Role of clinical pathologies in myocardial injury following ischaemia and reperfusion

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Abstract

The myocardial injury that occurs following a period of ischaemia and reperfusion is well recognised and the underlying mechanisms have been extensively investigated in healthy myocardial tissues; however, the influence of the risk factors associated with cardiovascular diseases on ischaemia/reperfusion injury remains unclear. In this article, the present knowledge on how clinical pathologies such as diabetes, hypercholesterolaemia, hypertension, myocardial hypertrophy and heart failure may influence ischaemia/reperfusion injury of the heart and the potential mechanisms involved are reviewed.

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1. Introduction

The mechanisms underlying ischaemia/reperfusion injury have been extensively studied using healthy tissues, yet less attention has been devoted to defining how the different risk factors for cardiovascular diseases may influence the degree of injury. The aim of this article is to review present knowledge on how clinical pathologies such as diabetes, hypercholesterolaemia, hypertension, myocardial hypertrophy and heart failure may influence ischaemia/reperfusion injury of the heart and the mechanisms involved. For discussion of the traditional mechanisms implicated with ischemia/reperfusion injury, the reader is referred to the numerous excellent reviews existing in the literature. Due to the scarcity of studies separating ischaemia and reperfusion injury in disease states, this review will not attempt to dissociate the two forms of injury.

2. Diabetes

There is overwhelming clinical data indicating that the diabetic heart is more sensitive to ischaemia/reperfusion injury. Patients with diabetes are at increased risk of ischaemic events [1–6] and diabetes also represents an important risk factor for poor outcomes after coronary revascularization [7–11]. Diabetes alters neutrophil adhesion, elicits endothelial and myocyte dysfunction, and influences oxidative stress and myocardial energetics [12–15], each of which alone or in combination may be responsible for the exaggerated reperfusion injury following a period of ischaemia. In contrast with the concept that the diabetic myocardium is more sensitive to ischaemia/reperfusion injury, our laboratory [16,17] has consistently demonstrated that the human diabetic myocardium obtained from the right atrial appendage at the time of elective cardiac surgery and subjected to in vitro conditions of ischaemia and reperfusion exhibits a similar sensitivity to injury as the myocardium from subjects without diabetes. It is recognised that diabetic patients have altered platelet and fibrinolytic function that may contribute to exacerbate ischaemia/reperfusion injury, and the lack of blood components in our model may provide a plausible explanation for
the apparent discrepancy between clinical observations and our in vitro studies.

Experimental studies are also divided on the susceptibility of diabetic myocardium to ischaemia/reperfusion injury reporting more [14,18,19], less [20–23] or similar [24,25] sensitivity than found in hearts without diabetes. A possible explanation for the differing results may be the use of different experimental conditions such as the duration and severity of the diabetic state, the nutritional status and cardiac function, the degree of ischaemia, and the type of metabolic substrate used. Another potential contributing factor could be the presence of a diabetic cardiomyopathy that may be responsible for the contractile dysfunction observed in diabetic patients [26,27]. In addition, several mechanisms, including: (i) alterations in the control of intracellular pH and Ca\(^{2+}\) with improvement in the recovery of contractile function during reperfusion [22]. However, during low flow ischaemia, the degree of intracellular acidosis is less because of the washout of lactate, and under these conditions and because of the lower glycolytic rates during ischaemia may result in reduced accumulations of Na\(^+\) and Ca\(^{2+}\) with improvement in the recovery of contractile function during reperfusion [22].

