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LEUKOCYTES AND ISCHEMIA-INDUCED MYOCARDIAL INJURY

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THE MEANS OF LIMITING INFARCT SIZE

In the early 1970s Maroko, Braunwald, and others demonstrated the importance of myocardial oxygen supply and demand as a major determinant of the extent of myocardial injury (1–3). A variety of agents that reduced myocardial oxygen demand, B-adrenoceptor antagonists and calcium channel blockers for example, were shown to be beneficial in animal models of myocardial ischemia. The time for clinical testing of agents that improve myocardial oxygenation was said to have come (4). A decade later, however, the limited clinical results have been far from exciting. This approach to reducing ischemia-induced myocardial injury suffers from three major problems.

Implicit in the concept of salvaging ischemic myocardium by altering myocardial oxygen supply and demand is the assumption that myocardial tissue injury results solely from an inability of the coronary vasculature to deliver necessary oxygen and nutrients to maintain myocyte viability. However, we believe that other pathophysiologic processes contribute to irreversible myocardial damage.

It is also assumed that the restoration of coronary blood flow to the ischemic myocardium would, of itself, prevent the development of irreversible damage. However, reperfusion of jeopardized myocardium may lead to cellular processes that extend the area of cell necrosis beyond that which could be attributed

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to the period of ischemia, alone, and produce long-term functional derangements of the heart (5–8).

Finally, if myocardial damage is solely the result of an imbalance between oxygen supply and demand, then maneuvers designed to limit infarct size will only be effective if given early after the onset of myocardial ischemia. While such treatment has practical limitations clinically, which has probably contributed to the tempered enthusiasm for infarct size limitation (9), the ability to salvage ischemic myocardium at later periods (10, 11), or even after a period of myocardial oxygen deprivation (i.e. after adequate reperfusion) (12, 13), suggests other processes have to be taken into consideration.

In the past three years, several investigative efforts (14–16, 12, 14–19) have independently proposed a role for leukocytes as contributors to ischemia-induced myocardial injury. The tissue damage resulting from myocardial ischemia activates a cascade of events that can broadly be defined as an inflammatory response that occurs independently of any improvement in myocardial oxygenation. If the premise is true, that the inflammatory response and invading leukocytes contribute to the ultimate extent of myocardial injury, then agents directed against the leukocytes may provide a novel means of limiting infarct size and may expand the time frame in which therapy can be initiated and still be effective, since neutrophil infiltration proceeds for up to 24 hr (20, 21).

CAN LEUKOCYTES EXACERBATE ISCHEMIA-INDUCED MYOCARDIAL DAMAGE?

The presence of leukocytes in infarcted myocardium has been demonstrated histologically at autopsy (20, 21) and by the use of radiolabeled cells to clinically define infarcts in patients (22, 23). Owing, in part, to methodological problems whereby the blood pool of radioactivity had to be cleared before accurate scanning, these studies suggested that polymorphonuclear leukocytes (PMN) do not appear until 24 hr after the start of the ischemia event and that they peak at about 72 hr before declining. This led to the belief that PMN infiltration was merely the result of necrosis and reflected the initiation of a repair process. However, the question still remains, can PMNs cause or exacerbate necrosis? For this to be true, neutrophils should be evident much earlier than 24 hr, before the final extent of the infarct has been delineated. Mullane and co-workers (17) found that the activation, margination, and diapedesis of PMNs already became apparent after 60 min of coronary artery occlusion, and over the ensuing 5 hr a large number of PMNs invaded the ischemic area. Enhanced capillary permeability after coronary occlusion is evident within 15–20 min (24). Edema formation appears and is dependent upon an *interaction between* chemotactic factors, such as complement fragment

C_{5a}, the presence of PMNs, and the local generation of a vasodilator prostaglandin (25). All of these requirements are fulfilled in the ischemic myocardium (17, 26–29) and compel one to consider that PMNs are recruited very quickly and may contribute to some early changes associated with myocardial ischemia. Thus temporally, PMN activation and subsequent influx do appear to be related to ischemia-induced myocardial necrosis. In the study of patients at the time of autopsy, Fishbein et al (21) observed a parallel development of PMN infiltration and tissue necrosis, but they did not associate the two processes in the progression of infarction. Although the evidence indicates, therefore, that PMN influx and the development of necrosis are temporally related, this association does not prove causality.

HOW CAN LEUKOCYTES PROMOTE TISSUE DAMAGE?

