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Mechanisms Involved in Secondary Cardiac Dysfunction in Animal Models of Trauma and Haemorrhagic Shock

Running Head

Mechanisms of Secondary Cardiac Dysfunction in Trauma

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Abstract

Clinical evidence reveals the existence of a trauma induced secondary cardiac injury (TISCI) which is associated with poor patient outcomes. The mechanisms leading to TISCI in injured patients are uncertain. Conversely, animal models of trauma haemorrhage have repeatedly demonstrated significant cardiac dysfunction following injury, and highlighted mechanisms through which this might occur. The aim of this review was to provide an overview of the animal studies describing TISCI and its pathophysiology.

Basic science models of trauma show evidence of innate immune system activation via Toll-Iike receptors (TLRs), the exact protagonists of which remain unclear. Shortly following trauma and haemorrhage, cardiomyocytes upregulate gene regulatory protein and inflammatory molecule expression including Nuclear factor kappa beta (NF-κB), tumour necrosis factor alpha (TNFα) and interleukin-6 (IL-6). This is associated with expression of membrane bound adhesion molecules and chemokines leading to marked myocardial leukocyte infiltration. This cell activation and infiltration is linked to a rise in enzymes that cause oxidative and nitrative stress and subsequent protein misfolding within cardiomyocytes. Such protein damage may lead to reduced contractility and myocyte apoptosis. Other molecules have been identified as cardioprotective following injury. These include p38 mitogen-activated protein kinases (MAPK) and heat shock proteins.

The balance between increasing damaging mediators and a reduction in cardio-protective molecules appears to define myocardial function following trauma. Exogenous therapeutics have been trialled in rodents with promising abilities to favourably alter this balance, and subsequently lead to improved cardiac function.

Keywords

Trauma, Secondary, Dysfunction, Cardiac, Mechanisms, Inflammation

Trauma Induced Secondary Cardiac Injury in Patients

There is mounting evidence to support the existence of a trauma induced secondary cardiac injury (TISCI), namely the development of cardiac injury and dysfunction in the absence of direct trauma to the heart (1-8). TISCI has been shown to occur acutely in at least ten percent of trauma patients, and is associated with adverse outcomes, including an increased risk of death (1, 2, 9).

Many studies have demonstrated a significant risk of adverse cardiac events (ACEs) following trauma that bear no association to the presence or severity of chest injury. In a retrospective review of over a million injured patients, the risk of post-traumatic myocardial infarction (MI) was found to nearly double (4). Of some 1,051,081 injury discharges, 32,616 (3.10%) acute MIs were diagnosed. The study revealed that abdominal or pelvic trauma led to a 65% (Odds Ratio [OR] 1.65, 95% Confidence Intervals [95% CI] 1.26-2.16) increase in the risk of acute MI in patients below the age of 46 years, regardless of confounders or coronary artery status. In patients over 46 years, abdominal or pelvic trauma alone nearly doubled the risk of acute MI (OR 1.93, 95% CI 1.42-2.62). When confirmed by coronary angiography (CA), the risk of MI was six times greater (OR 6.33, 95% CI 4.00-9.99).

Atrial arrhythmias have also been reported in trauma patients, and associated with increasing age, catecholamine use, the presence of a systemic inflammatory response (SIRS) and overall injury severity. Importantly, the presence of arrhythmias may be associated with a longer length of critical care stay and linked to higher mortality (10, 11).

Cardiac failure in severely injured patients has been observed at an average onset of two days following injury (12). When myocardial contractile dysfunction following trauma is identified with invasive monitoring, it may be treated effectively with inotropic support

(13). Decreased cardiac index (CI) following trauma is related to increased mortality, and conversely a higher CI with survival (14, 15).

