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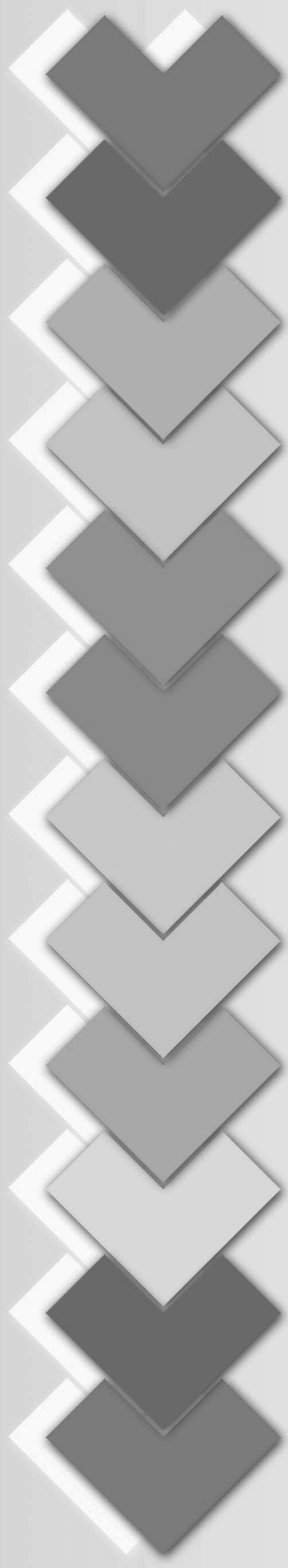
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Summary and general discussion



Sepsis is the non-specific whole-body response to infection and results, in adults, mainly from pneumonia or abdominal infection (1;2). It may progress into severe sepsis and septic shock which are associated with multiple organ failure (3), with the heart and lungs as most prominent failing organs (1;2). Myocardial dysfunction during severe sepsis can be induced through multiple, highly investigated, mechanisms, including local hypoxia, altered calcium handling and sensitivity, mitochondrial dysfunction and apoptosis (4). In 50% of all severe septic patients, acute respiratory distress syndrome (ARDS)(5) develops, for which mechanical ventilation (MV) is required. Surprisingly, the main cause of death in ARDS patients is not insupportable respiratory failure but rather multiple organ failure (6). Indeed, increasing evidence indicates that although MV improves oxygenation and therefore is a life-saving therapy, it also may have harmful effects, particularly when high pressures and/or high tidal volumes (V_t) are used (7). As a result, alveolar membranes may be disrupted and ventilator-induced lung injury (VILI) may develop which is histologically and functionally indistinguishable from ARDS (8). Inflammatory mediators may spill over from the lungs after VILI and may adversely affect distant organs (9), thereby potentially creating multiple organ failure. Although effects of injurious MV and VILI on other organs, such as the kidneys (10), are currently highly investigated, data on the effects on the heart are limited. As the inflammatory response elicited by injurious MV can be described as a sepsis-like syndrome (11), injurious MV and VILI may adversely affect the heart through similar pathways. In this thesis the effects on lung-injurious MV on yet compromised myocardial function during sepsis were studied.

Role of mitochondria in sepsis-induced myocardial dysfunction

Although most studies investigated functional alterations in the heart during sepsis, multiple findings suggest that structural alterations may occur which potentially can contribute to the development of sepsis-induced myocardial dysfunction and mortality (12). To identify common structural alterations and their potential role in myocardial dysfunction we reviewed clinical and experimental studies (chapter 2). We found that myocardial infiltration of inflammatory cells is universally observed in both clinical and experimental studies (table 1, chapter 2) and may contribute to myocardial edema and myocardial stiffness and subsequently decrease contractility. Next, we found mitochondrial injury in human autopsy specimens which was confirmed in numerous experimental studies, and also a decrease in mitochondrial number was seen (table 2, chapter 2). Importantly, mitochondrial injury was frequently associated with mitochondrial dysfunction and myocardial

dysfunction. Finally, we searched for cardiomyocyte cell death. Necrosis was only found in a small portion of human autopsy specimens and has been non-universally confirmed in experimental studies. Whereas apoptosis was not observed in human autopsy specimens, it was frequently observed in experimental studies and was associated with myocardial dysfunction (table 3, chapter 2). Apoptosis can be induced via the death receptor pathway and the mitochondrial pathway, the latter is activated by mitochondrial injury. As mitochondrial injury can thus affect myocardial function both by decreasing mitochondrial function as well as by inducing apoptosis, it is evident that mitochondrial dysfunction and injury may play an important role in sepsis induced myocardial dysfunction. As structural changes almost always result from molecular changes (figure 1, chapter 2), therapies aiming to prevent induction of these molecular pathways, may attenuate myocardial dysfunction by both addressing their original target as well as by preventing structural alterations.