In vitro studies show that during the process of phagocytosis, PMNs release a variety of mediators that can be considered toxic to tissues. These mediators include oxygen metabolites (free radicals), arachidonic acid metabolites, platelet-activating factor, and lysosomal enzymes.

Oxygen Metabolites and Free Radicals

The activation of neutrophils by a soluble or phagocytic stimulus initiates a "respiratory burst" with a sudden and large increase in oxygen consumption, activation of the hexose monophosphate shunt, and the generation of oxygen metabolites and toxic free radicals, which can be released into the external tissue environment (30, 31). Greater than 90% of the oxygen consumed by neutrophils during this period can be accounted for by superoxide anion (O_2^-) formation, through the action of a NAD(P)H-oxidase located in the cell membrane (32, 33). When two molecules of O_2^- react with each other, one is oxidized and the other is reduced forming hydrogen peroxide (H_2O_2) and oxygen in a dismutation reaction, which can be catalyzed by superoxide dismutase (also present in the leukocyte) (34). Formation of the hydroxyl radical (.OH) requires a trace metal, which, when reduced by O_2^-, can react with H_2O_2 to form .OH and OH^-. Lactoferrin is an iron-binding protein found in the specific granules of neutrophils, which can increase .OH production in vitro (35), presumably by its ability to provide iron for the O_2^-/H_2O_2 system. Lactoferrin is also released into the extracellular environment during neutrophil activation and could represent an important source of mediators of tissue injury. Finally, the reaction of H_2O_2 with the neutrophil myeloperoxidase (contained within the azurophilic granules) produces an enzyme substrate complex that can oxidize various halides, in particular chloride (Cl^-), to produce highly reactive toxic products such as hypochlorous acid (HOCl) (36, 37). For a more detailed

account of the formation and role of leukocyte-derived oxygen metabolites, the reader is referred to the excellent review by Fantone & Ward (38).

Oxygen metabolites can directly alter structural components of tissue, producing degradation of glycosaminoglycans, proteoglycans, and collagen (38), together with injury and lysis of a variety of cell types including endothelial cells (39), erythrocytes (40), fibroblasts (41), platetlets (42), and leukocytes (43). These highly reactive oxygen metabolites attack membrane phospholipids and act on unsaturated fatty acids to produce lipid peroxidation, a frequent manifestation of free radical generation, which results in increased membrane fluidity, increased permeability, and loss of membrane integrity (44, 45). Hess and co-workers (46) have described a depression of calcium uptake in the cardiac sarcoplasmic reticulum that is due to the generation of free radicals at pH 6.4. This effect can be reproduced by exogenously formed free radicals that are generated from a xanthine-xanthine oxidase system that, at pH 6.5, produces the superoxide anion and hydroxyl radical. Activated human leukocytes have been shown to depress canine cardiac sarcoplasmic reticulum calcium transport because of their ability to generate superoxide anion, hydrogen peroxide, and hydroxyl radical (47). Myocardial cells in culture show uptake of antimyosin, reflecting sarcolemmal damage, under conditions that promote free radical generation (48). The production of malonyldialdehyde (MDA), indicative of lipid free radical oxidation, is associated with reoxygenation-induced damage of the heart (49). The exogenous generation of superoxide anions via the xanthine-xanthine oxidase system at pH 7.4 also results in increased mitochondrial MDA formation and reduced mitochondrial respiration leading to an increase in cytosolic free calcium and a decrease in ATP (50). These changes in the sarcoplasmic reticulum and mitochondria correlate with the development of irreversible injury. Lipid peroxides can also react with proteins, leading to a loss of enzymatic activity, scission of polypeptide chains, and destruction of some labile amino acids including cysteine and lysine (51). In contrast, phospholipase activity may actually be enhanced (52), leading to the release of unsaturated fatty acids. Thus, the generation of free radicals, and the ensuing lipid peroxidation, can contribute to many different aspects of tissue damage.

The generation of free radicals may be particularly important during reoxygenation of ischemic myocardium, which often enhances the injury (5–8). However, free radicals can be produced even during the period of ischemia, since the pO_2 in the ischemic core is 5–10 mmHg, which is sufficient to sustain 83% of the free radical generation (53). Myocardial ischemia is associated with a decrease in superoxide dismutase and glutathione peroxidase activity (54–57), the two major intracellular enzymes that normally protect the heart from free radical-mediated damage by preventing the increased formation of toxic oxygen metabolites.