Hyperglycemia is an important risk factor for post-myocardial infarction mortality in subjects with and without diabetes [40]. This clinical effect may be the result of high ambient glucose-induced excessive cell death [41], which in turn has been reported to be due to high production of reactive oxygen species activating JNK and then caspase-3 [42]. Hyperglycemia has also been shown to acutely increase circulating inflammatory cytokines in humans [43]. Several studies have demonstrated elevated levels of IL-6 and TNF-\(\alpha\) among individuals both with features of the insulin resistance syndrome and with clinically overt type 2 diabetes mellitus [44–46] and a prospective study has found that two circulating markers of systemic inflammation, C-reactive protein and IL-6, are risk determinants for the development of type 2 diabetes mellitus in apparently healthy middle-age women [47]. There is in vitro evidence that the release of TNF-\(\alpha\) induced by high glucose may be mediated by reactive oxygen species [48] and that oxidative stress might be implicated in promoting a low-grade systemic inflammation in patients with type 2 diabetes mellitus [45]. Inflammatory factors may influence the sensitivity of the non-diabetic heart to ischaemia/reperfusion injury and when over-expressed they may exacerbate the injury in the diabetic heart. In this connection, diabetes has been associated with exaggerated leukocyte–endothelial cell adhesion and albumin leakage responses of postcapillary venules following ischaemia/reperfusion [49].

Nitric oxide (NO) when produced in large amounts (\(\mu\)M) is a toxic and damaging agent [50], whereas when produced in low concentrations (nM) is protective [51]. Impaired eNOS activity and endothelial dysfunction are seen in various disease states, including diabetes [52,53], hypertension [54], hypercholesterolaemia [55] and atherosclerosis [56]. The expression of eNOS is upregulated in diabetes [56] and this has been held responsible for the intrarenal vasodilatation that is characteristic of early diabetes [57]. A number of studies have reported decreased ischaemia/reperfusion injury following the administration of NO donors [58–60]; however, the manipulation of endogenous NO using pharmacological means or transgenic NOS knockouts have rendered conflicting results [61–64]. It is also controversial whether the increase or decrease in iNOS activity attenuates or exacerbates injury [63,65]. At present, limited evidence exists that the upregulation of eNOS or any other modification of the NO metabolism will protect myocardium from ischaemia/reperfusion injury under pathological conditions such as diabetes. Clearly, further investigations are required to fully elucidate the role of NO in ischaemia/reperfusion injury in diabetes.
Katp channels have been shown to play a role in cardioprotection but their place in the signal transduction pathway and whether they are triggers or mediators have not been fully elucidated [66–70]. Since diabetes alters the function of vascular and myocardial Katp channels [71], it may be speculated that these alterations may reduce cardioprotection of the diabetic heart to ischaemia/reperfusion injury. Our own laboratory has demonstrated, using an in vitro model of ischaemia/reoxygenation of human myocardium, that blocking the mitoKatp channel does not exacerbate the degree of injury in both diabetics and non-diabetics [16,17]. However, these studies also showed that whereas the application of ischaemic preconditioning and the mitoKatp channel opener diazoxide cardioprotected the non-diabetic myocardium the diabetic myocardium was not [16,17], thus suggesting that the failure to precondition the diabetic heart is due to a dysfunction of mitoKatp channels. The demonstration that protection of the diabetic myocardium can still be elicited by pharmacological activation of PKC and p38-MAPK suggests that the signal transduction pathway downstream of mitoKatp channels is still unaffected by the disease state [17], an observation that can be exploited to obtain cardioprotection in the diabetic heart.

An important question when considering the protection of the diabetic heart from ischaemia/reperfusion injury is whether the control of glucose metabolism, including that of insulin levels, can be of therapeutic utility. Insulin is cardioprotective of ischaemia/reperfusion events [72,73] and it re-instates protection by preconditioning in type 2 diabetic patients [74]. Furthermore, the combined treatment of glucose, insulin and potassium has been shown to be protective in non-diabetic animals [75,76] and to improve long-term survival following myocardial infarction in diabetics in the DIGAMI randomised trial [77]. From the existing literature, it is clear that more research is required to fully elucidate the role of diabetes in ischaemia/reperfusion injury and the underlying mechanisms in order to counteract tissue damage and to exploit the pathways of cardioprotection.

