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Review

Pharmacological possibilities for protection against myocardial reperfusion injury

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Abstract

Reperfusion through thrombolysis or percutananeous coronary angioplasty is standard treatment in impending acute myocardial infarction. Although restoration of blood flow to the jeopardised myocardial area is a perquisite for myocardial salvage, reperfusion itself may lead to accelerated and additional myocardial injury beyond that generated by ischemia alone. This is referred to as the ''reperfusion injury''. Since the reperfusion injury is initiated by the treatment of myocardial infarction, it is of importance to limit the extent of the injury. Several studies aimed at preventing reperfusion injury by means of pharmacological agents have therefore been conducted. The design of such studies is crucial for the results. Factors of importance are the timing of drug administration, animal species used, the degree of collateral flow and the duration of ischemia. A variety of pharmacological compounds have been investigated in different experimental models of myocardial ischemia and reperfusion. These include oxygen free radical scavengers, antioxidants, calcium channel blockers, inhibitors of neutrophils, nitric oxide, adenosine-related agents, inhibitors of the renin-angiotensin system, endothelin receptor antagonists, Na^+/H^+ exchange inhibitors, and anti-apoptotic agents. All these groups of pharmacological agents have been demonstrated to protect from reperfusion injury determined as limitation of infarct size, improved myocardial and endothelial function, and reduced incidence of arrhythmias. The mechanism behind the protective effect may differ between different groups of compounds, but some compounds may exert cardioprotection via common pathways. Such a pathway may be via maintained bioavailability of nitric oxide.

Keywords: Reperfusion; Free radicals; Nitric oxide; Endothelins; Na/H-exchanger; Angiotensin; Leukocytes

1. Introduction

Reperfusion through thrombolysis or percutaneous coronary angioplasty (primary PTCA) is standard treatment in impending acute myocardial infarction. Although restoration of blood flow to the jeopardized myocardial area is a prerequisite for myocardial salvage, reperfusion itself may lead to additional tissue injury beyond that generated by ischemia alone, 'reperfusion injury'. The manifestations of reperfusion injury include arrhythmia, reversible contractile dysfunction-myocardial stunning, endothelial dysfunction and cell death. Although debated [1,2] there are reasons to believe that irreversible reperfusion injury is a pathologic phenomenon by itself [3–5]. The mechanisms proposed to contribute include oxygen free radical formation, calcium overload, neutrophil-mediated myocardial and endothelial injury, progressive decline in microvascular flow to the reperfused myocardium, and depletion of high energy phosphate stores. Possible mechanisms behind reperfusion injury have been extensively reviewed elsewhere [6–8] and are accordingly not the topic of this article, which will focus on the possibility of preventing reperfusion injury by means of pharmacological agents. The outcome of many in vitro and in vivo studies, although not all, suggests that this is possible if treatment is initiated at the very onset of reperfusion. Most of these studies were carried out in uninjured, naive vessels in the absence of coronary atherosclerosis. Clinical studies on this topic are sparse, which probably relates to the technical difficulties

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in quantifying myocardial area at risk, final infarct size, and differences in duration from coronary occlusion to reperfusion. However, on the basis of the encouraging effects in experimental data, several clinical trials of various pharmacological strategies are under way. The purpose of the present review is to summarize experimental studies undertaken to elucidate the possibilities to decrease the impact of reperfusion injury by the use of pharmacological agents.

2. Pharmacological agents and targets

A variety of pharmacological compounds have been investigated, in particular oxygen free radical scavengers, antioxidants, calcium channel blockers, inhibitors of neutrophils, nitric oxide, adenosine-related agents, inhibitors of the renin–angiotensin system, endothelin receptor antagonists, Na^+/H^+ exchange inhibitors, and anti-apoptotic agents (Fig. 1). Some of these studies, however not all, demonstrated positive effects. Reasons for contradictory results are not readily apparent but several factors may contribute. Among them are differences in animal species, degree of collateral blood flow, duration of ischemia, timing of drug administration, drug delivery method, and different end-points of cardioprotection (infarct size, myocardial function, endothelial function or arrhythmia). For example, too short periods of ischemia may not cause significant reperfusion injury, while too long periods may have induced nearly complete ischemic myocardial injury, leaving a negligible component of reperfusion injury. It is also of importance that therapeutic concentrations of the pharmacological agent are present in the ischemic myocardium at the very onset of reperfusion. Drugs given intravenously during ischemia may not reach the ischemic area in the absence of collaterals. Even in the presence of collaterals, a large intravenous dose is usually needed to achieve significant concentrations in the ischemic area. Systemic effects may then induce negative hemodynamic side-effects. In contrast, local drug administration, such as antegrade intracoronary administration, has the advantage that the compound is selectively delivered into the targeted region reaching a sufficient level without affecting the non-ischemic myocardium. One technique is retrograde coronary venous drug delivery [9]. Intracoronary administration before the opening of an occluded artery is a clinically more feasible way.

