Role of toll-like receptor 4 in melatonin-induced cardioprotection

Abstract: Melatonin protects the heart against myocardial ischemia/reperfusion injury via the activation of the survivor activating factor enhancement (SAFE) pathway which involves tumor necrosis factor alpha (TNFα) and the signal transducer and activator of transcription 3 (STAT3). Toll-like receptor 4 (TLR4) plays a crucial role in myocardial ischemia/reperfusion injury and activates TNFα. In this study, we investigated whether melatonin may target TLR4 to activate the SAFE pathway. Isolated hearts from rats or mice were subjected to ischemia/reperfusion injury. Melatonin (75 ng/L) and/or TAK242 (a specific inhibitor of TLR4 signaling, 500 nM) were administered to the rat hearts before the induction of ischemia. Pre-ischemic myocardial STAT3 was evaluated by Western blotting. Lipopolysaccharide (LPS, a stimulator of TLR4) was administered to wild-type, TNFα receptor 2 knockout or cardiomyocyte-specific STAT3-deficient mice (2.8 mg/kg, i.p) 45 min before the heart isolation. Myocardial infarct size was measured as an endpoint. Compared to the control, administration of melatonin reduced myocardial infarct size (34.7 ± 2.8% versus 62.6 ± 2.7%, P < 0.01). This protective effect was abolished in the presence of TAK242 (49.2 ± 6.5%). Melatonin administered alone increased the pre-ischemic activation of mitochondrial STAT3, and this effect was attenuated with TAK242. Furthermore, stimulation of TLR4 with LPS pretreatment to mice reduced myocardial infarct size of the hearts isolated from wild-type animals but failed to protect the hearts isolated from TNFα receptor 2-knockout mice or cardiomyocyte-specific STAT3-deficient mice (P < 0.001). Taken together, these data suggest that cardioprotection induced by melatonin is mediated by TLR4 to activate the SAFE pathway.

Introduction

The role of melatonin in health and disease has attracted many researchers with new findings being far from the expectations [1]. Since its original identification in the bovine pineal gland [2], it is now established that melatonin plays a crucial role in many physiological functions, including among others, antioxidant, immunomodulatory, and anti-inflammatory effects [3, 4]. Besides its pineal production, melatonin is also locally produced in other tissues where it exhibits various actions [5]. Most importantly, melatonin is also present in food such as cherries, cereals, grapes, meats, and wine [6, 7], supporting the potential implication of the dietary melatonin in health conditions [8].

Reduced serum melatonin levels are associated with elevated incidence of cardiovascular diseases in humans [9, 10]. Solid experimental evidence supports the potential benefits of melatonin supplementation in myocardial infarction, cardiomyopathy as well as hypertensive heart diseases [11] and pulmonary hypertension [12]. However, the mechanism of cardioprotection by melatonin is complex and not yet established [13]. At a cellular level, it involves receptor-independent activities including free radical scavenging actions and/or receptor-mediated activities such as signaling effects via membrane and nuclear melatonin receptors [13]. Cardioprotection induced by melatonin is associated with the activation of the survivor activating factor enhancement (SAFE) pathway [13–15], an important cardiac survival pathway which is initiated by tumor necrosis factor alpha (TNFα) binding to its receptor 2 (TNFR2) leading to the activation of the signal transducer and activator of transcription 3 (STAT3) [16]. Interestingly, chronic oral administration of dietary melatonin in rats and mice protects the heart against ex vivo and in vivo myocardial ischemia/reperfusion (I/R) injury via the activation of the SAFE pathway [17]. However, how melatonin activates the SAFE pathway is still unclear.

Toll-like receptor 4 (TLR4) is a transmembrane receptor protein that can be activated by lipopolysaccharide (LPS) [18]. It is highly expressed in the heart and upregulated during myocardial I/R injury [19]. As a receptor protein of the innate immune system, the stimulation of TLR4 results in an increased production of pro-inflammatory cytokines such as TNFα as well as anti-inflammatory cytokines [20]. It is well established that excessive stimulation of pro-inflammatory cytokines associated with increased oxidative stress is detrimental to the heart and, accordingly, inhibition of TLR4 confers cardioprotection [20]. Paradoxically, activation of TLR4 prior to ischemic insult
protects the heart against myocardial I/R damage [21–23]. In this context, it is not clear whether this TLR4-mediated cardioprotection occurs via the activation of TNFα and STAT3 [24, 25]. In addition, whether melatonin may target TLR4 to protect the heart remains unknown.