In addition to adverse cardiac events, heart specific biomarkers have identified both the severity and clinical significance of secondary cardiac injury. Troponin I rises after trauma and may be associated with both an increased risk of ACEs and death (1, 5, 6). In Martin et al's (6) study of over a thousand critically injured patients, around a third of the cohort (29%) demonstrated a rise in Troponin I. When adjusted for, only the severity of injury, base excess and degree of physiological derangement were predictors of Troponin I increase. Neither the presence nor severity of thoracic injury, however, was associated with Troponin I. Elevated Troponin I levels were also a significant predictor of mortality (OR 2.1, 95% CI 1.4–3.1). Fifty patients (4.6%) had an ACE of some description and these were associated with increases in Troponin I. Beta blockers were linked to survival and a 50% decrease in mortality was seen in patients with Troponin I increases on these medications.

Furthermore, B-type natriuretic peptide (BNP) may be elevated in the absence of echocardiographic evidence of congestive heart failure (8, 16). Measured 24 hours after injury, levels of NT-proBNP correlate with a decreased CI and multiple organ dysfunction syndrome (MODS) (7, 8). Moreover, in 135 critically injured patients, BNP levels were found to be significantly higher in non-survivors compared to survivors in blood taken on admission, and again after 24 and 72 hours post admission (1). Elevations in heart specific fatty acid binding protein (H-FABP) over the same time frames were also associated with subsequent ACEs (1, 3).

Echocardiographic abnormalities, including regional wall abnormalities and reduced ejection fraction, have been shown in patients following isolated traumatic brain injury (TBI) (17). Cardiac dysfunction in TBI is again linked to raised plasma Troponin and mortality (8, 18).

Post-mortem studies have corroborated the existence of TISCI. A review of 125 trauma patients undergoing autopsy revealed lesions in 20% of the hearts examined. Lesions were apparent as early as four hours after injury, and typically located around the left ventricle. Histological changes included subendocardial haemorrhages, interstitial oedema, mononuclear infiltration, coagulative necrosis and evidence of hypoxic injury in the absence of existing coronary disease (19). An older post-mortem study of homicide trauma victims has supported these findings, and revealed myofibrillar degeneration, namely scattered clusters of myocytes with homogeneous eosinophilic transverse bands alternating with areas of fine granulation (20). Contraction bands were also observed, a process whereby hypercontraction (secondary to catecholamine surges) of the cardiac muscle leads to structureless masses of contractile protein. Accordingly, the data presented provided good evidence of TISCI, and suggested that such heart damage is the result of the cardiotoxic effect of catecholamines.

In spite of the clinical evidence revealing the presence and impact of TISCI, few patient studies have explored and verified the cause of this secondary cardiac injury. In contrast, animal models have repeatedly verified the deleterious effects of trauma and haemorrhage on the heart, and explored the mechanisms through which this injury may occur. The bulk of these studies implicate inflammation, often revealing that blocking the inflammatory process leads to an improvement in cardiac function.

The aim of this review was to provide an overview of the mechanisms involved in cardiac injury and dysfunction studied in animal models of trauma and haemorrhage.

Animal Trauma Models and Impaired Myocardial Physiology

Rodent models have consistently demonstrated cardiac dysfunction following trauma, haemorrhagic shock (H-S) and trauma-haemorrhage (T-H). Isolated trauma-induced cardiac injury, without blood loss, has been assessed in a minority of studies, and the impact of injury generally determined in terms of cardiomyocyte apoptosis and autophagy (Table 1). Isolated trauma is conducted using the Noble Collip drum, a technique where the rodent is placed inside a rotating drum and exposed to a pre-determined number of revolutions. They do not sustain life threatening trauma but instead a significant amount of soft tissue injury.

Haemorrhage alone has also been relatively less investigated, and research focused predominantly on the role of inflammation (Table 2.)

Conversely, models of combined T-H are extensively described in the literature, and report a variety of mechanisms leading to the development of secondary cardiac injury and dysfunction, with inflammation again frequently investigated (Table 3).

Both *in vivo* and *ex vivo* models have been reported in the literature, and used a number of invasive and non-invasive haemodynamic monitoring to diagnose cardiac dysfunction (21-69), including the use of LV micro-manometry (66). In studies where the impact of trauma and haemorrhage is investigated, injury was most commonly a laparotomy. Performing a laparotomy alone means that the animals can be recovered after surgery and cardiac dysfunction can be assessed at a later stage. However, one potential drawback of this approach is that it can limit the severity of the insult, and therefore the translational potential when considering severely injured polytrauma patients.