2.1. Free radical scavengers/antioxidants

The generation of oxygen free radicals is a key process in the development of reperfusion injury. Molecules involved in free radical reactions include superoxide anion, hydroxyl radical, hydrogen peroxide, peroxynitrite and hypochlorous acid. Free radicals contain an unpaired

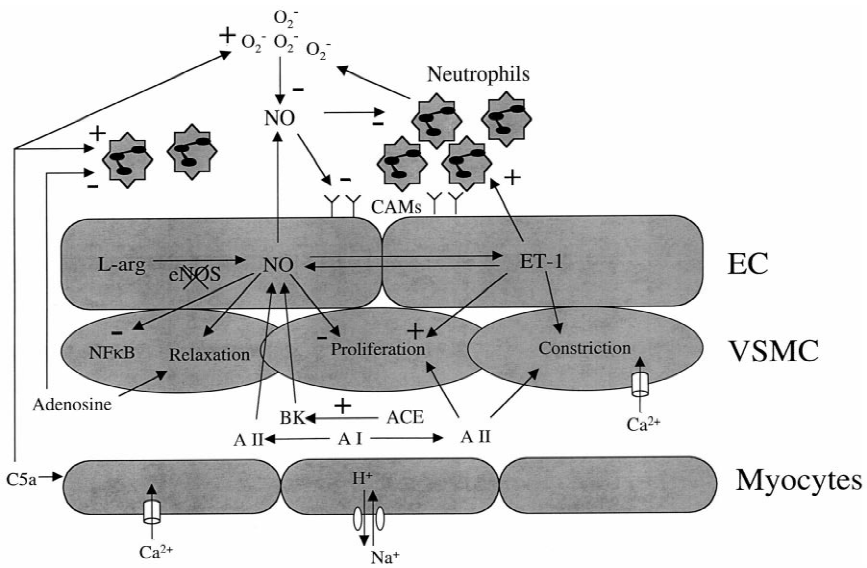

Fig. 1. Summary of the effect of several mediators during ischemia/reperfusion. Following reperfusion, the levels of nitric oxide (NO) are decreased due to reduced activity of the enzyme eNOS and enhanced levels of superoxide which are produced by neutrophils and other sources. This results in increased vascular tone due to reduced relaxation by NO and enhanced production of the vasoconstrictors endothelin-1 (ET-1) and angiotensin II (A II). In addition, the pro-inflammatory transcription factor nuclear factor kappa B (NFκB) is activated which results in increased expression of pro-inflammatory cytokines and cell adhesion molecules (CAM) which in turn stimulate the adhesion of neutrophils. Complement factors such as C5a contribute to membrane damage and stimulation of neutrophils and superoxide production. The reperfusion injury can be attenuated by enhancing levels of NO, blocking ET-1 receptors, blocking the production (ACE-inhibitors) or the receptors (AT1) of A II, stimulating adenosine receptors, blocking the complement system, blocking calcium channels, blocking Na^+/H^+ channels or inhibiting neutrophil adhesion via the CAMs. Important interactions seem to exist between several of these mediators. For instance, the cardioprotective effect achieved by blockade of ACE, A II receptors or ET-1 receptors seems to involve activation of bradykinin (BK) and/or NO.

electron and are accordingly highly reactive. Reintroduction of abundant oxygen at the very onset of reperfusion evokes a burst of free radicals as demonstrated in experimental settings as well as in humans with acute myocardial infarction undergoing thrombolysis [10] or PTCA [11] and also in patients undergoing open heart surgery [12]. The release of free radicals, in combination with the ischemia-induced decrease in antioxidant activity, renders the myocardium extremely vulnerable. Oxygen radicals react readily with cellular phospholipids and proteins, causing lipid peroxidation and oxidation of thiol groups with subsequent alteration of membrane ultrastructure and dysfunction of various cellular proteins.

Oxygen free radical scavengers or antioxidants tested include superoxide dismutase (SOD), the H_2O_2 degrading enzyme catalase, the xanthine oxidase inhibitor allopurinol, the iron-chelator deferoxamine, and antioxidants including vitamin E and vitamin C. The first to report on a beneficial effect were Jolly and coworkers [13] in a canine ischemia/reperfusion model. Left circumflex coronary artery occlusion for 90 min was followed by 24 h of reperfusion. Administration of SOD and catalase into the left atrium reduced final infarct size when the infusion started before ischemia or 15 min before reperfusion, but there was no effect when the infusion was started 40 min following reperfusion, suggesting that the oxygen free radical-mediated damage is an early event. This study serves as an example of the critical importance of proper timing of drug delivery. In another study, infarct size was reduced when SOD was delivered retrogradely via the great cardiac vein into myocardial tissue made ischemic via LAD occlusion in pigs [14]. No beneficial effect was observed when the same amount of SOD was given via the left atrium. This may serve as an example of the importance of collaterals. Pigs lack coronary collaterals making antegrade administration of the scavenger during persistent LAD occlusion ineffective [14].

Detrimental effects of oxidative stress have also been demonstrated by the cardioprotective effect of antioxidants such as vitamin E analogues and lipid peroxidation inhibitors. Due to its high lipophilicity and slow incorporation into tissue, vitamin E has limited value for administration in the acute situation. In contrast, vitamin E analogues that are more hydrophilic are effective in attenuating ischemia/reperfusion injury. In a rat model of 45 min of coronary artery occlusion followed by 5 h of reperfusion, Altavilla et al. [15] observed that IRFI 042, a novel vitamin E analogue, significantly reduced infarct size in a dose-dependent manner when given 5 min after the initiation of reperfusion. Lipid peroxidation is a major mechanism for oxygen free radical mediated injury. The lipid peroxidation inhibitor H290/51, given retrogradely by coronary venous infusion shortly before reperfusion, reduced myocardial infarcts in pigs subjected to 45 min of LAD occlusion followed by 4 h of reperfusion [16].