As mitochondrial function, number and injury may play an important role in sepsis-induced myocardial dysfunction, we aimed to identify their contribution in two different sepsis models (chapter 3 and 4). In chapter 3 we show myocardial inflammation and endothelial activation, indicated by increased tumor necrosis factor (TNF)- α and vascular cell adhesion molecule (VCAM)-1 expression, and myocardial dysfunction in the first 2-4 hours of LPS-induced peritonitis. Interestingly, mitochondrial function, mitochondrial integrity and mitochondrial biogenesis, were not altered at that time point. In chapter 4 we used a cecal ligation and puncture (CLP) model and found myocardial dysfunction at 48 hours after CLP which was associated with structural alterations that comply to those described in chapter 2; disrupted mitochondria, disruption of myofibrils, focal necrosis and edema. Treatment with resveratrol, a polyphenol whose beneficial effects has been implicated in numerous diseases (13;14) attenuated these alterations and improved myocardial function. In contrast to chapter 3, in which myocardial dysfunction was found independent of mitochondrial dysfunction, injury or altered biogenesis, in chapter 4 we found that resveratrol-induced prevention of myocardial dysfunction was associated with increased expression of genes regulated by the transcription factor proliferator-activated receptor gamma coactivator (PGC-) 1α , which is a key regulator in mitochondrial metabolism and biogenesis (15;16) and with increased expression of genes involved in bio-energy metabolism, as well as with a down-regulated the pro-inflammatory response and decreased oxidative stress.

The difference of mitochondrial involvement in chapter 3 and 4 is mainly due to the difference in time point at which end points were evaluated. In chapter 3 we used an acute model of lipopolysaccharide (LPS)-induced peritonitis and studied myocardial function and mitochondrial

parameters 2-4 hours after LPS-injection. As in this model a rather quick inflammatory response is elicited, this creates the opportunity to study the onset of myocardial dysfunction. In contrast, in chapter 4 we used a CLP model in which the development of sepsis is more comparable to the course of the disease seen at the bedside when compared with the LPS-induced peritonitis. As with these two models mitochondrial involvement can be studied at different time-points, our results may suggest that sepsis-induced myocardial dysfunction is not initiated by mitochondrial dysfunction which can be concluded from chapter 3, but that after initiation of myocardial dysfunction mitochondrial dysfunction plays a role in its prolongation, as shown in chapter 4. The differences in findings are not likely due to the different insults in the two models, as we have shown in chapter 2 that mitochondrial dysfunction and injury was found in both LPS and CLP models, when this was studied in a more subacute or chronic setting (table 2, chapter 2).

As myocardial dysfunction is initiated independent of mitochondrial alterations, other factors, such as cytokines and endothelial activation, are more likely to be involved in the onset of sepsis-induced myocardial dysfunction. Indeed, in chapter 3 we show that myocardial TNF- α and coronary VCAM-1 are increased already 2-4 hours after the septic insult, before mitochondrial alterations are observed. It is well known that TNF- α can affect the heart by multiple mechanisms, which includes altering calcium handling and sensitivity (17). We indeed showed that these were altered in another LPS-induced sepsis model in chapter 7. These data indicate that TNF- α induced changes in calcium handling and sensitivity may play a role in the onset of the observed LPS-induced myocardial dysfunction. Furthermore, endothelial activation, shown by VCAM-1 expression, may also have contributed.

Effect of VILI on sepsis-induced myocardial dysfunction

As sepsis can induce respiratory failure such that MV is required (5), we investigated the effects of MV on myocardial (dys-)function. After institution of positive pressure MV, cardiac output will decrease during inspiration due to a decrease in right ventricular (RV) preload, an increase in RV afterload and compression of the heart in the cardiac fossa, the space that is left for the heart when the lungs are inflated (chapter 5). Moreover, through variable effects on the autonomous nerve system, variable effects on contractility may also be observed. The ultimate effects of MV on myocardial function are however dependent on the underlying conditions of the lungs and the heart, which are known to be altered in sepsis.