The majority of the studies reviewed included a resuscitation phase in the study protocol. The administration of fluid after an antecedent period of hypotension, is not only important to allow animals to survive through a longer experimental phase, but also

allows for the development of reperfusion-related phenomenon in tissues previously subjected to a period of ischaemia. The inclusion of a resuscitation phase adds another level of complexity to these challenging small animal models, but is important in order to simulate the complex pathophysiology associated with ischaemia-reperfusion related inflammation and injury within the myocardium. Resuscitation protocols within the literature vary in terms of volume and nature of the fluid given. The majority of the models in the literature implemented a fixed-volume resuscitation strategy (based upon the volume of blood loss) with crystalloid solutions (70), rather than whole blood (31), or a combination of blood and crystalloid. One study adopted a targeted resuscitation approach, with intravenous fluid given in accordance with mean arterial pressure (53).

Complex *in vivo* studies have provided important insights into key effector molecules and mechanisms involved in the development of myocardial dysfunction. The role of sex hormones in rat models in particular have been widely reported, and manipulation of these pathways has been invaluable in characterising the cardio-protective effect of oestrogens. Genetically-modified mice have been used to investigate the contribution of inflammatory signalling pathways modulated by the Toll-like receptors (TLRs) (27, 28).

Cardiac dysfunction has manifested through a number of parameters including a decline in stroke volume (SV), or a reduction in either the maximum rates of rise and fall of ventricular pressure (-dP/dtmax, 34), left ventricular developed pressure (LVDP, 25) and ventricular peak systolic pressure, cardiac output (CO, 70) and CI (59). Research reveals cardiac impairment occurs both during systole and diastole, and electrophysiological and significant conduction abnormalities have been reported (31, 53). Cardiac dysfunction leads to a rise in plasma lactate levels, which reverse if myocardial function is restored (35). Elevations in cardiac-specific biomarkers such as troponin and creatine kinase in

response to trauma have also been reported. Indeed, these biomarkers may rise as early as two hours after injury (23).

In terms of timings, limited research has documented a significant, hyper-acute (<1 hour) reduction in cardiac function following trauma. Impaired contractility in cardiomyocytes has been demonstrated as early as 30 minutes after trauma without haemorrhage, perhaps suggesting that hyper-acute dysfunction is not necessarily dependent upon the combination of shock with injury (23). Overall, however, the onset of myocardial depression is usually between two and five hours following injury (33, 58). Later onset, namely up to 24 hours has been recorded (24).

Hearts excised from rats 24 hours following T-H demonstrated impaired cardiac function independent of coronary perfusion and calcium ion concentration (31). The decline in cardiac function following T-H does not reverse with fluid resuscitation and increased preload suggesting impaired myocardial contractility (53).

Studies have demonstrated the development of cardiac injury with biomarker rise, inflammation with local and systemic inflammatory cytokine release, and have characterised the nature of resultant myocardial dysfunction both *in vivo* and *ex vivo* and in isolated cardiomyocytes (23). Animal studies have also been used to assess potential cardio-protective pathways and molecules. Modulation of apoptotic and inflammatory pathways and effectors such as IL-6 and TNF-α, have been identified as being protective. Pre-induction of heat shock proteins was found to confer benefit in terms of subsequent cardiac performance (41), but the majority of studies assessed potential therapeutics delivered agents during a resuscitation phase.

Sensing Injury and the Role of the Innate Immune System

TLRs are central to the innate immune system and expressed on both immune and non-immune cells including cardiomyocytes (71, 72). TLRs respond to pathogen associated

molecular patterns (PAMPs) and endogenous Damage Associated Molecular Patterns (DAMPs) released during cellular stress (73). TLR activation represents a mechanism of cardiomyocyte traumatic injury recognition and subsequent activation of an innate immune response.