The aim of this study was therefore to investigate the role of TLR4 in melatonin-induced cardioprotection. We hypothesized that administration of melatonin prior to ischemic insult protects the heart against I/R injury by activating the TLR4 which, in turn, activates the SAFE pathway.

**Material and methods**

**Animals**

All animals were housed with free access to water and food, a 12-hr dark/light cycle (light from 6:00 a.m. to 6:00 p.m.) with temperature and humidity kept constant at 22°C and 40%, respectively. The experimental procedure was approved by the Animal Research Ethic Committee of the University of Cape Town (Ethical clearance no. 011/031). Animals were treated according to the revised South African National Standard (SANS) for the Care and Use of Animals for Scientific Purposes (South African Bureau of Standards, SANS 10386, 2008). This study used 61 rats and 48 mice.

**Isolated rat heart model**

Male Wistar rats (240–300 g) were anesthetized with sodium pentobarbitone (60 mg/kg, i.p.) and heparinized (200 IU i.v.). Hearts were rapidly removed and retrogradely perfused using the Langendorff technique at a constant pressure (100 cm) with oxygenated Krebs–Henseleit buffer [25]. After stabilization, hearts were exposed to one of four treatment protocols including control, melatonin (Sigma-Aldrich, St Louis, MO, USA), an inhibitor of TLR4 (TAK242, CLI-095; InvivoGen, San Diego, CA, USA) and melatonin with TAK242 (see Fig. 1A). The perfusion protocol for isolated perfused rat heart is represented in Fig. 1A.

After a 30-min stabilization period, hearts were subjected to 30 min of regional ischemia followed by 120 min of reperfusion. Hearts were perfused with melatonin at the physiological concentration of 75 ng/L for 15 min followed by a 10-min washout period before the ischemic insult, as previously described [15]. TAK242 (500 nM) was administered for 23 min followed by a 5-min washout period before ischemic insult. When TAK242 and melatonin were administered together, TAK242 was perfused for 3 min alone followed by 15-min perfusion with melatonin and 5 min on its own. Ischemia was induced by left anterior descending artery ligation [15]. Hemodynamic parameters including heart rate (HR, beat/min), left ventricular...
end diastolic pressure (LVEDP, mmHg), left ventricular end systolic pressure (LVESP, mmHg), and the coronary flow (mL/min) were monitored continuously. The left ventricular developed systolic pressure (LVDP, mmHg) was calculated as the difference between LVESP and LVEDP. At the end of the experiment, myocardial infarct and risk zone areas were assessed with blue Evans and 2,3,5 triphenyltetrazolium chloride (TTC) staining and determined, as previously reported [26]. Infarct size was expressed as a percentage of the risk zone area.

For Western blot analysis, heart tissues were collected after 7 or 10 min of melatonin or TAK242 administration, respectively; that is, at 18 min before the onset of ischemia (Fig. 1A) and a minimum of four hearts per group were used.

### Isolated mouse heart model

Mice (12–16 wk of age) were randomly divided into control and treatment groups. Male TNFR2 knockout (TNFR2−−/−) mice, cardiomyocyte-specific STAT3-deficient (STAT3−−/−) mice, and their respective wild type were used in this study. Mice were injected with a stimulator of TLR4, LPS (Sigma-Aldrich) (2.8 mg/kg, i.p.) or saline (pH 7.4) 45 min before heart perfusion. All mice were heparinized to eliminate clotting (200U) and anaesthetized with sodium pentobarbitone (60 mg/kg,i.p.) [27]. Hearts were removed, mounted on a Langendorff system, and perfused as previously described [27]. The perfusion protocol for isolated perfused mouse heart is represented in Fig. 1B. After a 30-min stabilization period, hearts were subjected to 35 min of global ischemia at 37°C followed by 45 min of reperfusion. Infarct size was assessed by TTC staining and determined with planimetry [27] and expressed as a percentage of the risk zone.

### Western blot analysis

Before the onset of ischemia (see Fig. 1A), hearts were freeze-clamped in liquid nitrogen and stored at −80°C until protein extraction was performed. Cytosolic and nuclear proteins were extracted as previously published [28, 29]. Mitochondrial proteins were extracted as described by Lewin et al. [30]. The protein content in each fraction was determined using the Lowry method [31], and samples were kept at −80°C until analysis.

