Beta 3-Adrenergic Receptor (β3-AR) Activation at the End of Sustained Ischemia and / or Early Reperfusion may Prove to be a Valuable Cardioprotective Strategy

Ruduwaan Salie1*, Erna Marais2, Amanda Lochner2

1South African Medical Research Council, Biomedical Research and Innovation Platform, Cape Town Western Cape, South Africa
2Stellenbosch University, Faculty of Medicine and Health Sciences, Division of Medical Physiology, Centre for Cardio-metabolic Research in Africa (CARMA), Cape Town, Western Cape, South Africa
*Correspondence should be addressed to Dr. Ruduwaan Salie, Ruduwaan.salie@mrc.ac.za

Received date: August 01, 2022, Accepted date: September 09, 2022

Citation: Salie R, Marais E, Lochner A. Beta 3-Adrenergic Receptor (β3-AR) Activation at the End of Sustained Ischemia and / or Early Reperfusion may Prove to be a Valuable Cardioprotective Strategy. J Clin Cardiol. 2022;3(2):47-50.

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Keywords: Myocardial ischemia; Reperfusion injury; Cardioprotection; β3-AR modulation

Introduction

The contribution of β3-AR activation towards ischemia-reperfusion (IR) damage largely depends on the disease stage, severity, experimental model as well as drug specificities which should be considered when investigating β3-AR pharmacology for potential therapeutic applications. These conceptions largely contribute to the discrepancies of the subsequent role of β3-AR activation in the cardiovascular disease process. The β3-AR delivers a sustained intracellular signal because of its resistance to short term agonist promoted desensitization, making this receptor an ideal target for therapeutic intervention; in this manner protecting the heart from catecholamine overstimulation.

Previous mechanical and /or pharmacological interventions applied at the end of ischemia or at the onset of reperfusion had varied cardioprotective outcomes. However, the current communication highlights the importance of the cumulative effect of transient β3-AR stimulation with selective agonist, BRL 37344 at the end stage of ischemia [BRL (PerT)] and continued BRL treatment at the onset of reperfusion [BRL (PostT)]. This undoubtedly illustrate the significance of the end stage of ischemia as well as the onset of reperfusion in the concept of ischemia-reperfusion damage and the importance of the application of cardioprotective interventions at these time periods. Subsequently, if cardioprotective regimens are initiated during the late phase of ischemia and notably continued into early reperfusion, it is likely that they will enhance protection, especially with longer durations of ischemia.

Cardioprotection not Limited to Ischemia or Reperfusion

The historical viewpoint of ischemia-reperfusion (IR) damage highlighted in the review of Turer and Hill, 2010 [1] emphasize the crucial observation of Jennings and colleagues, 1960 [2] that reperfusion following ischemia was associated with myocardial injury. In this milieu, reperfusion is additive to that component of cell death due to the ischemic event itself; on the other hand, reperfusion is an essential element of cardioprotection [3].

Since, ischemia as well as reperfusion forms part of the phenomenon of myocardial ischemia–reperfusion injury (IRI), the application of any cardioprotective strategy cannot be limited to either ischemia or reperfusion. For example, if drugs protecting against ischemic injury are administered after coronary occlusion in ST-segment elevation myocardial infarction (STEMI) patients, they may fail to penetrate severely ischemic areas with reduced or no collateral flow [4]. However, the application of antiplatelet therapy or dual antiplatelet therapy [5], confers greater antithrombotic efficacy and may result in moderately reperfused infarcts. Ideally, it would be better to apply any cardioprotective intervention as soon as possible, i.e. during ischemia that would guarantee adequate myocardial levels of the drug at the onset of reperfusion. Previous studies have investigated...
the capability of cardioprotective interventions or drugs administered at the onset of reperfusion to reduce infarct size [6,7] and it is established that transient β-adenrenergic activation with isoproterenol, formoterol or dobutamine before acute myocardial ischemia (AMI), elicits cardioprotection against subsequent ischemia [8,9]. However, many of these interventions rarely translate clinically, mainly due to variable clinical scenarios in patients, such as age, comorbidities, and co-treatments or therapies [10]. Subsequently, research of cardioprotective interventions should be aimed at more than a single cardioprotective signaling cascade but rather the convergence of cardioprotective signaling events.