Other studies have demonstrated that reperfusion arrhythmias and myocardial stunning are ameliorated by free radical scavengers or antioxidants. In dogs subjected to 90 min of coronary artery occlusion followed by 1 week reperfusion, recombinant SOD, administered before and 1 h after reperfusion, reduced reperfusion arrhythmias [17]. Administration of SOD before thrombolysis in an acute coronary thrombosis also reduced reperfusion arrhythmias and preserved myocardial function [18]. The postischemic recovery of contractile function has been shown to be enhanced by SOD, catalase, *N*-(2-merceptopropionyl)-glycine and other antioxidants. The amelioration of myocardial stunning was observed when the drug administration was started before ischemia or 1 min before reperfusion but not 1 min after reperfusion, suggesting that free radicals generated immediately after reperfusion are critical in myocardial stunning [19].

Some studies have failed to show any effects on myocardial infarct size. Thus, no infarct limitation was observed with SOD or the antioxidant *N*-(2-mercaptopropionyl)-glycine given shortly before reperfusion in dogs after 40 or 90 min of ischemia and 4 days of reperfusion [20,21]. Similarly, human recombinant SOD plus catalase, given before reperfusion by intravenous infusion or intraatrial bolus followed by intravenous infusion, failed to reduce myocardial infarct size in baboons subjected to 120 min of LAD occlusion followed by 22 h of reperfusion [22]. Interindividual variation of collateral flow and the technique for measuring infarct size were discussed as possible explanations for these conflicting results [23]. Moreover an ischemic time of 120 min may be too long to rescue any myocardium. In addition, the variable effects of SOD might be related to the limited cell penetration of the exogenous enzyme as suggested by a recent study in transgenic mice with overexpression of SOD [24]. In transgenic animals overexpressing SOD the infarct size was 50% smaller than in the nontransgenic animals after 30 min of global ischemia followed by either 45 or 120 min of reperfusion.

2.2. Calcium antagonists

Calcium overload may contribute to ischemia/reperfusion injury in several ways. It may for instance induce excessive myofilament activation at the moment of reoxygenation and in addition, the rise in intracellular calcium (Fig. 1) causes an increase in mitochondrial calcium. This leads to decreased mitochondrial ability to generate ATP limiting metabolic recovery of the myocyte. Finally, a number of calcium-activated proteases may destroy critical intracellular structures. Short periods of low calcium perfusion prior to ischemia or at reperfusion effectively preserves postischemic myocardial mechanical performance. The definite pathways for calcium entry are still undetermined, but the Na^+/Ca^{2+} exchanger and the voltage-gated calcium channel are the most likely [25].

Calcium antagonists are cardioprotective in the setting

of myocardial ischemia/reperfusion when administered prior to the induction of ischemia. Since long-acting calcium antagonists were used, these studies do not separate between protection against injury induced by ischemia or reperfusion in particular [26–28]. It was suggested that the beneficial effects of calcium antagonists were related to the negative inotropic and/or chronotropic, the energy-saving effects of these compounds. In addition, some calcium antagonists are antioxidants or nitric oxide synthase regulators. More recent studies have, however, revealed that calcium antagonists do not only have an anti-ischemic effect, they are also protective against myocardial injury caused by reperfusion in itself. A number of reports showed that calcium antagonists given immediately before or at the onset of reperfusion were protective, suggesting an effect against reperfusion injury [29–31]. Herzog et al. [31] demonstrated that a low dose of diltiazem delivered via LAD exclusively during early reperfusion diminished infarct size in the swine heart. They reported that the crucial time was within the first 12 min of reperfusion. In pigs subjected to LAD occlusion followed by 3 h of reperfusion, it was demonstrated that retrograde coronary venous infusion of the calcium antagonist diltiazem for 30 min beginning 5 min before reperfusion reduces final infarct size [32]. This effect was not simply due to a delay of infarct size development. A similar, beneficial effect of felodipine was observed when the reperfusion time was prolonged to 24 h [30]. On the other hand, one study by Klein et al. [33] demonstrated that although pretreatment with diltiazem prior to ischemia significantly reduced the infarct size in pigs subjected to 75 min ischemia followed by 4 h of reperfusion, intravenous diltiazem 30 min before reperfusion only resulted in an insignificant reduction in infarct size. One possibility for this lack of significant effect of diltiazem prior to reperfusion might be that an insufficient amount of diltiazem reached the ischemic myocardium at the time of reperfusion as discussed above.

Calcium antagonists have also been shown to reduce reperfusion arrhythmias and attenuate myocardial stunning. Verapamil decreased reperfusion arrhythmias in isolated rat hearts as well as in pigs [34,35]. In dogs subjected to 15 min of coronary artery occlusion followed by 3 h of reperfusion, intracoronary infusion of nifedipine from 2 min before to 5 min after reperfusion markedly enhanced the postischemic contractile function and diminished ventricular fibrillation during reperfusion [36], while infusion from 0 to 30 min after reperfusion failed to alter contractile function or ventricular fibrillation.