While it is apparent from the foregoing that the formation of free radicals can impair cell and mitochondrial membrane integrity, the effects of these chemical species on cardiac function have not been addressed. Recently Jackson and co-workers (58), using isolated hearts perfused with a buffer subjected to electrolysis to generate free radicals, demonstrated an increase in perfusion pressure and a decrease in myocardial contractility—changes indicative of ischemia.

The most persuasive evidence implicating oxygen-derived free radicals in myocardial injury stems from the use of a variety of free radical scavengers to suppress various indices of myocardial dysfunction or damage. These scavengers have included superoxide dismutase and catalase (46, 59–61), superoxide dismutase alone (48, 49, 58, 62), superoxide dismutase plus mannitol (46, 47, 63), dimethyl sulfoxide (48, 64, 65), allopurinol (66), co-enzyme Q10 (67), and glucose-insulin-potassium cardioplegia (68), together with antioxidants such as alpha-tocopherol, selenium, and ascorbate (49, 69, 70). These drugs prevent cardiac enzyme release (48, 49, 59, 68–71, 89), prevent sarcolemmal damage (48) and restore depressed calcium uptake and ATPase activity in the sarcolemma (46, 47, 68), maintain mitochondrial integrity and function (59, 61, 62), and prevent malondialdehyde formation and release (49, 57). In addition, these drugs improve cardiac function (58–60, 63, 72) and quantitatively reduce infarct size (13, 66). The study by Jolly and co-workers (13) in an occlusion-reperfusion model of myocardial damage in the anesthetized dog is particularly pertinent because these workers demonstrated that the administration of superoxide dismutase and catalase during the early reperfusion phase was associated with maximal protection against reperfusion injury that could be divorced from the damage that was the result of myocardial oxygen deprivation (coronary occlusion). These authors postulated that primary myocardial cellular damage due to ischemia is additive to the cardiac cell damage during the reperfusion phase; the latter form of injury is mediated, at least in part, by toxic metabolites of oxygen.

From the foregoing, it is clear that myocardial ischemia and, in particular, reperfusion of the ischemic tissue are accompanied by the formation of oxygen-derived free radicals, which overwhelm diminished endogenous protective mechanisms to exacerbate cell injury and tissue damage. It is important to recognize that cell death is probably a far more violent event than is often imagined, "an explosion rather than a dissolution" (73), since the generation of free radicals (the conversion of a stable to an unstable chemical species) requires an explosive release of energy. The question arises as to the source of oxygen-derived free radicals in myocardial ischemia and/or reperfusion. While it is known that activated neutrophils can generate and release substantial quantities of oxygen metabolites, it is certain that they are not the sole source of these products, since free radical generation and protection by various scaven-

gers is also observed in isolated hearts, perfused with a blood-free medium (49, 50, 54, 55, 57, 67). McCord (74) has demonstrated that ischemia in vivo results in the conversion of xanthine dehydrogenase to xanthine oxidase and that reperfusion of the tissue is accompanied by a burst of O_2^- and H_2O_2 production via this enzyme. Samples of ischemic myocardium biopsied 30 min after coronary occlusion show a greater than 300% increase in xanthine oxidase activity (66), indicating that this mechanism could be an important source of free radicals in myocardial tissue. The relative importance of myocardial xanthine oxidase and the neutrophil NAD(P)H oxidase system to free radical-mediated damage of the ischemic or reperfused myocardium remains to be determined.

Membrane Phospholipids and Lysolipids

A number of studies have focused on examining changes in the integrity of the sarcolemmal membrane since Jennings and co-workers (75, 76) demonstrated that changes in permeability of the sarcolemmal membrane led to the accumulation of tissue calcium, which correlated with the time course of the onset of irreversible injury. These alterations in permeability may be due to free radical generation (46, 77) or the degradation of membrane phospholipds (78). These two events are not mutually exclusive, and changes in membrane phospholipids may well result from free radical attack, while the enhanced lipid peroxidation increases membrane fluidity and permeability. Chien and co-workers (79) found that irreversible ischemic injury in the heart is accompanied by the degradation of membrane phospholipids because of activation of phospholipases and loss of reacylase activity. This can be seen as an increased accumulation of free arachindonate, which parallels the time course of irreversible injury. Moreover, in vitro treatment of sarcolemmal vesicles or cultured myocardial cells with exogenous phospholipases causes damage similar to that seen in vivo (78), while activated neutrophils also release phospholipase A_2 (which cleaves and releases arachidonate from membrane phospholipids) into the external environment (80, 81) and may contribute to this effect. These mechanisms may provide arachidonic acid for its subsequent metabolism to eicosanoids.