Phosphorylated states of STAT3 (phospho-STAT3) at tyrosine (Tyr) 705 and serine (Ser) 727 residues as well as total levels of STAT3 (Tot-STAT3) were analyzed by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE), as previously described [24]. Membranes were incubated overnight with the appropriate primary antibody and exposed to the diluted horseradish peroxidase-labeled secondary antibody for 1 hr. Thereafter, proteins were detected with enhanced chemiluminescence (ECL) reagents (Amersham Life Science, Buckinghamshire, UK) and captured electronically using a Genegnome imager (Syngene, Cambridge, UK). Relative densitometry was determined using Quantity One analysis software (Bio-Rad, Hercules, CA, USA). Actin, lamin, and voltage-dependent anion channel (VDAC) expressions were used as loading control for cytosolic, nuclear, and mitochondrial fractions, respectively. Purity was verified by absence of lamin and VDAC in mitochondria and nuclear fractions, respectively. The protein expression levels were normalized to the levels of the respective loading control. STAT3 activation was expressed as the ratio of levels of phosphorylated proteins to their total protein levels performed on a separate membrane but in the same samples and under the same conditions. All antibodies were from Santa Cruz Biotechnology (Dallas, TX, USA).

### Statistical analysis

All data are presented as mean ± standard error of the mean (S.E.M.). Comparisons between multiple groups were performed by one-way ANOVA followed by the Dunnett’s post hoc test (Graph Pad Software Inc, San Diego, CA, USA). A value of $P < 0.05$ was considered as statistically significant.

### Results

In isolated rat hearts subjected to regional I/R injury, administration of melatonin and/or TAK242 did not affect the LVDP, heart rate, and coronary flow of the perfused hearts prior to ischemia, after 30 min of ischemia, at 30, 60, and 120 min of reperfusion compared to control (Table 1). Importantly, after 120 min of reperfusion, rat hearts perfused with melatonin alone had a 43% reduction in infarct size (melatonin: 34.7 ± 2.8% versus control: 62.6 ± 2.7%, $P < 0.01$) (Fig. 2). This beneficial effect was partially abolished in the hearts perfused with melatonin and TAK242 compared to control hearts (49.2 ± 6.5% versus 62.6 ± 2.7%, $P < 0.05$) (Fig. 2). TAK242 alone had no effect on infarct size (68.6 ± 5.3%) (Fig. 2).

To evaluate whether the role of SAFE pathway in TLR4-induced cardioprotection, LPS was administered to wild-type mice, TNFR2−−/− mice, or cardiomyocyte-specific STAT3−−/− mice, and isolated hearts subjected to global I/R injury. At the end of reperfusion, the isolated hearts from wild-type mice treated with LPS had smaller infarct size compared to their controls (16.5 ± 0.3% versus 51 ± 2.8% and 16.7 ± 0.5% versus 49.8 ± 4.2%, $P < 0.001$) (Fig. 3A,B). However, there was no significant difference between infarct size of the hearts isolated from TNFR2−−/− or cardiomyocyte-specific STAT3−−/− mice treated with LPS and their respective wild-type controls (Fig. 3A,B).

To further explore whether TLR4 is involved in melatonin-induced activation of the SAFE pathway, phosphorylation of STAT3 (Tyr705 or Ser727 residues) was evaluated in hearts tissues collected prior to ischemic insult. Administration of melatonin increased significantly the mitochondrial phosphorylation of STAT3 at Tyr705 but not at Ser727 residue while TAK242 with or without melatonin had no effect (Fig. 4). In addition, melatonin with or without TAK242 had no effect on the cytosolic phosphorylation of STAT3 (at both Ser727 and Tyr705 residues) (Fig. 5) while TAK242 reduced significantly the cytosolic phosphorylation of STAT3 at Ser727 residue (Fig. 5). Furthermore, nuclear localization of phosphorylated STAT3 (both Ser727 and Tyr705) (Figure S1) as well as total STAT3 expression in all cellular fractions was not affected by melatonin and/or TAK242 treatment.