2.3. Inhibitors of neutrophils

Neutrophils are important for the development of reperfusion injury by releasing oxygen free radicals, proteases and pro-inflammatory mediators that further amplify the infiltration of neutrophils into the jeopardized myocardium [37,38] (Figs. 1 and 2). Attempts to diminish neutrophil-mediated injury have included depletion of neutrophils, direct inhibition of neutrophils and inhibition of cell adhesion molecules on neutrophils and endothelial cells. Depletion by administration of antibodies directed against neutrophils or neutrophil clearing filters attenuates the ‘no reflow’ area and reduces infarct size [39,40]. Conversely, addition of neutrophils to buffer-perfused

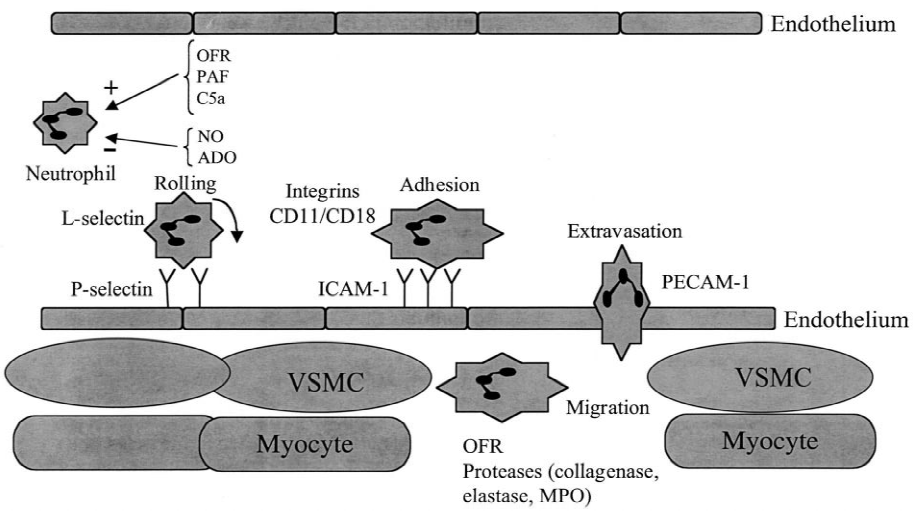

Fig. 2. Interaction between neutrophils and adhesion molecules. Circulating neutrophils are inhibited by nitric oxide (NO) and adenosine (ADO), whereas they are activated by oxygen free radicals (OFR), platelet activating factor (PAF) and various complement factors like C5a. Neutrophils are activated early during reperfusion, which results in rolling, adhesion to endothelial cells and transmigration. Rolling of neutrophils is mediated by P-selectin on endothelial cells. The counterligand for P-selectin on neutrophils may be L-selectin. The firm adhesion is mediated by intercellular adhesion molecule-1 (ICAM-1) on endothelial cells and the integrin complex (CD11/CD18) on neutrophils. The extravasation and migration is mediated by platelet endothelial cell adhesion molecule-1 (PECAM-1) located at the endothelial cell junction. Following transmigration neutrophils release OFR and proteases which contribute to tissue injury and further recruitment of neutrophils. VSMC, vascular smooth muscle cell; MPO, myeloperoxidase.

hearts at the onset of reperfusion significantly aggravates myocardial dysfunction [41].

A critical event in the recruitment of neutrophils during reperfusion is the interaction between adhesion molecules on the neutrophils and on the endothelium. This interaction results in rolling, adhesion and transmigration of neutrophils [37,42]. The adhesion molecules are categorized into three families: selectins, β2-integrins and the immunoglobulin superfamily which are illustrated in Fig. 2. Several studies have evaluated the effect of blocking adhesion molecules as a tool to inhibit neutrophil-mediated injury and thereby reducing reperfusion injury. Administration of a monoclonal antibody against CD11b/CD18 i.v. to dogs 45 min following coronary artery ligation for 90 min followed by 6 or 72 h of reperfusion resulted in significant reductions in infarct size [43,44]. This was associated with reduced neutrophil accumulation in the jeopardized myocardium. In addition, i.v. administration of antibodies against ICAM-1 [45], P-selectin [46], L-selectin [47], and PECAM-1 [48], given 10 min before reperfusion to cats significantly reduced neutrophil adhesion or transmigration and attenuated infarct size by more than 50%. These studies support the notion that infiltrating neutrophils play an important role in the development of the reperfusion injury.

In this perspective, it must be emphasized that reperfusion injury is not entirely dependent on neutrophil-mediated effects. Marked myocardial damage (stunning and infarction) is induced by ischemia/reperfusion in buffer-perfused hearts in which no blood cells are present. Furthermore, there are studies in which reductions in myocardial neutrophil accumulation in vivo are not associated with any reduction in infarct size [49] or improvement in myocardial function [50]. In addition, it was recently reported that mice with genetic deletions of the adhesion molecules ICAM-1 and P-selectin have impaired neutrophil accumulation during ischemia/reperfusion without any difference in infarct size compared to wild-type mice [51].