Phospholipase attack on membrane phospholipids will not only cleave a fatty acid, such as arachidonic acid, but will leave a lysophospholipid remaining. Normally, reacylation enzymes prevent the accumulation of lysolipids (82); however, during ischemia reacylase activity is low (83), due in part to the depletion of ATP which is required to form the fatty acyl CoA derivatives of the fatty acids to be reincorporated. Consequently, an increase in lysophosphoglycerides has been detected in ischemic tissue in vivo as well as in effluents from ischemic regions (84–86). Corr and co-workers (87, 88) have obtained

evidence implicating these lysolipids in electrophysiological derangements accompanying myocardial ischemia.

Neutrophils contain high concentrations of 1-O-alkyl-2-aryl-sn-glycero-3-phosphocholine within the membrane phospholipids (89). Stimulation of the neutrophils activates phospholipase A_2 to liberate arachidonic acid, leaving 1-O-alkyl-2-lyso-sn-glycero-3-phosphocholine, which can subsequently be acetylated to form platelet-activating factor (PAF-acether) (90, 91). PAF-acether promotes neutrophil activation and degranulation and has been implicated in anaphylaxis and inflammation, where it induces vascular leakage and smooth muscle contraction (90, 91). In addition, this mediator can cause cardiac dysfunction and promote arrhythmias (92, 93), and it can exacerbate ischemia-induced myocardial damage (94). Recently, 24-hr infarcted myocardium has been demonstrated to generate PAF-acether, which may reflect the earlier influx and continued presence of functioning neutrophils (95).

Arachidonic Acid Metabolism

The activation of PMNs is accompanied by the release of arachadonic acid (AA) and the formation of a variety of pro-inflammatory metabolites. Arachindonic acid can activate the neutrophil NADPH oxidase, leading to the generation of superoxide anions (96). PMNs metabolize AA primarily via a 5-lipoxygenase enzyme to form 5-HETE and LTB_4 (97, 98). This latter compound is chemotactic for neutrophils (99, 100), promotes leukocyte adhesion to the vascular endothelium (101), and, at high concentrations, acts as a calcium ionophore to increase intracellular calcium and to promote free radical generation and the release of lysosomal enzymes (102). Moreover, in the presence of neutrophils and a vasodilator prostaglandin such as PGI_2, LTB_4 can produce edema (25). LTC_4 may also be formed, which induces coronary vasoconstriction and impairs contractility (103–106). Neutrophils contain a number of other enzymes capable of metabolizing AA, including 15- (107) and 12-lipoxygenases (17) and a cytochrome P450-dependent monooxygenase (108). The importance of the products of these pathways is not clear. Metabolites of AA can be re-esterified into the cell membrane (109), however, and, if this occurs in the myocytes, it may result in alterations in membrane function and permeability. Superoxide anions can also oxygenate arachidonic acid to a potent chemotactic factor (110).

The heart is unusual in that it has a very low capacity to metabolize AA when compared to other organs (7). Indeed almost all of the cyclo-oxygenase activity that is detected is localized to the coronary blood vessels (111) and the epicardial membrane (112), while the myocytes have either nondetectable or negligible cyclo-oxygenase or lipoxygenase activites. Consequently, the finding (17) that infarcted myocardium had an increased capacity to metabolize

exogenous AA suggested either the unmasking of dormant enzymes already contained with the tissue or an influx of cells with a very active metabolic pathway. This latter possibility is the most likely, since the profile of metabolites formed by the infarcted myocardium was similar to that observed in purified preparations of neutrophils, whereas the enhanced metabolism could be prevented by pharmacologic interventions that suppressed the neutrophil infiltration into the heart (17). Thus, migrating cells can alter the metabolic profile of the tissue that they invade and give rise to products not normally found in the host tissue and which may influence the activities and function of that tissue. We feel that this is a potentially important concept, the significance of which has yet to be examined.