3. Hypercholesterolaemia

Elevated serum cholesterol has long been associated with an increased risk for coronary artery disease and the development of myocardial infarction [78,79]. In experimental studies, hypercholesterolaemia results in larger myocardial infarcts in the rabbit subjected to ischaemia and reperfusion after short-term cholesterol diet [80,81], but in mice, short-term (2-week) exposure to high cholesterol levels does not alter the development of infarct size in the wild-type animal although it markedly enhances infarct size in animals deficient in the low density lipoprotein receptor [82]. However, prolonged (> 6 weeks) exposure to elevated circulating cholesterol levels protects the myocardium from ischaemia/reperfusion injury in the rabbit [83] and in the wild-type and low density lipoprotein receptor deficient mice [82]. A possible explanation for these apparently opposed findings may be a reduction of myocardial GSH levels after 2 weeks of the start of the cholesterol diet but with increased GSH levels after 12 weeks [82].

One of the earliest events associated with elevated serum cholesterol is a diminution in NO release by the vascular endothelium [83], an effect that may be responsible for the impaired endothelium-dependent vascular reactivity reported in animal models of hypercholesterolaemia [84–87] and in man [88,89]. Microvascular dysfunction can be central in the development of atherosclerosis and can also exert a profound influence in ischaemia/reperfusion injury. The observation that hypercholesterolaemic rats exhibit enhanced leukocyte adhesion and microvascular protein leakage in the setting of mesenteric ischaemia/reperfusion [90] suggests that vascular alterations may be responsible for the enhanced ischaemia/reperfusion injury in the setting of hypercholesterolaemia. In the heart, a decrease in NO also exacerbates ischaemia/reperfusion injury due to an increase in the accumulation of leukocytes in the myocardium [91,92]. On the other hand, the administration of the NO donor S-nitroso-N-acetylpenicillamine during 30 min of ischaemia and for the first 30 min of reperfusion effectively reduces infarct size and the myocardial accumulation of leukocytes in rabbits with acute hypercholesterolaemia after 24 h of reperfusion [93].

Another potential cause for the exacerbation of ischaemia/reperfusion injury by hypercholesterolaemia is increased oxidative stress. In this connection, it has been shown that the production of superoxide by arterial endothelial cells is enhanced in hypercholesterolaemic rabbits [94] and that a marked increase in oxidant production occurs after ischaemia/reperfusion from mesenteric venules of hypercholesterolaemic rats [95]. Furthermore, the observation that an increased oxidative stress can be blunted by treatment with allopurinol and oxyypurinol suggests that xanthine oxidase is a major source of free radicals in hypercholesterolaemic animals [94,95], although the role and existence of this enzyme in some animal species such as the rat [96], rabbit [97] and man [98] have been questioned.

The cholesterol-lowering agents known as statins extend the half-life of nitric oxide synthase mRNA in endothelial cells [99]. This increase in bioavailability of NO by statins has resulted in inhibition of leukocyte–endothelium interactions in the microcirculation of normocholesterolaemic animals [99,100] and in protection of the ischaemic and reperfused myocardium from polymorphonuclear neutrophil-mediated injury [101,102]. Statins also increase PI3-K activity [103] and adenosine production [104], suggesting that the cardioprotective effect of statins may be mediated by pleiotropic mechanisms. The beneficial actions of statins in normocholesterolaemic subjects also suggest that these agents exert their action independently of their cholesterol-lowering effects.
4. Hypertension

Hypertension is a complex syndrome in which the primary symptom is a chronic elevation in blood pressure. Although it is well established that the renin–angiotensin system is central to hypertension and that it contributes to ischaemic coronary events \([105–107]\), little is known about the influence of hypertension on ischaemia/reperfusion injury. AT1 receptor expression is increased markedly following ischaemia and reperfusion in the rat heart \([108–110]\) and receptor expression and activation have been suggested to play a critical role in the genesis of ischaemia/reperfusion injury. Thus, both ACE inhibitors and angiotensin II receptor antagonists have been shown to protect the myocardium from ischaemia/reperfusion injury in experimental animal models by reducing infarct size and reperfusion arrhythmias and by improving LV function \([111–115]\). Interestingly, the combined use of ACE inhibitors and angiotensin II receptor antagonists may act synergistically to further reduce infarct size in the pig \([116]\). Clinical studies have also demonstrated that the use of ACE inhibitors and angiotensin II receptor blockers in patients with ischaemic heart disease improves LV function and prevents geometric remodelling, thereby suppressing cardiovascular events following a myocardial infarction and ultimately increasing the survival of patients \([117,118]\).