2.4. Adenosine

Adenosine is present in low concentrations in the normal myocardium but increases during ischemia and reperfusion by hydrolysis of high-energy phosphates such as ATP, ADP and AMP. Adenosine mediates a variety of responses via specific receptors on myocytes, vascular smooth muscle cells, endothelial cells and neutrophils (Fig. 1). In addition to being involved in ischemic preconditioning, adenosine has been demonstrated to mediate protection against reperfusion injury. Intracoronary adenosine given at the onset of reperfusion to dogs following coronary artery ligation preserves endothelial structure, attenuates neutrophil accumulation, improves regional ventricular function and reduces infarct size [52]. Agonists specific for the adenosine A1, A2 and A3 receptor subtypes reduce infarct size when administered just prior to or in connection with reperfusion in vivo [53–56]. Adenosine inhibits adherence of neutrophils to the endothelium, inhibits degranulation of neutrophils and reduces the generation of superoxide by neutrophils, factors which all may contribute to the cardioprotection [37,57].

It should be noted, however, that some studies have demonstrated no protective effects against reperfusion injury. Homeister et al. [58] found that an intracoronary infusion of adenosine to dogs did not reduce infarct size per se, but reduced infarct size when it was administered together with lidocain. In another study in dogs, i.v. adenosine did not reduce infarct size induced by 1 h of coronary artery occlusion followed by 24 h reperfusion [59]. On the other hand, the potent adenosine A1 and A2 receptor agonist AMP 579 significantly reduced infarct size in that study, indicating that the dose of adenosine may have been insufficient. Two other studies using adenosine or an A1 agonist found no effect on infarct size [60,61]. In both these studies, the agonists were administered i.v. before the onset of reperfusion to rabbits which lack coronary collaterals. It is therefore possible that the agonists had not reached the myocardial area at risk in sufficiently high concentrations at the onset of reperfusion as discussed above (Section 2).

The interpretation of the role of adenosine during reperfusion is further complicated by results demonstrating that adenosine A1 receptor antagonists may be protective during ischemia/reperfusion [62,63]. The protective effect of an A1 receptor antagonist was suggested to be due to interference with neutrophils, an effect possibly mediated during the later phase of the reperfusion period [63].

2.5. Inhibitors of the complement system

The complement system is activated during ischemia and reperfusion (Fig. 1). This results in the formation of the anaphylatoxins C3a, C4a, and C5a, as well as the terminal complement complex, the membrane attack complex, which is deposited in cell membranes [8]. The complement factors induce direct cell injury by increasing cell permeability and release of histamine and platelet activating factor. In addition, complement factors, especially C5a, are potent stimulators of neutrophil adherence and superoxide production [37]. Inhibition of the complement cascade by inhibition of C1 esterase [64], a monoclonal antibody against C5a [65] and a C5a receptor antagonist [66] reduces infarct size following myocardial ischemia and reperfusion.

2.6. Nitric oxide (NO)

Endothelium-derived NO produces a variety of biological actions that indicate a protective role during myocardial ischemia and reperfusion (Fig. 1). NO is a powerful vasodilator and may improve blood flow during

reperfusion. In addition, NO inhibits adherence of neutrophils to the vascular endothelium. NO also scavenges superoxide, which is formed in excessive amounts during reperfusion. Reperfusion following an ischemic period is associated with impaired bioavailability of NO, most likely due to enhanced inactivation of NO by superoxide and to reduced production of NO. The development of endothelial dysfunction occurs rapidly and is observed within the first minute following initiation of reperfusion [67]. In isolated rat hearts subjected to global ischemia followed by reperfusion, calcium-dependent NO synthase activity was markedly impaired [68] indicating reduced NO production by the constitutive form of endothelial NO synthase. Based on the reduced NO bioavailability during reperfusion, restoration of NO formation or administration of exogenous NO is likely to result in protection from ischemia/reperfusion injury. Accordingly, administration of NO dissolved in aqueous medium or NO donors reduce infarct size in various experimental models of ischemia and reperfusion in the cat and dog [69–71]. Cardioprotective effects determined as reduction in infarct size in vivo and enhanced recovery of myocardial function in vitro have in addition been obtained with the NO precursor L-arginine [72–74]. The cardioprotective effect of L-arginine could not be reproduced by D-arginine [75] and was blocked by an inhibitor of NO synthase [73] supporting the view that it was mediated by enhanced NO formation. L-Arginine and NO donors protect from myocardial injury when the drugs are administered immediately prior to or at the onset of reperfusion, indicating that NO protects from reperfusion injury. The infarct size was larger and the post-ischemic myocardial functional recovery was lower in mice lacking the gene for endothelial NO synthase than in wild-type mice [76–78], supporting the notion that endogenous NO protects against ischemia/reperfusion injury.

There are also studies indicating that NO may exert anti-arrhythmic actions during ischemia/reperfusion. A NO donor was shown to suppress arrhythmias in pigs subjected to coronary artery occlusion [79], and inhibition of NO synthase increased the incidence of reperfusion-induced arrhythmias in isolated rat and rabbit hearts [80,81].