Lysosomal Enzymes

Wildenthal (113, 114) proposed a "lysosomal hypothesis" whereby myocardial ischemia enhanced the fragility of cardiac lysosomes leading to the leakage of lysosomal enzymes into the cell cytosol, thereby promoting cellular damage. Certainly increased lysosomal permeability and the release of lysosomal-derived cathepsin D can be related temporally to the process of irreversible injury (115). This hypothesis can be extended to consider the lysosomal enzymes released by activated neutrophils that are capable of proteolytic attack on ischemic myocytes as has been demonstrated for the neutrophil-derived lysosomal enzymes that contribute to the degenerative tissue damage associated with rheumatoid arthritis (116). However, the extent of myocardial cell injury caused by proteolytic enzymes is not clear. It appears unlikely that they contribute to a significant extent, since drugs shown to stabilize lysosomes in vitro and prevent the release of cardiac enzymes do not uniformly reduce ischemia-induced myocardial damage. For example, PGE_2 (117), PGI_2 (117), naproxen (118), and ibuprofen (119) suppress the ischemia-induced release of cardiac lysosomal enzymes to a similar extent, whereas only PGI_2 (120, 121) and ibuprofen (122–124), but not PGE_2 (120) or naproxen (125), reduce infarct size. Measuring the formation of tyrosine (an amino acid that is neither synthesized nor degraded by cardiac muscle) as an index of lysosomal protease activity in the myocardium, Bolli and co-workers (126, 127) found that proteolysis actually decreased during 3 hr of ischemia. The subsequent increase in proteolysis correlated with the leukocyte infiltration that increased over 24 hr and was thought to reflect leukocyte-mediated digestion of the necrotic debris rather than an extension of the myocardial injury. Moreover, inhibition of lysosomal protease activity with leupeptin, antipain, pepstatin, and chymostatin totally suppressed proteolysis but did not influence infarct size (128, 129), thus suggesting that lysosomal enzyme activity and the extent of myocardial damage are not linked.

WHAT CAUSES LEUKOCYTE ACTIVATION?

The complement system of plasma proteins is thought to play an important role in the pathogenesis of ischemia-induced myocardial damage. Activated components of complement are potent chemotactic and stimulatory agents for neutrophils and may initiate leukocyte infiltration into the extravascular myocardial tissue (26, 27, 130). Hill & Ward (130) found that coronary artery ligation led to the stimulation of a tissue protease, present in the myocardium, that cleaves the third component of complement into chemotactically active fragments. Subsequently, the localization of complement components has been studied in the ischemic myocardium of the baboon, using immunohistochemical techniques (26, 27). C_3, C_4, and C_5 were found extensively throughout the infarcted myocardium within the myocytes and arteriolar smooth muscle. Electron microscopy revealed C_3 associated with the contractile elements of myocytes as well as nuclear, mitochondrial, and sarcoplasmic reticular membranes (27). Moreover, human heart subcellular membranes, in particular mitochondrial membranes, bind and activate the same complement components in vitro (131). While myocardial infarction in patients is accompanied by a reduction in circulating complement components after 24–48 hrs (132), the complement components are already present in infarcted myocardium within 4 hr (26). Unfortunately, earlier time points have not been examined to determine if there is a temporal correlation with neutrophil activation and infiltration. The presence of complement components within the myocytes places these chemotactic mediators in an ideal position to attract neutrophils from the circulation. The interaction of C_{3b} with a specific receptor on the surface of the neutrophil triggers phagocytosis and the formation of neutrophil-derived mediators (133, 134). Moreover, complement-induced superoxide anion generation can occur independently of phagocytosis (135), thereby promoting free radical-mediated damage. The concept that the complement system is responsible for the neutrophil accumulation is supported by the observation that C_3, C_4, and C_5 localization is more intense at the periphery of the infarct and tends to decrease towards the center of the damaged area (27), a transmural distribution similar to that observed by Mullane et al (136) for the neutrophils using an assay for the neutrophil-specific myeloperoxidase enzyme.

If the complement system does account for the neutrophil activation and infiltration, the question arises as to what triggers complement activation? It is important to recognize that the immunohistochemical demonstration of C_3 in the myocardial fibers was accompanied by histological evidence of ischemic damage with myofibril disintegration, nuclear fragmentation, mitochondrial dense body formation, and the presence of albumin within the same fibers (27).

Thus damage initiated by the ischemic insult activates the complement system, which in turn promotes neutrophil infiltration. The influx of neutrophils into the myocardium is not observed if the ischemia is not of a sufficient duration to initiate the damage. However, marginating neutrophils in the vascular compartment may contribute to some early consequences of ischemia, such as edema (25), but remain within the vasculature until irreversible damage occurs.