Trauma causes soft tissue injury and cellular damage leading to release of DAMPs, thereby activating TLRs independent of exogenous pathogen involvement, so called 'sterile inflammation' (Figure1)(74). TLR4 is unregulated following injury and is likely to play a crucial role in the subsequent cardiac dysfunction by sensing tissue damage and activating cardiomyocyte inflammatory pathways (75). T-H induced cardiac dysfunction is dependent on TLR4 as shown by significant reduction in dysfunction with TLR4 gene knock-out rodents, and when using TLR4 antagonists (27, 58). Ameliorating the TLR4 response following injury led to reduced cardiac activity of the transcription factor nuclear factor kappa beta (NF- κB) and reduced cytokine production (27). TLR9 has also been studied following T-H and a reduction in cardiac dysfunction following TLR9 ligand binding has been shown (28).

Hearts and cardiomyocytes exposed to lymph from T-H exposed animals demonstrated an initial dose dependent inotropic effect immediately after exposure, which was followed by progressive loss of contractile function (31). A recent study revealed a reduction in cardiac contractile dysfunction following H-S in animals subject to mesenteric lymph drainage (65). The lymph studied was sterile and contained similar levels of cytokines when compared to lymph from control animals, thereby suggesting a role for ALARMIN and DAMP type molecules in the process (31, 32).

Cardiomyocyte Amplification of the Immune Response to Injury

Following T-H, trauma and isolated haemorrhage in animal models, there are rises in plasma and cardiomyocyte tumour necrosis factor alpha (TNFα, 21, 22) and interleukin

6 (IL-6, 33, 35) levels which are repeatedly associated with cardiac dysfunction. Reductions in TNFα (23) and IL-6 (76) are conversely linked to improved cardiac function.

Cardiomyocytes exhibit intrinsic leukocyte-like function as demonstrated by significant gene expression of TNFα and IL-6 messenger ribonucleic acid (mRNA) in cardiomyocytes following T-H (33, 36, 77). Myocardial TNFα production begins as early as 30 minutes following injury (53). Increased IL-6 expression following trauma is linked to a rise in the stress associated transcription factor hypoxia-inducible factor-1α (HIF-1α) (78).

The transcription factor NF-κB is present in cell cytoplasm bound to the protein inhibitory kappa B (IκB). Following T-H there is an early cytokine dependent rise in NF-κB activity with an increase in cardiomyocyte IκB-alpha phosphorylation, NF-κB transcription factor and both nuclear levels of NF-κB and NF-κB DNA-binding activity (57). This is associated with a decline in myocardial contractility and cardiac output (35). Attenuation of the NF-κB pathway leads to a reduction in IκB-alpha phosphorylation, NF-κB expression and activity and a reduction in cardiomyocyte TNFα, IL-6 and leukocyte adhesion molecule production (36).

Cardiomyocyte activation following trauma leads to a cytokine dependent rise in cardiac adhesion molecules, including intracellular adhesion molecule-1 (ICAM-1), cytokine induced chemoattractant-1 (CINC-1) and CINC-3 (29, 35). Consequently, a marked increase in cardiac tissue neutrophil infiltration may occur as early as two hours following injury with a subsequent decline in contractile function (30, 36, 64). Pharmacological reduction in expression of the chemoattractants, adhesion molecules and subsequent reduced neutrophil infiltration have been associated with preservation of cardiac output after T-H (26, 44, 48).

Following innate immune sensing of injury, cardiomyocytes intrinsically amplify the inflammatory response with marked expression of cytokines and adhesion molecules leading to neutrophil infiltration.

Cardiomyocytes Stressors

The rise in myocardial cytokines, chemoattractant and adhesion molecules and leukocytes leads to an increase in myocardial enzymes capable of producing oxygen and nitrogenous radicals (25). These include Myeloperoxidase (MPO), Nicotinamide Adenine Dinucleotide Phosphate-oxidase (NADPH) and inducible nitric oxide synthase (iNOS) (22, 35, 61).