**β3-AR a potential target in the treatment of AMI and / or heart disease**

In the past, the emphasis was largely on the β1- and β2-adrenergic receptors but of late the β3-AR has been explored as a potential target in the treatment of AMI and / or heart disease, which highlights a possible cardioprotective role of β3-AR activation in reperfusion injury. The β3-AR is identified as the third isoform of human β-AR [11] and is recognized for its role in smooth muscle cell relaxation in the bladder, gastrointestinal and urinary tract [12,13]. It is documented to be involved in cardiovascular control, due to its expression in vascular endothelial cells [14,15] as well as atrial and ventricular cardiac myocytes [16]. Normally in myocardial tissue, the β1-AR (80%) and β2-AR (17%) are more abundantly expressed compared to the low levels of β3-AR (3%) [17]. Notably, the percentage of β3-AR is increased at high catecholamine levels in myocardial ischemia or heart failure [16], which produces negative inotropic effects opposite to that of β1-and β2-AR and subsequent loss of function which may hold true, particularly during the end-stage heart failure.

β3-AR activates the conventional NOS/NO/cGMP pathway and subsequently attenuate residual influences of β1/β2-AR on contractility and remodeling [18]. The β3-AR lacks serine and threonine residues sequences on the third intracellular loop and C-terminal tail targeted by G protein-coupled receptor kinases (GPCR kinases) or cAMP-dependent protein kinase (PKA) phosphorylation making this receptor resistant to agonist-induced desensitization [19]. It was documented that longer periods of ischemia accompanies increased sympathetic drive and excessive catecholamine release into the extracellular space of the myocardium which peaks at the onset of reperfusion [20], setting the stage for the up-regulation of the β3-AR population [21] and a synchronized β1- and β2-ARs down-regulation [17]. However, the contribution of β3-AR activation towards cardiac damage in myocardial ischemia or heart failure largely depends on the disease stage, severity, experimental model, drug specificities as well as duration of drug exposure, which should be considered when investigating β3-AR pharmacology for potential therapeutic applications. These conceptions may largely contribute to the discrepancies of the subsequent role of β3-AR activation in the cardiovascular disease process and cardioprotection.

**β3-AR Modulation at Various Stages of Ischemia / Reperfusion**

This was evidently illustrated, using either an isolated rat heart model of acute myocardial ischemia / reperfusion, which allows for definitive evaluation of a specific mechanism / signaling pathway in the heart without systemic interference, or an in vivo swine model of ischemia-reperfusion. The application of β3-AR agonist (BRL37344, 1 µM) before ischemia as a pre-treatment (PreT), or at the end stage of ischemia, labelled as per-treatment (PerT), or at the onset of reperfusion as a post-treatment (PostT), all of which reduced infarct size of isolated rat hearts [28]. However, this cardioprotective effect could not be reproduced in a in vivo swine model of ischemia-reperfusion with mirabegron, an β3-AR agonist with high specificity for the human β3-AR. The drug was applied only near the end stage of ischemia before reperfusion and did not improve left ventricular (LV) functional recovery or reduce infarct size after 7 or 45 days post-IR [22]. In the in vivo swine heart failure model, continuously high systemic catecholamine levels from cardiac postganglionic fibers followed by high systemic catecholamine levels may result in unremitting β3-AR activation, which may ultimately culminate in dilated cardiomyopathy, remodeling and fibrosis and perhaps transient β3-AR inhibition should have been considered at the onset of reperfusion. Particularly, when it was reported that sustained β3-AR activation in heart failure (HF) could contribute to impaired cardiac function due to its cardiodepressant effect [23]. By contrast with this hypothesis is the β3-AR /NO-cGMP/ PKG signaling axis, which seems to be a robust cardioprotective mechanism that can be beneficial in failing myocardium [16,24]. However, whichever befitting premise for β3-AR mediated cardioprotection would be appropriate; It is well established that the β3-AR delivers a sustained intracellular signal [25] because of its resistance to short term agonist promoted desensitization [19], making this receptor an ideal target for therapeutic intervention and in this manner protecting the heart from catecholamine overstimulation [9].

