma NO₂⁻ levels²⁵.

In the heart, NO₂⁻ can be reduced to NO and N₂O₃ by myoglobin. In addition, mitochondrial amidoxime reducing components reduce NO₂⁻ to NO in various

tissues^{26,27}. NO and S-nitrosothiols formed from nitrite inhibit complex I of the respiratory chain during reperfusion. This attenuates the increased production of ROS in response to IRI, and indirectly improves the functionality of complex II^{28,29}.

In the vascular endothelium, at low concentrations, NO reacts with certain target proteins mainly through post-translational S-nitrosylation, thus regulating cell survival, smooth muscle tone and immune signaling³⁰. Nevertheless, NO reactions in the setting of apoptosis appear to be double-faced. The nitrosative stress, similar to the oxidative stress, can potentially trigger cell death processes such as DNA fragmentation and lipid oxidation³¹. However, it can also have a protective role involving nitrosation of caspases and Poly-ADPribose-Polymerase, leading to inhibition of apoptosis³².

Role of FB

On the contrary to the pro-inflammatory effects of immobilized FB/fibrin, sFB has the ability to inhibit lymphocytic antigen 1-dependent binding to ICAM-1 through a direct interaction with ICAM-1⁷, and to reduce IL8-activated neutrophils binding to ICAM-1-expressing cells, in addition to reducing the binding of neutrophils to TNF α -activated endothelium to 40%, under flow conditions³³.

In addition, the interaction between FB and α M β II-integrin receptor has been reported to result in the activation of Rap-1, Talin-1, and CaMKII signaling. Rap1 is activated by adenosine diphosphate, hyperosmotic and cold stresses, interleukin-1, adenosine, and TNF α , where its active form bind to GTP³⁴. The increased expression/activity of Rap-1 leads to NF- κ B induction, while Rap-1 depletion leads to NF- κ B decreased activity as Rap-1 is also important for the phosphorylation of p65 subunit of NF- κ B. Remarkably, similar to inhibiting NF- κ B, knockdown of Rap-1 sensitizes some cells to apoptosis³⁵⁻³⁷.

While Talin-1 interacts with Rap-1 and is essential for adhesion, migration, and phagocytosis, NF- κ B activity is enhanced by ROS, TNF α , and IL-1 β . Active NF- κ B turns on the expression of genes that keep the cell proliferating and protect the cell from the conditions that would otherwise initiate apoptosis. Defects in NF- κ B results in increased susceptibility to apoptosis leading to increased cell death. This is because NF- κ B regulates anti-apoptotic genes especially the TRAF1 and TRAF2 and, therefore, checks the activities of the caspase family of enzymes, which are central to most apoptotic processes^{38,39}. In conclusion, FB has the

potential to play an anti-apoptotic role, especially during increased ROS production and inflammation.

CaMKII is activated by Ca⁺⁺ and phosphorylation of its Thr287. Its inactivation is achieved through the phosphorylation of Thr 306/307. Independently of Ca⁺⁺, it can also be activated by ROS through O-linked glycosylation of Ser280, by O-linked N-acetylglucosamine and through nitrosylation of Cys116, Cys273, or Cys290. When activated, it activates L Type Ca⁺⁺ channels, slows the inactivation of Na⁺ channel and activates K⁺ channels⁹. The activation of K⁺ channels has been strongly suggested to be a key protective mechanism against IRI.

Taken together, NO and the circulating nitrite, which can be reduced to NO, have been reported to be involved in mediating the action of the IPC and RIPC techniques, and have potential antiapoptotic activities. However, according to the above discussed findings, the activation of Rap1-NF-κB and CaMKII signaling, by sFB during inflammation (acute phase), has potential anti-inflammatory and anti-apoptotic activities, in addition to the ability to activate K⁺ channels, which are protective against IRI.

The interaction between NO and FB

While the vascular endothelial uptake of FB was reported to increase by the inhibition of NO, this effect seems to be related to the secondary induced hypertension as the effect was not reproducible when the inhibition of NO did not alter the normotensive status⁴⁰. In addition, the exposure of FB to ProlinONOate, a donor of NO, affects the structural formation of the fibrin clot, resulting in lower density, but thicker fibers⁴¹. Such structural variations can have significant impact on embolization and fibrinolysis, where the platelets can retract low density more than high density fibrin fibres⁴². On the other side, FB has been reported to decrease the efflux of NO from the erythrocytes, and increase the NO oxidative metabolites and S-nitroglutathione⁴³.

Conclusion

While FB is a risk factor for cardiovascular and inflammatory diseases that is also able to decrease the efflux of NO, and increase the NO oxidative metabolites and S-nitroglutathione, the increased sFB during the acute phase reaction might have other protective aspects that should be carefully investigated. Determining the exact signaling and functions of sFB, and the

incorporated functional domains responsible for various actions, has the potential to open the gate for new pharmacological innovations to protect and or treat certain vascular and inflammatory diseases.

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Conflicts of interest

The author declares no conflicts of interest.

Ethical disclosures

Protection of human and animal subjects. The authors declare that no experiments were performed on humans or animals for this study.

Confidentiality of data. The authors declare that no patient data appear in this article.

Right to privacy and informed consent. The authors declare that no patient data appear in this article.

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