Cardiac MPO levels rise shortly following injury. MPO is stored in neutrophil granulocytes and catalyses the formation of volatile reactive oxidative and nitrating substances which are thought to contribute to cardiomyocyte protein misfolding and damage with subsequent reduced contractility (44, 48, 66).

iNOS is found in leukocytes, endothelial cells and cardiomyocytes and its expression and activation is dependent on stress and cytokine induced activation prior to producing significant quantities of Nitric Oxide (NO) (79). Increased cardiac iNOS expression has been shown five hours after tissue injury (80) and associated with increased NO content and cardiac tissue peroxynitrite formation. This is a marker of nitrative stress and linked to impaired left ventricle developed pressure and cardiac function (25, 26). The increase in NO, superoxide production and protein nitrotyrosine levels can be blocked by iNOS inhibition, leading to preservation of cardiac function (21).

Cardiomyocyte Apoptosis

The rise in myocardial oxidative and nitrative radicals following injury leads to a protein misfolding and DNA damaging milieu which in turn activates apoptotic machinery within cardiomyocytes. A rise in myocardial Caspase-3 activity and TUNEL-positive cells has

been demonstrated six hours after trauma, peaking at 12 hours, but ongoing at 24 hours. This caspase dependent dysfunction was associated with reduced CO. This was significantly attenuated by Caspase inhibition or pharmacological inhibition of pro-apoptotic factors and preservation of anti-apoptotic proteins (64, 81). There was no concurrent and significant increase in plasma Troponin levels, suggesting cardiac depression was not due to necrosis. A rise in caspase-3 activation has been shown in cardiomyocytes exposed to plasma from injured animals but not from control animals, implicating dependence on plasma circulated mediators (22). Increases in other pro-apoptotic protein expression has been observed following T-H, including bcl-2-like protein 4 (bax) (64). Furthermore, T-H is associated with a decrease in anti-apoptotic factors such as protein B-cell lymphoma 2 (bcl-2) (64). The activation of apoptosis is cytokine, oxidative and nitrative stress dependent (66, 82).

Cardiomyocyte Protectors

The mediators discussed thus far are upregulated by injury with resultant myocardial damage. There are, however, mediators which offer myocardial protection. Following injury, these can become depleted or downregulated, and the resultant imbalance between cardiac stressors and cardiac protectors is an important component in trauma induced secondary cardiac injury and dysfunction (Figure 2).

There is a significant decrease in the function of the cell signalling mediators Akt and p38 mitogen-activated protein kinase (MAPK) in the myocardium following T-H as demonstrated by a reduction in phosphorylated cardiac Akt and p38 MAPK (45, 48). This is thought to lead to myocardial dysfunction due to increased leukocyte infiltration and cardiomyocyte apoptosis. Indeed, the rise in cardiac inflammatory molecules following T-H can be abolished by the administration of exogenous substances that prevent reduction in pAkt, such as Oestrogen (38, 44, 83) and Tropisetron (30). Preservation of

cardiac pAkt is then linked to reduced neutrophil infiltration, apoptosis and restoration of cardiac glycogen levels with subsequent attenuation in cardiac contractile dysfunction (84). Similarly maintaining cardiac phosphorylated p38 MAPK levels is associated with preservation of cardiac function following T-H (29). Conversely, p38 MAPK has been shown to play a contributory role to myocardial inflammation following haemorrhage with inhibition of p38 MAPK phosphorylation associated with reduced cytokine production and dysfunction (77).

Heat shock proteins (HSPs) minimise damage caused to cells from stressors such as heat, hypoxia and ischaemia (85). Induction of HSPs is controlled by transcription factors called heat-shock factors (HSFs). T-H is associated with an increase in cardiac haem oxygenase 1 (HO-1, HSP-32) and a reduction in HSP-60 (49) and HSP-90 (86). Pharmacological preservation of myocardial levels of HSP-60 and HSP-90 and increased levels of HSF-1, H0-1, HSP-70 (87), HSP-27 and the HSP αβ-crystallin concentration expression following T-H lead to a reduction in cardiac cytokine concentration and improved cardiac function following injury (38, 44, 79).