The complement-derived anaphylatoxins C3a and C5a may themselves contribute to the cardiac dysfunction accompanying myocardial ischemia. C3a, apart from eliciting chemotaxis, enhancing vascular permeability, and provoking the release of mediators such as histamine, leukotrienes, prostaglandins, and PAF-acether (137), also produces coronary vasoconstriction, left ventricular contractile failure, tachycardia, and impaired atrioventricular conduction when injected into the isolated heart of the guinea pig (138). This profile of ischemia-like responses is attributed to the release of mediators from the heart, including histamine, leukotrienes, and a cyclo-oxygenase product (138).

Finally, the generation of oxygen-derived free radicals can also lead to the formation of chemotactic factors in plasma (139), if the oxygen metabolites are liberated and come into contact with plasma as a result of cellular damage. The importance of free-radical mediated activation of a chemotactic factor is currently unknown.

DOES LEUKOCYTE INHIBITION MODULATE THE DEVELOPMENT OF MYOCARDIAL NECROSIS?

A corollary of the proposal that neutrophils exacerbate ischemia-induced myocardial injury is that agents that abrogate leukocyte activation or infiltration, or prevent the release of specific neutrophil-derived mediators, should similarly reduce the extent of myocardial damage.

That a "heterolytic" or inflammatory process contributes to myocardial injury in addition to the autolytic process of oxygen deprivation has been recognized for over two decades (2, 3). However, results with anti-inflammatory drugs have been inconsistent (11, 12, 123–125, 140–146), which has made it difficult to appreciate the importance or extent of the damage provoked by the inflammatory response. Frequently, studies utilizing anti-inflammatory agents were not accompanied by any monitoring of the inflammatory response to demonstrate the effectiveness of the drug. Identification of the invading leukocytes as a major culprit of the heterolytic damage stemmed from studies by two independent groups of investigators (12, 14, 15, 17). Mullane & Moncada (12) and Jolly & Lucchesi (147) observed a reduction in infarct size with an experimental nonsteroidal anti-inflammatory drug (NSAID), BW755C. Since myocardial protection is not a general property of other NSAIDs, such as aspirin (140), naproxen (125), meclofenamate (141),

zomepirac (142), or indomethacin (12, 143), it could not be attributed to an inhibition of the enzyme system cyclo-oxygenase, a known general mechanism of action of NSAIDs (148). However, these latter drugs do not inhibit leukocyte infiltration into an inflammatory lesion (149, 150), and, while they are thought to provide some symptomatic relief in chronic inflammation, by reducing the pain and swelling for example, it is generally considered that they do little to ameliorate the underlying progression of the disease and tissue destruction (150). BW755C, in contrast, suppresses the neutrophil infiltration into inflammatory lesions (149, 151), probably as a result of its ability to inhibit the lipoxygenase pathway of arachidonic acid metabolism (151, 152). Mullane & Moncada (12) suggested that this effect could underly the myocardial protective effects of BW755C, and subsequently they provided evidence to support this proposal (17). Other drugs that inhibit the lipoxygenase enzyme (and consequently neutrophil infiltration), including nafazatrom (19) and dipyridamole (153), also reduce infarct size (19, 154–156).

Meanwhile, Lucchesi and co-workers were examining the effects of another NSAID, ibuprofen. This drug reduces infarct size, independently of any hemodynamic changes, which suggests that it is acting by a mechanism other than to alter myocardial oxygenation (122–124). Lucchesi's group, recognizing that ibuprofen is capable of influencing both platelet and leukocyte behavior, assessed the ability of ibuprofen to alter the accumulation of these blood elements in the ischemic myocardium. They found that the myocardial protection afforded by ibuprofen is accompanied by a selective suppression of neutrophil infiltration (14). Subsequently, the ability of ibuprofen to inhibit the release of a variety of neutrophil-derived mediators, and consequently neutrophil activation and ischemic damage, was demonstrated (157).

Direct studies whereby leukocytes were depleted with either specific anti-neutrophil antiserum (15) or hydroxyurea (17) confirmed that myocardial damage induced by coronary artery occlusion and reperfusion is reduced when leukocytes, in particular neutrophils, are prevented from invading the ischemic myocardium. Recognition of leukocyte inhibition as an effective means of salvaging ischemic myocardium helps explain the protection observed with a variety of chemically unrelated drugs.