TLR4 in melatonin-induced cardioprotection
Table 1. Effect of melatonin and TAK242 on myocardial function of isolated rat hearts subjected to regional ischemia/reperfusion

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Group</th>
<th>Pre-ischemia</th>
<th>Ischemia (30 min)</th>
<th>Reperfusion</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>5 min</td>
</tr>
<tr>
<td>LVDP (mmHg)</td>
<td>Control</td>
<td>89.73 ± 2.14</td>
<td>59.07 ± 5.58</td>
<td>77.15 ± 7.68</td>
</tr>
<tr>
<td></td>
<td>Melatonin</td>
<td>88.84 ± 1.71</td>
<td>48.8 ± 6.60</td>
<td>65.34 ± 13.33</td>
</tr>
<tr>
<td></td>
<td>TAK242</td>
<td>92.84 ± 6.94</td>
<td>55.84 ± 14.66</td>
<td>63.14 ± 16.52</td>
</tr>
<tr>
<td></td>
<td>Melatonin + TAK242</td>
<td>91.57 ± 4.95</td>
<td>55.39 ± 6.23</td>
<td>67.27 ± 13.87</td>
</tr>
<tr>
<td>Heart rate (beats/min)</td>
<td>Control</td>
<td>305.27 ± 6.43</td>
<td>272.62 ± 18.36</td>
<td>281.01 ± 18.93</td>
</tr>
<tr>
<td></td>
<td>Melatonin</td>
<td>305.54 ± 15.46</td>
<td>303.31 ± 22.22</td>
<td>250.56 ± 47.70</td>
</tr>
<tr>
<td></td>
<td>TAK242</td>
<td>307.84 ± 16.36</td>
<td>251.72 ± 65.34</td>
<td>262.82 ± 68.17</td>
</tr>
<tr>
<td></td>
<td>Melatonin + TAK242</td>
<td>300.57 ± 16.69</td>
<td>275.94 ± 25.44</td>
<td>209.66 ± 48.14</td>
</tr>
<tr>
<td>Coronary flow (mL/min)</td>
<td>Control</td>
<td>9.07 ± 0.32</td>
<td>5.3 ± 0.61</td>
<td>8.4 ± 0.77</td>
</tr>
<tr>
<td></td>
<td>Melatonin</td>
<td>10.29 ± 0.62</td>
<td>5.49 ± 0.58</td>
<td>6.97 ± 1.31</td>
</tr>
<tr>
<td></td>
<td>TAK242</td>
<td>9.6 ± 0.96</td>
<td>6.56 ± 1.50</td>
<td>7.2 ± 2.11</td>
</tr>
<tr>
<td></td>
<td>Melatonin + TAK242</td>
<td>10.11 ± 1.13</td>
<td>4.81 ± 1.17</td>
<td>5.83 ± 1.72</td>
</tr>
</tbody>
</table>

Parameters measured prior to ischemia and after ischemia at 5, 30, 60, and 120 min of reperfusion; LVDP, left ventricular developed pressure; n = control: 18, melatonin: 7, TAK242: 5, melatonin + TAK242: 7.

Discussion

Our aim was to investigate the role of TLR4 in melatonin-induced cardioprotection. The major findings in our study showed that: (i) administration of melatonin to isolated rat heart prior to ischemic insult increased mitochondrial STAT3 activation and protect the heart against myocardial I/R injury, (ii) co-administration of TLR4 inhibitor with melatonin attenuated the cardioprotective effect of melatonin and inhibited melatonin-induced STAT3 activation, and (iii) administration of LPS (TLR4 stimulator) protected the isolated hearts from wild-type mice but failed to protect the hearts from TNFR2−/− and STAT3−/− mice. Collectively, these data demonstrate for the first time to our knowledge that melatonin confers cardioprotection via the stimulation of TLR4 which, in turn, activates the SAFE pathway.

The protective effect of melatonin against myocardial I/R injury is well established [13]. In our setting, the amount of melatonin given directly to the heart was equivalent to a physiological nocturnal concentration of melatonin (plasma melatonin concentration in rats varies from 5 to 160 ng/L) [32]. An increase in melatonin levels in the blood can be obtained during the day via dietary ingestion of melatonin. Hence, moderate and chronic consumption of beer increases blood melatonin levels in humans within the physiological ranges (10–200 ng/L) [33]. Similarly,

Fig. 2. The effect of an acute administration of melatonin with or without TAK242 on infarct size of isolated rat hearts subjected to regional ischemia/reperfusion injury, Mel, melatonin; TAK, TAK242; **P < 0.01 (versus control); *P < 0.05 versus control; n: control: 18, melatonin: 7, TAK242: 5, melatonin + TAK242: 7.