Female rats in the pro-oestrus phase have the highest oestrogen level and demonstrate a significant degree of cardio-protection following injury (34). There is a decrease in oestrogen receptor (OR) expression in cardiomyocytes following T-H, and preservation of these receptors by increased exogenous or endogenous oestrogen and testosterone antagonists is again associated with improved cardiac output (88-90). Furthermore, this reduction in ORb expression is associated with a rise in cardiac TNFα, IL1-6, chemokines and adhesion molecules (29, 34, 40). Oestrogen mediated cardio-protection is also dependent on Akt and MAPK (29, 42, 45).

T-H induced rises in cardiac TNFα, MIP-2, CINC-1, ICAM-1 and MPO are abolished by administering exogenous oestrogen (29, 44). ORs are classically considered to initiate

the activity of a downstream intra-nuclear ligand dependent transcription factor. However, due to the rapid speed of onset and inability of transcription inhibitors to negate the effects of OR agonists, additional oestrogenic effects are thought to occur independent of gene up-regulation (90).

Limitations in Applying the Basic Science to the Clinical Context

There are restrictions to the experimental studies, however, which limit both their applicability and their relevance to the clinical setting. Experiments are designed to create damage to the heart using scenarios that are not usually reflective of clinical patterns of injury. In trauma-haemorrhage (T-H) models, injury often takes the form of laparotomy, with the Noble-Collip drum used as an alternative in models of isolated injury in the absence of haemorrhage (22-26). H-S is performed in a pressure-dependent or volume-dependent fashion with controlled blood withdrawal (33-35). In the majority of haemorrhage and T-H models, animals undergo resuscitation (53, 62), though some haemorrhage models are non-resuscitated (58). It is therefore difficult to apply these uniform models to heterogeneous trauma populations, who present with a wide variety of injuries and a broad spectrum of physiological derangement. Much of the clinical evidence is from retrospective cohort studies, and primarily limited to small numbers of critically injured patients only. Clinical manifestations of secondary cardiac injury are restricted to isolated diagnoses, whilst biomarker studies have investigated single markers. There exists some further difficulty, therefore, in translating the findings of interventional animal experiments to the observational research which forms the bulk of the current clinical evidence.

Furthermore, the animals are not exposed to the same degree and nature of interventions as trauma patients are such as medications, blood products, multiple operations and superimposed factors such as infection.

The effects these have on the human heart are therefore inevitably neglected.

Animals typically do not suffer co-morbidity or poor background physiology, due to advanced age or smoking for example. This further limits the applicability of the experimental evidence to the clinical context and highlights the need for more patient based research.

However, the animal models have been invaluable in demonstrating cardiomyocyte damage and myocardial dysfunction following trauma. They have provided important insights into the key molecular pathways of cardiac pathophysiology in the context of the complex, multi-factorial scenario of sterile inflammation resulting from injury combined with haemorrhagic shock. Research has identified novel therapeutic strategies including the administration of sex hormones, anti-inflammatory and antibody blocking agents capable of damping down the inflammatory drive behind cardiac injury and dysfunction. The greater understanding of these processes has provided platforms on which to base future clinical studies.

Future Directions

There is an urgent need to develop treatment strategies which will not only prevent acute cardiovascular collapse, but also ameliorate cellular injury and prevent the cardiac dysfunction seen after resuscitation. The focus should now shift toward taking promising therapeutic studies into the clinical setting with well-designed translational studies. This field of trauma research offers an exciting opportunity for clinical and pre-clinical researchers to collaborate with the aim of developing translatable models in which to investigate new therapeutics.

Injury and surgery has been shown to be associated with the formation of neutrophil extracellular traps (NETs) (91). It is thought that NET formation represents an innate immune system response, which, via inflammatory pathways has a detrimental impact

upon organ function after trauma (92). The role of this NET formation and their subsequent release of inflammatory mediators upon cardiomyocytes and cardiac function after trauma has yet to be determined, and further work in this area is therefore required.

There are a number of highly sophisticated, clinically relevant imaging technologies, which could also be applied in this area. Non-invasive imaging technologies are developing which have applications in pre-clinical trauma research. Micro-imaging modalities such as echocardiography and magnetic resonance, for example, are available with improved resolution, which would allow for detailed *in vivo* assessment of rodent hearts under conditions of trauma-haemorrhage. Application of these imaging techniques would be of benefit in mechanistic studies and in monitoring responses to therapeutics.