Complement depletion with cobra venom factor attenuates leukocyte infiltration into the heart and diminishes the area of damage (145, 158), thereby further implicating complement fragments as the important chemotactic factors in this response. Aprotinin (Trasylol®), a serine protease inhibitor, also reduces infarct size (145, 159). Hill & Ward (130) provided evidence that a protease was involved in complement activation in the ischemic myocardium, and aprotinin subsequently was found to prevent the release of a chemotactic factor into the coronary sinus blood during ischemia (145). Thus, aprotinin may suppress complement activation and, in turn, leukocyte infiltration and may result in a

reduction in ischemic damage. More recently, aprotinin was found to prevent the increased production of oxygen-derived free radicals by PMNs sensitized by exposure to a low oxygen tension (160), thereby offering another leukocyte-dependent mechanism for the beneficial effects of this drug.

Epidemiologic studies have recognized that Greenland Eskimos have a low incidence of cardiovascular diseases such as myocardial infarction (161, 162). This intrinsic myocardial protection is attributed to the diet rich in eicosapentaenoic acid (EPA, C20:5), obtained from seal meat and fish (161–164). Feeding dogs a diet enriched with fish oil resulted in a smaller infarct size after being subjected to coronary artery occlusion and reperfusion (165). Although not recognized at the time, this myocardial protection may have resulted from an effect on leukocytes. Terano and co-workers (166) found that rats fed EPA accumulated the fatty acid in their neutrophils. Upon stimulation, the EPA is released together with AA. The EPA, which competes with AA, is readily metabolized by the 5-lipoxygenase enzyme to LTB_5. Similar results recently have been obtained in human volunteers fed EPA (167). LTB_5 exhibits only one fifth to one tenth the biological potency of LTB_4 in stimulating neutrophil chemotaxis and aggregation (167, 168), whereas EPA-fed rats also exhibit a diminished inflammatory response (166). Thus EPA also has an "anti-inflammatory" action involving the leukocytes, which could account for its beneficial actions in acute myocardial ischemia.

In summary, a variety of chemically unrelated drugs can reduce infarct size, independently of changes in myocardial oxygenation. The common effect of all of these drugs is to reduce leukocyte infiltration and the ensuing inflammatory response, which suggests that this action accounts for the myocardial protective effects of this diverse group of agents.

MYOCARDIAL SALVAGE OR DELAY?

It was concluded by Chambers and co-workers (144) using another NSAID, flurbiprofen, that although infarct size was reduced at 6 hr, it was not reduced after 24 hr, so the drug merely delayed the development of damage rather than limiting its ultimate size. Although drugs that delay myocardial injury may be important in their own right and may extend the time frame in which other strategies can be gainfully employed to limit infarct size, it is apparent that many of the drugs demonstrated to influence leukocyte behavior do actually reduce the area of necrosis. Ibuprofen persistently reduces infarct size at 6 hr, 24 hr (124), 48 hr (123), and 72 hr (169). Neutropenia maintained with specific antiserum reduces the extent of damage induced by circumflex coronary artery occlusion for 90 min, whether measured 6 hr (15) or 24 hr after reperfusion (16)—as does BW755C (12, 147). Finally, complement depletion in the rat with cobra venom factor retarded the development of necrosis for 21 days

(170). These studies indicate that drugs that are directed against the inflammatory response and that suppress leukocyte infiltration can permanently salvage the ischemic myocardium. The failure of flurbiprofen to reduce infarct size at 24 hr may have resulted from an ineffective dose to inhibit leukocyte invasion, since no means was used to determine the effective (anti-inflammatory) concentrations of the drug.

MECHANISMS UNDERLYING THE MYOCARDIAL PROTECTIVE EFFECTS OF DRUGS DIRECTED AGAINST LEUKOCYTES

The initial studies highlighting the ability of drugs directed against the leukocytes to limit the area of ischemic damage utilized an occlusion-reperfusion model of myocardial injury (12, 14, 15, 17, 18, 154). Reperfusion is thought to exacerbate some biochemical and functional derangements of ischemia (5–8). Moreover, reperfusion of the ischemic region improves the likelihood of leukocytes reaching the area of injury and may accelerate their influx. However, the participation of the leukocytes in myocardial damage is not restricted to reperfusion models. Total occlusion of a coronary artery also is accompanied by a rapid leukocyte infiltration (17, 171), in particular at the periphery of the infarct, while infarct size is still reduced by drug-induced leukopenia (172).