Fig. 3. The effect of LPS on infarct size of isolated mouse hearts subjected to a global ischemia/reperfusion insult, (A) TNFR2−/−: tumour necrosis factor receptor 2 knockout, (B) STAT3−/−: signal transducer and activator of transcription 3 knockout, LPS: lipopolysaccharide; vehicle: saline, Mel, melatonin; ***,P < 0.001 (versus vehicle); n: 6 hearts per group.
consumption of walnuts increases blood melatonin levels from 11.5 ng/L to 38 ng/L in rats [34]. If the concentration of melatonin, as used in our setting, corresponds to the concentration of melatonin in wine (concentration in wine varies from 50 to 230 ng/L) [8, 35], we acknowledge that the concentration of melatonin given directly to the heart in our system may be higher than the amount of melatonin that the heart would have received after ingestion of wine. The cardioprotective effects of physiological and pharmacological doses of melatonin as well as the role of endogenous melatonin secretion in cardioprotection have been previously demonstrated [9, 11, 13]. As a small amphiphilic molecule, melatonin is able to enter in the cardiomyocyte and other cardiac cells and confer cardioprotection via its antioxidant activities [36]. Other mechanisms include anti-adrenergic [37], anti-inflammatory [38], and anti-excitatory [38] effects as well as inhibition of the mitochondrial permeability transition pore opening [39]. Importantly, the role of melatonin receptors was also demonstrated in cardioprotection by melatonin [37]. In this regard, administration of melatonin protects the heart via various intracellular signaling including, among others, the SAFE pathway and the RISK (reperfusion injury salvage kinases) pathway which involves the activation of protein kinase B (PKB), extracellular signal-regulated kinase (ERK1/2) during reperfusion [13]. The involvement of the SAFE pathway in cardioprotection by melatonin was previously demonstrated in our laboratory [15] and confirmed by others [13, 14, 40]. TNFα can activate two types of receptors, TNF receptors 1 and 2 (TNFR1 and TNFR2), but only TNFR2 is involved in the activation of the SAFE pathway [41]. Previous studies suggest that upon activation of TNFR2 [15], melatonin induces STAT3 nuclear translocation and subsequent transcriptional activities [13, 42]. Surprisingly, administration of melatonin in our setting did not affect the levels of
nuclear phosphorylated STAT3 in the present study. This may be due, in part, to the difference in study protocol such as the time point to collect heart tissue for Western blot analysis which may not have been optimal to detect phosphorylated STAT3. However, hearts perfused with melatonin had a significant increase in activation of mitochondrial STAT3 (Tyr705 residue), indicating the possible role of mitochondrial STAT3 in the observed cardioprotection. As potential mechanism of action, activation STAT3 may directly limit reactive oxygen species generation, induce anti-apoptotic capacity [14], inhibit the mitochondrial permeability transition pore opening [43], and improve mitochondrial function [44]. Today, there is no consensus regarding the exact site of mitochondrial STAT3 phosphorylation associated with improved mitochondrial function and cardioprotection. Previous reports in isolated mitochondria from the perfused hearts show that the site of STAT3 phosphorylation varies depending on the type of animal model as well as the study protocol [43, 45, 46]. Our data suggest that although phosphorylation of STAT3 at Ser727 residue appears to be more pronounced than Tyr705 residue, only Tyr705 residue was significantly increased in mitochondria isolated from melatonin-treated hearts.

Apart from its well-documented role in infectious disease, TLR4 is also a pro-inflammatory receptor in noninfectious disease processes such as I/R injury in several organs [20, 47, 48]. However, previous data on the role of TLR4 in cardioprotection are controversial, having both detrimental (for example, enhanced inflammation) [49, 50] or protective (for example, anti-apoptotic) [22, 51, 52] effects. The reason behind this controversy remains unclear, but it might be explained by differences in study protocols as well as animal strains leading to differences in TLR4 signaling. In the present study, we used TAK242, which is a selective inhibitor of TLR4 signaling [53]. Contrary to previous findings in rats [49] and mice [54], our data show that inhibition of TLR4 had no effect on myocardial function and infarct size of the isolated heart. We also used LPS, which is a well-known activator of TLR4 [18]. Consistent with the previous reports in mice heart [22], liver [55], brain [56], and kidney [57], stimulation of TLR4 with LPS pretreatment to wild-type mice induced tolerance to myocardial I/R injury (Fig. 3). This finding is supported by previous observation that LPS (low dose) induces cardiac preconditioning [22, 51, 52].

