Beta3-Adrenergic Receptor (β3-AR) Modulation Prior to and / or at the end of Sustained Ischaemia and / or Early Reperfusion May Prove to be a Valuable Cardioprotective Strategy

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Beta3-Adrenergic Receptor (β3-AR) modulation prior to and / or at the end of sustained ischaemia and / or early reperfusion may prove to be a valuable cardioprotective strategy

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Abstract

β3-AR activation contributes partly or may be solely responsible for ensuing cardiac damage in myocardial ischaemia or heart failure. This would largely depend on 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 and in this manner protecting the heart from catecholamine overstimulation. The current communication highlights the importance of the cumulative effect of BRL (PerT) treatment, at the end stage of ischaemia as well as BRL (PostT) treatment, at the onset of reperfusion. This undoubtedly illustrate the significance of the end stage of ischaemia as well as the onset of reperfusion in the concept of ischaemia-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 ischaemia and continued into early reperfusion, it is likely that they will enhance protection, especially with longer durations of ischaemia.

The historical viewpoint of ischaemia-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 ischaemia was associated with myocardial injury. In this milieu, reperfusion is additive to that component of cell death due to the ischaemic event itself; on the other hand, reperfusion is an essential element of cardioprotection [3]. Previous studies have investigated the capability of cardioprotective interventions or drugs administered at the onset of reperfusion to reduce infarct size [4, 5] and it is established that transient β-adrenergic activation with isoproterenol, formoterol or dobutamine before acute myocardial ischaemia (AMI), elicits cardioprotection against subsequent ischaemia [6, 7]. In this context, 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 acute myocardial infarction (AIM) 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 [8] and is recognized for its role in smooth muscle cell relaxation in the bladder, gastrointestinal and urinary tract [9, 10]. It is documented to be involved in cardiovascular control, due to its expression in vascular endothelial cells [11, 12] atrial and ventricular cardiac myocytes [13]. Normally in myocardial tissue, the β1 (80%) and β2-AR (17%) are more abundantly expressed compared to the low levels of β3-AR (3%) [14]. However, the percentage of β3-AR is increased in myocardial ischaemia or heart failure [13], activates the conventional eNOS/NO/cGMP pathway which decreases residual β1/β2-AR effects on contractility and subsequently protecting the heart from β1-AR overstimulation [15]. 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 [16]. It is important to note whether subsequent activation of the β3-AR contribute partly or solely responsible for the ensuing cardiac damage. This would largely depend on 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 may largely contribute to the inconsistencies of the subsequent role of β3-AR activation in the cardiovascular disease process.

This was evidently illustrated when the β3-AR agonist (BRL37344, 1 µM) was administered before ischaemia as a pre-treatment (PreT) or at the end stage of ischaemia, labelled as per-treatment (PerT) or at the onset of reperfusion as a post-treatment (PostT), all of which reduced infarct size in isolated rat hearts [17]. However, this cardioprotective effect could not be reproduced with mirabegron, an β3-AR agonist with high specificity for the human β3-AR in a swine model of ischaemia-reperfusion in-vivo; when applied in the same context near the end stage of ischaemia before reperfusion, did not improve LV functional recovery or reduce infarct size after 7 or 45 days post-IR [18]. In the in-vivo swine heart failure model, continuously high systemic catecholamine levels may result in unremitting β3-AR activation, which will ultimately culminate in dilated cardiomyopathy, remodeling and fibrosis and perhaps transient β3-AR inhibition should have been considered at the onset of reperfusion. This was clearly illustrated when BRL (PerT) was followed by β3-AR inhibition with SR59230A (SR) at the onset of reperfusion in the BRL (PerT)+SR (PostT) treatment group, which was associated with reduce infarct size, significant extra cellular regulated kinase (ERKp44/p42), phosphatidylinositol 3-kinase-protein kinase B (PKB/Akt), endothelial nitric oxide synthase (eNOS) as well as GSK-3β phosphorylation [17]. Contrary to the in-vivo swine heart failure model, the isolated working rat heart model is a well-regulated model of myocardial ischaemia-reperfusion with adequate nerve supply, independent of overall extraneuronal catecholamine release or central sympathetic drive [19].

However, increased catecholamine levels in the event of ischaemia [20], results in the up-regulation of the β3-AR population and a synchronized β1- and β2-ARs down-regulation. In this setting the β3-AR delivers a sustained intracellular signal [21] because of its resistance to short term agonist promoted desensitization [16], making this receptor an ideal target for therapeutic intervention and in this manner protecting the heart from catecholamine overstimulation [22]. Subsequently, β3-AR modulation prior to and/or at the end of sustained ischaemia and/or early reperfusion may prove to be a valuable cardioprotective strategy [17]. Even though cardioprotection was achieved in this context with transient β3-AR activation, the correlation between infarct size reduction and activation of cardioprotective signaling pathways were not always comprehensible [17]. For example, the cardioprotective effect of β3-AR stimulation with selective β3-AR agonist BRL37344, applied as BRL (PreT), BRL (PerT) or at the end stage of ischaemia as BRL (PostT), 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 [23]. 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) [24] as well as the role of ERKp44/p42 and p38 signalling pathways in eNOS activation [25].
The possible connection between elevated cGMP levels and PKB/Akt activation found with the cardioprotection of BRL (PreT) or BRL (PerT) [17], may be consistent with an eNOS-dependent pathway where cGMP levels, a downstream effector of NO are elevated in smooth muscle cells in which constitutively-active Akt (myr-Akt) is expressed [26]. 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 [27] 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.

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. However, combining BRL (PostT) with BRL (PerT) in the experimental group BRL (PerT+PostT), followed the conventional β3-AR/eNOS/NO/cGMP signaling cascade and produced a synergistic effect in terms of cardioprotection, significant PKB/Akt, ERKp44/p42 as well as GSK-3β phosphorylation [17]. The addition of BRL (PostT) at the onset of reperfusion to BRL (PerT) at the end stage of ischaemia, undoubtedly illustrate the significance of the end stage of ischaemia as well as the onset of reperfusion in the concept of ischaemia-reperfusion damage and importance of the application of cardioprotective interventions at these time periods. Subsequently, if cardioprotective regimens are initiated during the late phase of ischaemia and continued into early reperfusion, it is likely that they will enhance protection, especially with longer durations of ischaemia.

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**Conflict of interest to disclosure**

All co-authors have read and approved the manuscript for submission to the journal of Cardiovascular Drugs and Therapy. We the authors of this manuscript, declare that we have no conflict of interest.

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**Author contributions**

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Experimental Animal Ethics
The handling of laboratory animals (male Wistar rats) used in the previous study was in accordance to ethical guidelines as set out by the University of Stellenbosch, Faculty of Medicine and Health Sciences Ethics Committee and the South African National Standard for Care and Use of Animals for Scientific Purpose (SANS 588 10386: 2008).

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