**β3-AR Mediated Cardioprotective Signaling Pathways**

**BRL (PreT) and BRL (PerT)**

Though, in the isolated rat heart model of acute myocardial ischemia / reperfusion, cardioprotection was achieved with β3-AR modulation at various stages of ischemia / reperfusion protocol; the correlation between infarct size reduction and activation of cardioprotective signaling pathways were not always coherent [13]. For example, the cardioprotective effect of β3-AR stimulation with selective β3-AR agonist BRL 37344, applied before [BRL (PreT)] or during [BRL (PerT)] ischemia were achieved without significant activation of eNOS, ERKp44/ p42 or GSK-3β phosphorylation but significantly increased cGMP levels and PKB/Akt activation. This may be due to partial activation of the Reperfusion injury salvage kinase (RISK)
pathway, namely PKB/Akt and not ERKp44/p42, both of which features prominently in the cardioprotection of ischemic preconditioning [26]. In this instance, it can also be speculated that the absence of eNOS activation with BRL (PreT) or BRL (PerT) may be due to the lack of significant ERKp44/p42 activation, since similar findings shows the involvement of ERKp44/p42 in the regulation of eNOS at Ser602 in bovine aortic endothelial cells (BAECs) [27-29] as well as the role of ERKp44/p42 and p38 signaling pathways in eNOS activation [14].

The involvement of atrial natriuretic peptide (ANP) should also be considered, since it was found that the application of ANP at reperfusion protected against I/R injury [30,31] and exerted antiapoptotic effects in rat cardiomyocytes through cGMP-PKG by inducing PI3K/Akt signaling. Similarly, it can be ventured to say that the cardioprotection achieved with BRL (PreT) or BRL (PerT) may have had an element of ANP activation, subsequent PKB/Akt activation and accompanying increased cGMP levels. However, this idea and the way cardioprotection was achieved in the absence of GSK-3β phosphorylation remain to be explored.

**BRL (PostT) and BRL (PerT+PostT)**

On the other hand, β3-AR stimulation at the beginning of reperfusion, BRL (PostT), significantly reduced infarct size, marginally increased eNOS activation, significantly increased cGMP levels, PKB/Akt as well as ERKp44/p42 phosphorylation. Furthermore, combining BRL (PostT) with BRL (PerT) in the experimental group BRL (PerT+PostT), followed the conventional β3-AR/NOS/NO/cGMP signaling cascade along with significant PKB/Akt, ERKp44/p42 as well as GSK-3β phosphorylation [13].

**BRL (PerT+PostT) a merger of cardioprotective signaling events**

Although basic research provided the theoretical framework for and has identified many targets for ischemia-reperfusion therapy, the application of these targets to human disease are often disappointing in large scale clinical trials due to profound disparities between laboratory-based animal studies and human disease. The clinical consequences of IR injury are numerous and may include myocardial stunning, arrhythmias and no-reflow that could lead to further extended periods of secondary ischemia and infarction [32], which should be kept in mind when considering any cardioprotective strategy to curtail IR cardiac damage. In the past numerous mechanical and/or pharmacological cardioprotective interventions were applied at the end of ischemia or at the onset of reperfusion, which had largely diverse outcomes. Notably, cardioprotection was achieved with β3-AR activation at different stages of the perfusion protocol, namely BRL (PreT), BRL (PerT) or BRL (PostT). However, combining transient β3-AR activation with BRL at end stage of ischemia with BRL at the onset reperfusion (BRL (PerT+PostT)), undoubtedly illustrate the significance of the end stage of ischemia as well as the onset of reperfusion in the concept of ischemia-reperfusion damage. Importantly, this strategy (BRL (PerT+PostT)) may have clinical significance, since the convergence of cardioprotective signaling events as well as inhibition of lethal cell death pathways during these time periods signifies the importance of cardioprotective interventions at these stages of IR. Subsequently, if cardioprotective treatments are initiated during the late phase of ischemia and continued into early reperfusion, it is likely that they will enhance protection, especially with longer durations of ischemia.

**Ethics Approval and Consent to Participate**

Not applicable.

**Consent for Publication**

All authors read and approved the final manuscript for submission to *The Journal of Clinical Cardiology*.

**Available Data and Materials**

Not applicable.

**Competing Interests**

We the authors of this manuscript, declare that we have no conflict of interest.

**Funding**

Not applicable

**Author Contributions**

The first draft of the manuscript was written by Dr Ruduwaan Salie and all co-authors commented and agreed on previous versions of the manuscript.

**Acknowledgements**

Not applicable.

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