As we regard technological advancement in cardiovascular research to apply to these studies, we should also take opportunities arising from advances from other areas of resuscitation research. For example, the development of selective aortic arch perfusion (SAAP) methods may represent, in the future, a targeted way of delivering cardio-protective drugs directly to the coronary circulation.

Building upon the existing knowledge gained from animal models of cardiac injury and dysfunction and developing future models in this way will optimise translational potential, and allow effective cardio-protective interventions identified in the laboratory to be assessed in the clinical setting. Ultimately, such research will be directed at further reducing the burden of injury, both in terms of the mortality and morbidity associated with trauma induced secondary cardiac dysfunction.

Conclusion

Clinical studies of injured patients reveal a secondary cardiac injury and dysfunction associated with poorer outcomes. Animal models of trauma have repeatedly demonstrated this entity, and revealed many of the mechanisms that lead to it.

Substances with therapeutic promise in animal models either reduce damaging molecules, or prevent reduction in protective agents, or both. Further clinical evidence should substantiate the findings of the pre-clinical experiments. In turn, this could lead to improved survival and function after trauma.

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Figures

Figure 1. Mechanisms of Cardiac Dysfunction in Trauma.

Numbered stages showing sensing of injury by innate immune system, inflammatory activation and leukocyte infiltration, upregulation of oxidative stress inducing enzymes and finally cell damage, apoptosis and impaired function.

Figure 2. The Shifting Balance in Protective Versus Stress Inducing Factors in the Heart After Trauma.

Altering this balance pharmacologically in favour of protective factors has been repeatedly shown to prevent myocardial dysfunction and injury following trauma.

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Table 1. Synopsis of the Animal Model Data of Secondary Cardiac Injury Following Isolated Trauma

Animal Model (& Reference)	Cardiac Monitoring	Substances Measured	Therapeutic Intervention(s)	Nature of Cardiac Dysfunction	Summary of Main Findings
Noble Collip drum (21,22)	Not performed	TNFα NO Caspase 3 IL-1B IFNγ iNOS NADPH	Anti-TNFα Anti-INOS Anti-NADPH	Not Measured	Oxidative, nitrative stress associated with TNFα led to apoptosis
Noble Collip drum (26)	*In-vivo* arterial blood pressure via transducer	MPO, ICAM-1, nitrotyrosine		Reduction in +dp/dtmax and –dp/dt max.	Leukocyte infiltration, nitrative and oxidative stress
Noble Collip drum (25)	*In-vitro* Langendorff, balloon transducer in left ventricle	NO iNOS superoxide	Caspase inhibition (Z-VAD-FMK)	Reduction in +dp/dtmax and –dp/dt max.	Free radicals and nitrative damage linked to apoptosis
Noble Collip Drum (23)	Left ventricle *in-vivo* pressure transduced	TNFα H_20_2 PI3K Akt	Insulin	Reduction in +dp/dtmax and –dp/dt	TNFα, reactive oxide species led to apoptosis
Noble Collip Drum (24)	*In-vitro* Langendorff, balloon transducer in left ventricle	Beclin LC3	Rapamycin (promotes autophagy)	Reduction in +dp/dtmax and –dp/dt max.	Reduction in autophagy post trauma
Noble Collip drum (66)	*In-vivo* Left ventricle pressure transduced	Reactive oxide species TNFα apoptotic markers	Quercetin	Reduction in +dp/dtmax and –dp/dt max.	Inflammation and ROS associated with apoptosis

Table 2. Synopsis of the Animal Model Data of Secondary Cardiac Injury Following Isolated Haemorrhage