As the inflammatory responses in VILI are similar to those in sepsis (11) VILI may decrease myocardial function through (increased activation of) identical molecular pathways. In chapter 3 we found that mitochondrial injury is not yet present at 4 hours after LPS-administration, whereas endothelial activation, shown by VCAM-1, was present, and in chapter 2 and 4 we found that myocardial edema can occur and may play a role in myocardial dysfunction. Moreover, it was shown previously that MV may induce endothelial activation and increase microvascular permeability (18;19) in distant organs, such that we hypothesized that MV may increase myocardial edema superimposed on yet existing sepsis-induced edema. Consequently, sepsis-induced myocardial dysfunction may be further augmented. We found that in the aforementioned LPS-induced peritonitis model, lung-injurious ventilation with high tidal volumes (V_t) (20;21) induced mild VILI and, surprisingly, attenuated rather than deteriorated LPS-induced myocardial dysfunction (chapter 6). LPS induced endothelial activation thereby increasing vascular permeability in the coronary arteries, but this was not altered by MV. Although MV did not affect endothelial activation and subsequent permeability, transmural pressure of the coronary veins was decreased in high V_t ventilation, such that edema formation during sepsis was, at least in part, prevented, which likely resulted in the attenuated decrease in myocardial function.

In chapter 7 ventilatory settings were adjusted to prevent the differences in coronary transmural pressure between the two ventilation strategies as seen in chapter 6. In a model of LPS-induced pneumonia, high V_t ventilation created VILI resulting in premature death in LPS-treated animals ventilated with high V_t . LPS decreased myocardial function through a calcium-dependent mechanism and high V_t ventilation further deteriorated LPS-induced myocardial dysfunction. This deterioration was mediated through a calcium-independent pathway. Expression of myocardial toll like receptor (TLR)-2 increased upon LPS exposure and expression of pulmonary heat shock protein (Hsp)-70 was increased upon high V_t -ventilation during pneumonia. We suggest that during severe VILI pulmonary Hsp70 may have spilled-over and bound to the myocardial TLR-2 thereby inducing the expression of chemokine (C-X-C motif) ligand (CXCL)-1 (22).

The opposite myocardial responses to high V_t ventilation in these studies are most likely caused by differences in severity of VILI. In order to understand these differences, we need to explain the experimental set-ups of the studies. First, the models that were used to mimic sepsis and to induce myocardial dysfunction have different effects on the lungs. LPS-induced peritonitis (chapter 6) provides an indirect insult to the lungs targeting the pulmonary vascular endothelium and relatively

sparing intra-alveolar spaces. In contrast, intratracheal administration of LPS (chapter 7) provides a direct hit to the lungs by targeting the alveolar epithelium thereby creating intrapulmonary inflammation (23). The different lung pathophysiology in the two models may contribute to different pulmonary responses to injurious MV with subsequent more severe VILI in the pneumonia model. These data are in alignment with data by Kuiper et al., who showed that VILI developed only after a direct hit with pulmonary acid installation but not after sepsis (24). Next to this, the levels of positive end-expiratory pressure (PEEP) are different between the studies. To prevent differences in myocardial edema-formation as seen in chapter 6, the level of PEEP was lowered in chapter 7 such that airway pressures were similar between the groups. As PEEP can protect against VILI, the lower level of PEEP may account in part for the more severe VILI that is seen in chapter 7. However, as in lung injury resulting from a direct hit, application of PEEP may be less effective as in lung injury resulting from an indirect hit (11), the contribution of the lowered PEEP to the increase in lung injury is debatable.

Conclusions and future directions

We may conclude that sepsis-induced myocardial dysfunction is potentially initiated by altered calcium sensitivity and handling induced by inflammatory mediators such as TNF- α . Mitochondrial injury and dysfunction develop later in the course of sepsis and contribute to the further development and continuation of the induced myocardial dysfunction. Inflammation and mitochondrial dysfunction may cause structural alterations that contribute to myocardial dysfunction. Resveratrol can prevent mitochondrial injury as it can increase expression of down-regulated PGC-1 α and other genes related to mitochondrial biogenesis.

The effects of lung-injurious MV on myocardial dysfunction depend on the severity of VILI which itself depends on the origin of pulmonary dysfunction. Adverse effects of VILI on myocardial dysfunction are mediated through a calcium-independent mechanism, which may involve pulmonary Hsp-70.