Several possible mechanisms behind the cardioprotective effect of NO may exist (Fig. 1). It has been demonstrated in several studies that NO donors and L-arginine attenuate neutrophil accumulation in the reperfused myocardial area [82] due to inhibition of the expression of adhesion molecules such as P-selectin, ICAM-1 and VCAM-1 [37,82]. However, since L-arginine and NO-donors exert cardioprotection in isolated buffer-perfused heart preparations [74], other mechanisms are likely to be involved as well. Such a mechanism may be inactivation of superoxide radicals. NO rapidly reacts with superoxide, which is formed in large amounts during ischemia and reperfusion. This reaction yields peroxynitrite, which is highly reactive and cytotoxic in high concentrations, but has also been suggested to exert cardioprotective effects at low concentrations [83]. Since NO is a potent vasodilator, it is possible that improved blood flow contributes to the protective actions of NO. Accordingly, intracoronary NO improves transmural blood flow during reperfusion in vivo [84] and to reduce the area of no-reflow in vitro [74]. However, it is not clear whether this effect is a contributing factor to the protective effect of NO or a consequence of the cardioprotective effect.

There are also studies that indicate a detrimental effect of NO during ischemia/reperfusion. Thus, blockade of NO production has been demonstrated to limit infarct size, improve myocardial function and reduce the incidence of arrhythmias in different models of ischemia/reperfusion [85–88]. In addition, the functional recovery following ischemia was significantly better in endothelial NO synthase knockout mice [89]. The divergent results in different studies may depend on several factors including unspecific effect of available NO synthase blockers, different amounts of NO produced under various experimental conditions and which isoform of NO synthase (endothelial or inducible) that is expressed. The results may also depend on the amount of peroxynitrite that is formed by NO and superoxide as discussed above.

2.7. Endothelin-1 (ET-1) receptor antagonists

ET-1 is a potent vasoconstrictor peptide originally found in vascular endothelial cells [90], but has subsequently been demonstrated to be produced in other cells including vascular smooth muscle and myocytes. The production of ET-1 and its vasoconstrictor effects are enhanced during myocardial ischemia and reperfusion [91]. It was initially demonstrated that a monoclonal antibody directed against ET-1 reduced myocardial infarct size in the rat [92]. The subsequent development of selective ET receptor antagonists permitted further investigations in this field. In a dog model of 90 min of coronary artery ligation and 5 h reperfusion, local intracoronary infusion of the peptide-based ET_A receptor antagonist BQ123 reduced infarct size by 40% [93]. Reductions in infarct size have also been obtained with low molecular non-peptide selective ET_A and mixed ET_A/ET_B receptor antagonists such as LU135252 and bosentan [94–96]. The same degree of cardioprotection is obtained with both types of antagonists and with local and systemic administration. Thus, it is likely that the effect is mediated via the ET_A receptor located in the jeopardized myocardium.

The mechanism underlying the ET receptor antagonists is not fully clarified. Since ET-1 is a potent vasoconstrictor, the receptor antagonists may induce vasodilatation, which attenuates the ‘no-reflow’ phenomenon. A second interesting possibility is that the ET receptor antagonists inhibit neutrophils (Fig. 1). The ET_A receptor antagonist LU 135252 significantly improved post-ischemic ventricular function in hearts reperfused with neutrophils [97]. The

accumulation of neutrophils in the heart was enhanced by ET-1 and inhibited by LU 135252. These findings indicate that ET-1 stimulates neutrophil accumulation during reperfusion and that the ET_A receptor antagonist attenuates the neutrophil-mediated injury. The ET receptor antagonists may in addition inhibit ET-1-induced production of myocardial superoxide [98] and increase intracellular calcium due to stimulation of phospholipase C activation [91]. Finally, it is interesting to note that the cardioprotective effect of ET receptor blockade seems to be coupled to production of NO. The reduction in infarct size by the ET_A receptor antagonist LU 135252 in pigs subjected to coronary artery ligation followed by reperfusion was blocked by inhibitors of NO synthase [96,99]. In addition, the inhibitory effect of the NO synthase blocker on the cardioprotective effect of LU 135252 was reversed by L-arginine [99] indicating that the cardioprotective effect was dependent on NO production.

There are also studies indicating that endogenous ET-1 exerts pro-arrhythmic actions during ischemia and reperfusion. The ET_A receptor antagonist LU 135252 reduced the incidence of ventricular fibrillation during ischemia in pigs [100], and the mixed ET_A/ET_B receptor antagonist TAK 044 reduced reperfusion arrhythmias in rats [101].

Some controversy exists concerning the role of ET-1 during ischemia and reperfusion. First, exogenous ET-1 has been demonstrated to reduce infarct size and the incidence of arrhythmia when administered prior to ischemia [102,103]. This preconditioning effect of ET-1 is mediated by activation of protein kinase C via the ET_A receptor [102]. Second, some studies have failed to demonstrate any protective effects of ET receptor antagonists in various models of ischemia/reperfusion injury [104–106]. The reason for the lack of protective effects in these studies remains unclear. It may relate to the use of short acting peptide compounds, route and timing of administration or the experimental model as discussed above.

2.8. Inhibitors of the renin–angiotensin system (RAS)

The key product of RAS, angiotensin II, increases intracellular calcium levels of myocytes and smooth muscle cells, leading to positive inotropism, impairment of diastolic function, and coronary vasoconstriction (Fig. 1). Angiotensin II is an important regulator of noradrenaline release from sympathetic nerve terminals, modulating local cardiac and vascular sympathetic activity. At pathophysiological levels angiotensin II is cardiotoxic and induces myocyte necrosis. These effects of angiotensin II may be detrimental in the process of myocardial ischemia/reperfusion.