Angiotensin II may contribute to reperfusion injury by increasing oxidative stress and inflammatory factors. Indeed, there is experimental evidence suggesting that increased oxidative stress is related to activation of the renin–angiotensin system \([119,120]\). Spontaneous augmentation in the production of reactive oxygen metabolites has been reported in the vasculature of hypertensive rats \([121]\) and hypertensive Dahl salt-sensitive rats \([122]\). Recently, it has been shown that oxidative stress is also elevated in patients with hypertension \([123]\) and this is a factor that may contribute to a greater sensitivity of the heart to ischaemia/reperfusion injury; however, the relevance of this mechanism in the context of hypertension remains undefined.

The elevated blood leukocyte count and the greater proportion of activated granulocytes circulating in spontaneous hypertensive rats as compared to Wistar–Kyoto rats \([124]\) may result in an enhanced inflammatory response during reperfusion and in exacerbation of ischaemia/reperfusion injury. Indeed, published reports suggest that arterial hypertension could enhance \([124,125]\) the inflammatory responses elicited by ischaemia/reperfusion, thereby increasing the vulnerability of the microvasculature to ischaemia/reperfusion injury, although these findings have been disputed \([126,127]\). The demonstration of an exaggerated albumin leakage response in the mesenteric postcapillary venules of spontaneously hypertensive rats during reperfusion after only 10 min of ischaemia (an action mediated by the interaction between beta2 (CD18) integrins on leukocytes and ICAM-1 on endothelial cells) \([128]\) would support the view that inflammatory factors play a role in intensifying ischaemia/reperfusion injury in hypertension.

5. Myocardial hypertrophy

Cardiac hypertrophy affects 15–20% of adults in the general population \([129]\) and is an adaptive response to increased pressure or volume by which myocardial stress is normalised \([130]\). However, the response is also mal-adaptive in that hypertrophied hearts develop greater left ventricular dysfunction than non-hypertrophied hearts following ischaemia \([131–134]\). The underlying mechanisms responsible for this increased susceptibility remain unclear; however, three major factors might contribute to exacerbate ischaemia/reperfusion injury: (i) alterations in ionic calcium handling, (ii) alterations in myocardial energy metabolism, and (iii) anatomic and functional abnormalities of the coronary bed such as a decrease in capillary density and in coronary reserve (for further discussion on this subject, see Refs. \([135,136]\)).

Calcium overload during reperfusion is one of several mechanisms postulated to be responsible for myocardial dysfunction following ischaemia and reperfusion in the normal heart \([137,138]\). Alterations in Ca\(^{2+}\) handling with overload of Ca\(^{2+}\) during reperfusion have been reported in hypertrophied hearts \([139]\) and this has been shown to play a significant role in post-ischaemic left ventricular dysfunction \([140]\). Experimental studies in the hypertrophied rat heart have demonstrated that verapamil, administered for 3 days before a period of 25 min of coronary artery ligation, causes a reduction of injury after 1 h of reperfusion \([141]\). The increased incidence of reperfusion-induced ventricular fibrillation in the hypertrophied heart after 15 min of coronary artery occlusion in the awake, unsedated dog \([142]\), and in the rat \([143]\), and their attenuation by calcium antagonists when administered before the onset of ischaemia \([144]\), further support the concept of Ca\(^{2+}\) playing an important role in ischaemia/reperfusion injury of the hypertrophied heart. Left ventricular hypertrophy has been coupled with electrophysiological alterations, such as increased transmembrane inward calcium current \([145]\), decreased cesium-sensitive early outward currents resulting in a net increase in slow inward calcium current \([146]\), and early and delayed afterpotentials resulting in triggered activity \([147]\), which might predispose hearts to arrhythmias and to increased likelihood of sudden death in man \([148,149]\).