Therapeutic potential of resveratrol in sepsis

In this thesis we show that mitochondrial alterations in sepsis-induced myocardial dysfunction are likely modulated by downregulation of PGC-1 α , and can be reversed with resveratrol. Interestingly, sepsis-induced acute kidney injury was recently also found to be associated with decreased PGC-1 α expression, swollen renal mitochondria with impaired function and reduced oxygen consumption by

kidney cells in both LPS- and CLP-induced sepsis (25). In addition, induction of PGC-1 α by adenovirus restored oxygen consumption and the expression of target genes in tubular cells treated with TNF- α . These data further confirm the importance of PGC-1 α and mitochondrial dysfunction in sepsis-induced organ failure and underline the therapeutic potential of resveratrol.

We found that treatment with resveratrol not only increased PGC-1 α expression, but also downregulated the pro-inflammatory response, which may have also contributed to the observed beneficial effects on mitochondrial and structural parameters. Indeed, resveratrol has several mechanisms of action, including modulation of apoptosis and mitochondrial activity, balancing redox status and suppression of inflammation (26;27), which may all contribute to attenuate myocardial dysfunction. These areas need to be further investigated.

Despite the beneficial effects of resveratrol on sepsis-induced myocardial dysfunction, mortality was not changed in our model (chapter 4). However, as other studies have shown beneficial effects of attenuated mitochondrial and myocardial dysfunction on mortality (28-31) prevention of sepsis-induced myocardial dysfunction is very likely to decrease mortality. Finally, positive effects of resveratrol in sepsis were also shown in other organs, in particular in the lungs and kidneys, through various pathways (32-39). Together with the multiple mechanisms by which resveratrol may attenuate myocardial dysfunction, these data indicate that resveratrol has a high therapeutic potential in sepsis and sepsis-induced organ dysfunction.

VILI and myocardial dysfunction

In this thesis we suggested that VILI-mediated myocardial dysfunction is caused by spill-over of Hsp-70 which may bind to myocardial TLR-2 and induces the expression of myocardial CXCL-1. This mechanism results in three potential targets for therapy. First, one might try to prevent pulmonary Hsp-70 expression. However, as Hsp-70 serves a protective effect in the lungs during VILI (40), therapies targeted against Hsp-70 may cause severe adverse effects. A better strategy may be to decrease the TLR-4-mediated myocardial TLR-2 expression, making the heart less susceptible for systemic Hsp-70 or to inhibit the down-stream pathway of TLR-2. First, the cyclohexene derivative, TAK-242, was shown to selectively suppress TLR-4-mediated cytokine production (41) and may therefore also decrease TLR-4-mediated TLR-2 expression. Also, eritoran tetrasodium, a second generation synthetic lipodisaccharide designed to antagonize the toxic effect of endotoxin, was shown to block LPS-mediated activation of nuclear factor κ B (NF- κ B) and cytokine production by direct

antagonism of TLR-4 (42;43). Decreased TLR-2 expression after treatment with eritoran was shown in the aorta of septic mice (44). Although in a recent worldwide phase 3 randomized trial eritoran failed to improve the primary end point of 28-day all-cause mortality in a cohort of 2000 patients with severe sepsis (45;46) eritoran may still be used as an additive treatment superimposed on other treatment to prevent VILI-mediated myocardial dysfunction. Furthermore, sparsolonin, a polyphenol found in a traditional Chinese herb, was shown to selectively suppress multiple pathways downstream of TLR-2 and TLR-4 but not of other TLRs (47). It could therefore be of use for reducing both the TLR-4 mediated TLR-2 induction as well as the TLR-2 mediated CXCL-1 expression, without interfering with pathways of other TLRs. Finally, due to its anti-inflammatory capacities, resveratrol may also be of use in VILI-mediated myocardial dysfunction as it can both decrease myocardial inflammation, potentially rendering the heart less susceptible for Hsp-70, as well as decrease the inflammatory response in VILI.

Future research may be aimed at the exact mechanism by which VILI affects myocardial dysfunction. As this may, at least initially, be mediated through other pathways than seen in sepsis, therapies directing these targets may decrease myocardial dysfunction synergistically with current therapies. However until then, as the severity of VILI seems the most predominant factor, prevention or reduction of VILI may seem one of the best strategies to prevent MV-induced myocardial dysfunction.

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