Angiotensin converting enzyme (ACE) inhibitors reduce reperfusion arrhythmia and infarct size and result in improved functional recovery. Captopril exerts a protective role against ischemia/reperfusion injury which might relate to free radical scavenging [107,108]. There may, however, be other mechanisms [109]. ACE does not only stimulate the conversion of angiotensin I to angiotensin II. It is also responsible for the degradation of bradykinin, which has been shown to be cardioprotective. Bradykinin B_2 receptor antagonist abolishes the cardioprotective effect of ACE inhibition [110].

It has not been easy to investigate the role of endogenous angiotensin II until recently when specific angiotensin II receptor blockers have become available. Two subtypes of angiotensin II receptors, AT_1 and AT_2, have been identified. The cardiovascular actions of angiotensin II are believed to be mediated mainly via activation of the AT_1 receptor. In anesthetized pigs, the AT_1 receptor blockers candesartan [111–113] and EXP3174 [114] clearly reduce infarct size. These infarct-limiting effects are present both when the blocker is given intravenously prior to ischemia and when it is given locally into the ischemic myocardium just prior to reperfusion. The effect of AT_1 receptor blockers was not due to a hemodynamic effect or improvement of regional myocardial blood flow during reperfusion [111]. Thus, it is most likely that candesartan or EXP3174 protected the heart through inhibition of local cardiac angiotensin II. In a recent study Preckel et al. were not able to detect any effect of the AT_1 receptor blocker irbesartan on infarct size in dogs subjected to 1 h of LAD occlusion followed by 3 h of reperfusion despite the fact that preischemic administration of irbesartan increased the collateral blood flow to the ischemic region [115]. The two bolus injections of angiotensin II before LAD occlusion in the study by Preckel et al. most probably contributed to the conflicting results. Short episodes of exposure to angiotensin II prior to ischemia mimic ischemic preconditioning, rendering the myocardium resistant to ischemic damage [116]. It is likely that the AT_1 blocker could not provide additional cardioprotection. Blockade of the AT_1 receptor also clearly reduces reperfusion arrhythmias. In AT_{1a} receptor knockout mice the number of ventricular premature beats after reperfusion is much less than in wild-type mice [117].

Blockade of the AT_1 receptor causes an increase in the angiotensin II level due to the removal of angiotensin II negative feedback. Angiotensin II subsequently activates the AT_2 receptor in endothelial cells, resulting in enhanced formation of bradykinin [118]. This may contribute to cardioprotection. Indeed, the infarct size reduction afforded by candesartan in anesthetized pigs has been reported to be abolished by the AT_2 receptor blocker PD 123319 or the bradykinin B_2 receptor antagonist icatibant [112,113]. Similarly, in isolated rat hearts subjected to global ischemia/reperfusion, the beneficial effect of the AT_1 receptor blocker losartan on postischemic functional recovery is diminished by the bradykinin B_2 receptor antagonist HOE 140. Activation of bradykinin B_2 receptors results in a stimulation of cyclo-oxygenase and NO synthase with subsequent increase in the synthesis/release of prostacyclin [119] and NO [120], which are possibly

responsible for the cardioprotective effects obtained with AT_1 receptor blockers.

2.9. Inhibitors of Na^+/H^+ exchanger

Inhibition of Na^+/H^+ exchange (NHE) in cardiac myocytes is believed to play an important role in regulating intracellular pH and sodium and calcium ion homeostasis [121,122]. It has been hypothesized that pharmacological inhibition of Na^+/H^+ exchanger during early reperfusion can be beneficial due to two factors (Fig. 1). First, under inhibition of the Na^+/H^+ exchanger, H^+ efflux from myocardial cells may be reduced and, thereby, the low ischemic intracellular pH maintained during the early phase of reperfusion. A continuation of intracellular acidosis during early reperfusion is believed to protect against reperfusion injury [123]. Second, inhibition of Na^+/H^+ exchanger reduces Na^+ influx with subsequent reduction in Ca^{2+} influx, thus attenuating the Ca^{2+} overload.

Pretreatment with the NHE inhibitor HOE-694 significantly reduced infarct size in pigs [124,125]. When given shortly before reperfusion, HOE-694 also reduced infarct size [125]. Supporting this latter finding, Linz et al. [126] and Gumina et al. [127] observed a similar beneficial effect when the NHE inhibitor cariporide or EMD-85131 was given shortly before the initiation of reperfusion in rabbits and dogs. Three other studies failed to detect an infarct reduction in pigs and rabbits with cariporide given just prior to reperfusion [128–130], not supporting NHE inhibitors as the optimal pharmacological approach to limit reperfusion-induced lethal injury. Similarly, both positive and negative results have been reported regarding whether NHE inhibitors given at the time of reperfusion attenuate myocardial stunning [131–133].

Several studies have consistently demonstrated beneficial effects of NHE inhibitors on reperfusion-induced arrhythmias. In rat hearts subjected to regional ischemia and reperfusion HOE-694 or cariporide remarkably suppressed the incidence of reperfusion-induced ventricular fibrillation in a dose-dependent manner, regardless of whether the compound was administered during both ischemia and reperfusion or during reperfusion alone [134,135]. A similar effect of NHE inhibition on reperfusion-induced ventricular fibrillation was also observed in pigs and dogs [136,137].