However, how LPS protects the heart against I/R damage is complex and not fully elucidated. It activates TLR4 and its downstream adaptor protein myeloid differentiation primary response gene 88 (MyD88) as well as the nuclear factor-κB (NF-κB) signaling pathway which results in the production of pro-inflammatory cytokines TNFα as well as anti-inflammatory cytokines (for more details, see [58]). It is well established that low concentration of TNFα (0.5 ng/mL) administered prior to ischemic insult protects the heart [24, 25]. Therefore, the lack of protection of isolated hearts from TNFR2−/− and STAT3−/− mice following LPS pretreatment may indicate the involvement of TNFα and STAT3 (SAFE pathway) in cardioprotection mediated by TLR4 stimulation. TLR4 was previously reported to mediate activation of STAT3 via the crosstalk between STAT3 and the mitogen-activated protein kinase (MAPK) signaling in bladder epithelial cells [59]. In isolated mouse heart model, LPS improves bone marrow-derived mesenchymal cells (MSCs)-mediated posts ischemic myocardial function via MyD88-dependent pathway and STAT3 activation [60]. However, it is unclear whether the cardioprotection induced with LPS results from the direct or systemic effect of LPS on the heart.

Co-administration of a TLR4 inhibitor with melatonin significantly attenuated cardioprotection. This observation suggests a crucial role of TLR4 signaling in melatonin-induced cardioprotection. Considering the pro-inflammatory effects of TLR4 stimulation [18], our finding is surprising in view of the well-known anti-inflammatory activities of melatonin [4]; however, it is supported by the current view of melatonin as an immune system buffer acting as a stimulant under basal or immunosuppressive conditions or as an anti-inflammatory compound in the presence of exacerbated immune responses (for review, see [61]). In apparent contradiction with our findings, studies carried out by Kang et al. [48, 62] showed that protection with melatonin of the liver subjected to I/R injury was associated with a decrease in hepatic TLR4 expression after 1 and 5 hr of reperfusion. However, it is important to note that in these studies: (i) melatonin was administered intraperitoneally in the rats and the concentration of melatonin may have been much higher than the concentration used in our system (ex vivo), (ii) melatonin was given both prior to ischemia and during the reperfusion period, and (iii) the authors did not demonstrate the causal role for TLR4 in the protection achieved by melatonin. Furthermore, our data suggest that the stimulation of TLR4 prior to ischemia is required for melatonin-induced cardioprotection and it is possible that TLR4 levels may also be downregulated at the time of reperfusion in our model. Hence, we have administered melatonin as a pharmacological preconditioning agent, and it is well known that most preconditioning stimuli lead to an upregulation of different signaling proteins prior to ischemia, but the same proteins are downregulated at the time of reperfusion (for review, see [16]). While melatonin or TLR4 stimulation prior to ischemic injury confers cardioprotection via the SAFE pathway, our data further show that administration of TLR4 inhibitor with melatonin abolished melatonin-induced mitochondrial STAT3 activation. As mentioned above, acute activation of myocardial STAT3 before the onset of ischemia plays a critical role in early phase of TNFα preconditioning [24]. In this context, the effect of melatonin on TNFα production levels, TLR4 protein expression, and the role of melatonin receptors are needed to better characterize the link between TLR4, melatonin, and the SAFE pathway.

In view of the present data, melatonin which is a regulatory molecule with immunomodulatory and anti-inflammatory activities [61] may be exploited as a new perspective for cardioprotection. However, more clinical studies are needed to validate the benefits of melatonin largely demonstrated in animal studies [13]. Only two clinical trials, namely, MARIA [63] and IMPACT [64], are currently investigating the effects of melatonin in patients with acute myocardial infarction.
In summary, our data suggest that melatonin confers cardioprotection via TLR4 stimulation which, in turn, activates TNFα/STAT3 pathway. The present findings support the use of melatonin as an effective, safe, and inexpensive therapy against cardiovascular disease.

Conflict of interest
There are no conflicts of interest.

Funding
This study was supported by the South African National Research Foundation, the South African Medical Research Council, Winetech South Africa, and the University of Cape Town.

Author contributions
NF: generated and analyzed data, wrote the manuscript; LK: generated and analyzed data, AZ: generated and analyzed data; OLH: data interpretation and critical review of the manuscript; LS: principal investigator, coordinated the study, and critical review of the manuscript. All authors reviewed and approved the final manuscript.

References


32. Dispersyn G, Pain L, Toutou Y. Propofol anesthesia significantly alters plasma blood levels of melatonin in rats. Anesthesiology 2010; 112:333–337.


Supporting Information

Additional Supporting Information may be found in the online version of this article:

Figure S1. Effect of acute administration of melatonin with or without TAK242 on nuclear STAT 3 activation, (A) Phospho-STAT3 (tyrosine 705), (B) phospho-STAT3 (Serine 727), Mel: melatonin, TAK: TAK242, blots are representative; n: 4 hearts/group.