Alterations in myocardial energy metabolism, particularly glucose metabolism, are thought to contribute to this increased susceptibility to injury \([150,151]\). It has been shown that the uncoupling of glycolysis from glucose oxidation during reperfusion is detrimental \([152]\) and the stimulation of glucose oxidation, which improves coupling between glycolysis and glucose oxidation, is beneficial \([153–155]\). In hypertrophied hearts, the uncoupling of glycolysis from glucose oxidation during reperfusion is
more exaggerated than in nonhypertrophied hearts which makes the hypertrophied heart more susceptible to ischaemia/reperfusion injury [150,151], an observation that is consistent with the finding that a number of glycolytic enzymes are elevated in hypertrophied hearts [155]. As expected, activation of pyruvate dehydrogenase with dichloroacetate at the time of reperfusion to stimulate oxidation also improves the metabolic coupling and the post-ischaemic recovery of cardiac function in the hypertrophied heart [156] to a degree similar to that seen in non-hypertrophied hearts [154]. The metabolic imbalance in the hypertrophied heart can result in accelerated H⁺ production and this has been proposed as an important factor contributing to the increased susceptibility to ischaemia/reperfusion injury in hypertrophied hearts [157]. Since acidosis per se has been shown to interfere with myofilament function [158], the exaggerated H⁺ load may be directly responsible for the greater contractile dysfunction observed in the hypertrophied heart. Thus the elevated production of H⁺ may contribute to significant disturbances in myocardial ion homeostasis and tissue injury during ischaemia/reperfusion caused by Na⁺ and Ca²⁺ overload via the Na⁺/H⁺ and Na⁺/Ca²⁺ exchangers [159]. Intracellular Na⁺ and Ca²⁺ accumulation during reperfusion is greater in the hypertrophied heart than in the normal heart and this has been found to be associated with impaired functional recovery [139,160]. It has been reported that the hypertrophied heart also has alterations to key ion transport proteins that can potentiate the effect of H⁺ overproduction. Thus, for example, it has been reported that the phenotypic expression of the Na⁺/H⁺ exchanger is altered in the hypertrophied myocardium, which may lead to exaggerated intracellular Na⁺ accumulation during ischaemia and reperfusion [161]. The demonstration of lower sarcolemmal Na⁺/K⁺-ATPase activity in the hypertrophied heart than in the normal heart [162] can also contribute to the increased accumulation of Na⁺ under these conditions. Both increased [163] and decreased [164] activity and/or expression of the Na⁺/Ca²⁺ exchanger have been reported in the hypertrophied heart and it is unclear what role this exchanger may play; although the two activity states of the exchanger could potentiate Ca²⁺ overload by increasing influx on the one hand, and decreasing efflux on the other. The described reduction in sarcoplasmic reticulum Ca²⁺-ATPase activity in the hypertrophied myocardium [165] could also contribute to ionic imbalance by exacerbating Ca²⁺ overload. These metabolic and ionic changes may be responsible, at least in part, for the greater susceptibility of the hypertrophied heart to ischaemia/reperfusion injury.

To conclude this section, it should be noted that hypertrophy can be the result of hypertension and, although they are two distinct clinical entities, they can share common mechanisms of injury following ischaemia and reperfusion. A growing body of evidence suggests that the renin–angiotensin system plays an important role in the development of cardiac hypertrophy induced by haemodynamic overload and left ventricular remodelling after myocardial infarction and, as shown in the previous section, this mechanism may also account, at least in part, for ischaemia/reperfusion injury in the hypertrophied heart.

6. Heart failure

The number of patients with chronic heart failure is constantly increasing, a growth due in part to an increase in the overall life expectancy. It is believed that the failing heart is more susceptible to injury when subjected to ischaemia and reperfusion and there is experimental evidence that rabbits with pronounced morphological signs of chronic heart failure have an enhanced susceptibility to ischaemia/reperfusion-induced arrhythmias [166]. An increase in oxidative stress together with the sustained expression of proinflammatory mediators in heart failure are strong candidates for the causes of the exacerbation of injury in heart failure.