2.10. Anti-apoptotic agents

Increasing evidence suggests that lethal reperfusion injury possibly consists of two forms of cell death, necrosis and apoptosis (programmed cell death). The apoptotic process is initiated shortly after the onset of ischemia, and becomes markedly enhanced during reperfusion due to replenishment of high-energy phosphates, cytosolic and intramitochondrial calcium overload, as well as oxygen free radical production [138]. Inhibition of the apoptotic process should then attenuate the irreversible injury in connection with reperfusion. This hypothesis has received support in a recent study by Mocanu et al. [139]. Inhibition during early reperfusion of caspase, a key protease involved in the apoptotic process, markedly reduced infarct size in isolated rat hearts subjected to 35 min of regional ischemia and 120 min of reperfusion. Apoptosis during ischemia/reperfusion was also abolished by administration of SOD and catalase, indicating that apoptosis in this situation is dependent on oxidative stress [140]. In addition, ischemia/reperfusion-induced apoptosis can also be inhibited by a monoclonal antibody against the C5 complement component, an effect related to neutrophil infiltration [141]. These results indicate that apoptosis may be an important target for the limitation of reperfusion-induced lethal injury.

2.11. Combination of drugs

It may be speculated that myocardial reperfusion injury is a net result of a complex process including several mechanisms. It is not clear whether these mechanisms interact. The pharmacological diversity of the compounds that have been shown to attenuate reperfusion injury suggests that either multiple initiating factors or a cascade of events are responsible. One way to test this is to compare the effect of a combination of compounds of different pharmacological properties with that of each individual compound. In a porcine model of myocardial ischemia/reperfusion, our laboratory studied the infarct size-limiting effect of a cocktail of the calcium antagonist felodipine, the lipid peroxidation inhibitor H290/51 and the AT_1 receptor blocker candesartan [142]. Each individual compound, administered locally via coronary venous retroinfusion at the time of reperfusion reduced infarct size to a similar extent. Interestingly, the reduction of infarct size was significantly larger in pigs given the cocktail than that in pigs given individual compound. In another study, Weidenbach et al. [113] demonstrated that in pigs subjected to 90 min low-flow ischemia and 120 min of reperfusion, both the ACE inhibitor ramiprilat and AT_1 receptor blocker candesartan reduced the infarct size by approximately 50%. Combination of ramiprilat and candesartan further reduced the infarct size compared to each of these compounds alone.

The exact mechanism for the additive effect is not known. One possibility is that these compounds exert a cardioprotective effect though discrete pathways, thus having an additive effect when combined. Another possibility is potentiation of one common protective mediator. One such mediator is bradykinin/NO, as indicated by the emerging evidence suggesting their involvement in the cardioprotective action of ACE inhibitors, AT_1 receptor blockers, and ET-1 receptor antagonists (Fig. 1).

2.12. Possible impact of risk factors and coronary artery disease on pharmacological protection

The large majority of data demonstrating protection against ischemia/reperfusion injury have been obtained in experiments on healthy animals with normal vascular function. However, most patients with an acute myocardial infarction have several risk factors for coronary artery disease such as hypercholesterolemia, diabetes, hypertension and atherosclerosis. It is not obvious that the pharmacological agents discussed above will afford a similar degree of cardioprotection under such conditions. As mentioned above, NO may be of importance for the cardioprotective action of ACE inhibitors and ET-1 receptor antagonists. In patients with coronary risk factors, NO bioavailability may be attenuated, decreasing the efficacy of some pharmacological agents as addressed in some studies. Simvastatin attenuates ischemia/reperfusion injury in normocholesterolemic animals via a mechanism related to NO production [143]. In apolipoprotein E-deficient mice fed with high cholesterol, simvastatin attenuated neutrophil accumulation and reduced infarct size [144]. This indicates that statin treatment was effective also in this experimental situation. It is interesting to note that pravastatin restored the infarct limiting effect of ischemic preconditioning blunted by hypercholesterolemia in the rabbit [145]. Furthermore, carvedilol which is a beta adrenoceptor blocker with antioxidative effects, reduced infarct size in rabbits with hypercholesterolemia [146]. In a study on diabetic mice, a monocolonal antibody against the CD18 complex inhibited neutrophil accumulation and reduced ischemia/reperfusion injury [147]. These observations suggest that at least some of the pharmacological agents discussed above evoke cardioprotective effects in the experimental setting in the presence of different risk factors for coronary artery disease.

3. Conclusion

The present summary demonstrates that it is possible to attenuate myocardial ischemia/reperfusion injury by several different types of pharmacological interventions in experimental settings. The protection is not only achieved in experimental models with ‘normal’ uninjured blood vessels but also in experimental models of hypercholesterolemia and atherosclerosis. An important question is whether these effects can be achieved also in the clinical setting. Most of the compounds described in the present review are already in clinical use. Therefore, application of these compounds locally, for example via the balloon catheter during primary PTCA, would shed light on the feasibility to limit myocardial reperfusion injury pharmacologically in the clinical setting. Recently, a few such clinical studies have been carried out with encouraging beneficial effects of intracoronary adenosine [148] and cariporide [149] as adjunct therapy. Given the small size of these studies, larger clinical studies are needed to determine the definite importance of these pharmacological agents in limiting myocardial damage in acute myocardial infarction.

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