Animal Model (& References)	**Cardiac Monitoring**	**Substances Measured**	**Therapeutic Intervention(s)**	**Nature of Cardiac Dysfunction**	**Summary of Main Findings**
Volume targeted (30% circulating volume venesection (58)	*In-vitro* Langendorff method, intraventricular pressure balloon	TNF	TLR-4 TNFα knockout	Reduction in +dp/dtmax and –dp/dt max	Dysfunction linked to TLR4 and p55 TNFα receptor
MABP of 50mmhg, resuscitated with Ringers lactate and autologous transfusion (53)	*In-vitro* Langendorff method, intraventricular pressure balloon	TNFα	Prazosin	Reduction in +dp/dtmax and –dp/dt max	Dysfunction linked to alpha adrenoceptor
Targeted MABP of 35mmhg, resuscitated with Ringers lactate (57)	None	TNFα NF-κB	None		Rise in TNFα and NF-κB in cardiomyocytes post HS
MABP target 40mmhg, reinfusion of shed blood (62), (65)	*In-vitro* Langendorff method, intraventricular pressure balloon	TNFα	Dipyrimadole Lymph drainage	Reduction in +dp/dtmax and –dp/dt max.	Reduced inflammatory cytokines and lymph drainage associated with cardioprotection

Table 3. Summary of the Animal Model Data of Secondary Cardiac Injury Following Combined Trauma-Haemorrhage

Animal Model (& References)	Cardiac Monitoring	Substances Measured	Therapeutic Intervention(s)	Nature of Cardiac Dysfunction	Summary of Main Findings
Blood pressure target. Thoracotomy. Non-resuscitated (27-28)	*In-vivo* Arterial and left ventricle pressure transducer	NF-κB PI3K/Akt TNFα	TLR-9 ligand (CPG-ODN1826) TLR-4 antagonist	Reduction in SV and reduction in +dp/dtmax and –dp/dt max	TLR-9 ligand led to PI3K, MEK/ERK dependent cardioprotection
MABP target of 40mmhg and maximum bleed out. Laparotomy. Fluid resuscitate (29-30, 64)	*In-vivo* Left ventricle pressure transducer.	P38 MAPK eNOS Nitrate Nitrite ICAM-1 IL-6 TNF α CINC-1 MIP-2 MPO Akt	17B estradiol Tropisetron Hydrogen sulphide	Reduction in +dp/dtmax and –dp/dt max	Increased cytokine levels, decrease in p38MAPK. Akt cardioprotective limiting apoptosis
Fixed pressure shock, MABP 35 mmhg for 90 minutes. Laparotomy. Autologous transfusion (31-32)	*In-vitro* Langendorff method, intraventricular pressure balloon	None	Lymph duct ligation	Reduction in +dp/dtmax and –dp/dt max.	Dysfunction limited by lymph duct ligation
Laparotomy, fixed pressure shock model and resuscitation (33-35, 38-39, 41-45, 47-49, 69)	*In-vivo* Indocyanine green fluorescence dilution technique and left ventricle pressure transducer	Estradiol Progesterone IL-6 ICAM-1 MPO CINC-1 & 3 HSPs P38, Glycogen Akt TNFα, HO-1 iNOS, PI3K Akt, Oestrogen receptors Cyclooxygenase	Il-6 antibody 17B estradiol Progesterone PPAR-gamma Diarylpropionitrile (beta oestrogen agonist)	Reduction in CO, SVR, +dp/dtmax and –dp/dt max	Cardiac IL-6, NF-κB, TNFα, ICAM-1, MPO associated with neutrophil infiltration, dysfunction and apoptosis Cardioprotection from HSP, PI3K, Akt, HO-1, E2

Laparotomy, fixed pressure shock model and resuscitation. (36, 76)	*In-vivo* Left ventricle and arterial pressure transducer. Radioactive microsphere cardiac output monitor	NF-κB ICAM-1 MPO Il-6 CINC-1 & 3	Glucosamine PUGNAc Anti IL-6 antibody	Reduction in CO, +dp/dtmax and –dp/dt max	Rise in cardiac NF-κB, IL-6, TNFα, ICAM-1, MPO associated inflammation and cardiac dysfunction
Laparotomy, fixed pressure. Resuscitation (78)	Not performed	Il-6 NF-κB HIF-1α	YC-1 (anti HIF-1 α)		Il-6 rise in cardiomyocytes induced by HIF-1α