Oxidative stress, as measured by markers of lipid peroxidation and levels of antioxidant defenses, is increased in patients with congestive heart failure [167,168]. It appears that the degree of oxidative stress correlates with the functional class—the worse the degree of heart failure the greater the oxidative stress—implying that free radical mediated injury may play a role in the progression of heart failure. In the hypertrophied and failing guinea pig heart, there is a relative deficiency of antioxidant enzymes [169]; however, the first direct evidence that superoxide levels are increased in heart failure has been provided in the canine pacing model of heart failure [170]. In this model, it was shown that mitochondria isolated from cardiac myocytes produce 2.8-fold more superoxide than controls. This effect was due to a reduction of the enzymatic activity of complex I which results in a functional block in the flow of electrons through the mitochondrial chain transporter. In additional studies, the same authors demonstrated that the enhancement of superoxide and hydroxyl radicals in failing hearts correlated with the level of myocardial dysfunction [171]. Therefore, it is possible to argue that an increased oxidative stress may be one of the factors responsible for the greater susceptibility of the failing heart to post-ischaemic injury.

Cytokines, such as TNF-α, are augmented in failing hearts and can induce cardiac and vascular responses that appear to be modulated by specific redox-sensitive intracellular pathways and contribute to the increased sensitivity to ischaemia/reperfusion injury [172]. TNF-α upregulation leads to production of the second messenger ceramide [172], a condition also present in heart failure [173–175]. Ceramide inhibits complex III of the mitochondrial electron transport chain [174] causing an increase in the production of reactive oxygen species [175], which in turn predisposes cells to the mitochondrial-driven apoptotic pathway [175,176]. As mentioned earlier, mitochondria play an important role in the death of myocytes via an increased
oxidative stress and the above results reinforce the view that reactive oxygen species may be central to an increased susceptibility to ischaemia/reperfusion injury in congestive heart failure.

As seen earlier in this article, neurohormones, such as angiotensin II, can influence myocardial ischaemia/reperfusion injury and their increase in heart failure may exacerbate tissue damage. This thesis is supported by the observation that the acute administration of the ACE inhibitor quinaprilat in a rat model of heart failure results in the improvement of post-ischaemic systolic and diastolic function, coronary perfusion and the content of high energy phosphates [177].

Despite the evidence in the literature that the failing heart may be more sensitive to ischaemia/reperfusion injury, our laboratory has shown that when the human myocardium obtained from the right atrium of patients with a left ventricular ejection fraction less than 30% is subjected to 90 min of ischaemia and 120 min of reoxygenation, it exhibits a similar degree of injury as the myocardium of patients with a greater ejection fraction [16]. The same studies also demonstrated that although the myocardium from patients with poor heart function cannot be cardioprotected by ischaemic preconditioning, protection was obtained by the mito KATP channel opener diazoxide [16]. These results suggest that the problem with the failing heart lies not in the sensitivity to ischaemia/reperfusion injury but in the mechanism of protection, a defect that can be overcome by bypassing some elements of the signal transduction pathway responsible for cardioprotection.

7. The future

There is scarce data on the role of clinical pathologies on ischaemia/reperfusion injury yet the existing working hypotheses are controversial. A major problem is the lack of reliable animal experimental models of human diseases. An additional challenge is the ethical constraint of performing clinical studies and the superimposed co-morbid conditions that serve to complicate both the execution of the investigations and the interpretation of the results. It is clear that the search for the development of animal models of human diseases is much needed and remains as important as ever. This together with the increasing availability of powerful research diagnostic tools such as proteomics and genomics may yet prove to be the springboard to unravel the particular aspects of injury caused by ischaemia and reperfusion and the specific role of clinical pathologies. Not until then will we be in a position to design therapeutic interventions to counteract ischaemia/reperfusion injury in a comprehensive and effective manner. In investigating the role of disease states in ischaemia/reperfusion injury it is important to elucidate whether there are one or more pathways of injury, which is the dominant one for each of the diseases and whether and how they alter the innate mechanism of protection.

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