A number of possible mechanisms could explain the beneficial effects of the anti-leukocyte agents. The release of pro-inflammatory mediators potentially deleterious to the ischemic myocardium has been addressed. Clearly, drugs that suppress either the activation or infiltration of leukocytes into the ischemic myocardium will prevent the formation and release of all these mediators at the site at which they could do harm. Indeed, it has not been possible to delineate the importance of any one group of leukocyte-derived mediators in the process of myocardial tissue injury, since it appears that cellular infiltration is the first property to be lost when drugs directed against leukocyte-derived mediators are administered in vivo (17, 19).

Another mechanism that could account for the beneficial effects of drugs directed against the leukocytes is the improvement of blood flow to the ischemic myocardium. Jacob and co-workers (173, 174) have proposed that the aggregation of leukocytes could block coronary vessels and contribute to myocardial ischemia. Engler and co-workers (175) showed that leukocytes obstructed 60% of the capillaries within the ischemic myocardium, thereby preventing the full restoration of blood flow to the previously ischemic zone upon reperfusion. This "no-reflow" phenomenon could exacerbate myocardial damage by maintaining the ischemia despite attempts to restore the oxygen supply. Thus damage due to oxygen deprivation and that induced by activated leukocytes cannot be separated totally but rather are interrelated. Capillary

plugging and leukocyte aggregation indicate an activated state of the cells that can be prevented by various anti-leukocyte drugs such as dexamethasone, which attenuate the "no-reflow" phenomenon (176). Leukocyte depletion prevents the progressive increase in coronary vascular resistance observed during ischemia and thus enhances blood flow to the ischemic zone (177). Consequently, leukocytes can exert deleterious effects on the coronary microcirculation during both myocardial ischemia and reperfusion.

INFLAMMATION, LEUKOCYTES, AND MYOCARDIAL REPAIR

The primary role of the invading neutrophils is not to destroy host tissue but rather to clear the cellular debris as the first step in a healing process in which a fibrous scar tissue of great tensile strength is deposited to replace the nonfunctional necrotic tissue (20, 21, 178). If site clearance is impaired, the necrotic mass becomes surrounded by a fibrous caspsule that prevents healing and can lead to ventricular wall thinning, dysfunction, and risk of ventricular wall aneurysm or rupture (170, 179–181). Measurements of proteolysis, as a reflection of the digestion of necrotic debris by neutrophils, show a dramatic increase within 48 hr of coronary occlusion, which correlates with leukocyte infiltration. Proteolysis may be prevented by the steroidal anti-inflammatory agent, methylprednisolone, implying retardation of the removal of necrotic tissue (182). Leukopenia, induced by whole body irradiation, reduces collagen degradation in the infarct zone after 24 hr of coronary occlusion (127). High doses of methylprednisolone, which suppress the inflammatory response induced by myocardial injury, slow the removal of necrotic myocytes, resulting in "mummification" of the infarct, and impaired myocardial healing (179). Moreover, multiple doses of methylprednisolone administered to patients with acute myocardial infarction have been associated with an increased incidence of ventricular aneurysm and rupture (183, 184)—effects attributed to inadequate repair of the infarct. The NSAIDs ibuprofen (185) and indomethacin (186) also were found to increase the incidence of scar thinning after myocardial infarction in experimental models, suggesting that retardation of the healing process may be a common property of drugs that inhibit the inflammatory process.

If anti-inflammatory or anti-leukocyte agents impair the healing process in the myocardium, they will be of limited use in the management of patients with acute myocardial ischemia and an evolving myocardial infarct. However, scar thinning does not correlate with the ability of the drug to suppress leukocyte infiltration, since only ibuprofen, and not indomethacin, attenuates the cellular response (14, 17, 157). Cobra venom factor and methylprednisolone attenuate neutrophil invasion and diminish myocardial damage to the same extent, yet only the latter is associated with impaired healing (170). Even agents that

provoke scar thinning—ibuprofen and methylprednisolone—can be administered for a shorter period to achieve the same degree of myocardial protection without producing wall thinning and the associated ventricular dysfunction (170, 185).

These studies indicate that there is no clear relationship between inhibition of the early neutrophil infiltration and subsequent impairment of the healing process. Rather, it appears that the neutrophil response can be manipulated successfully without automatically resulting in scar thinning. Recently, Roberts and co-workers (187) suggested that inhibition of the mononuclear cell infiltration, not the neutrophil invasion, is associated with left ventricular scar thinning. Consequently, the chronic inflammatory resonse, characterized by mononuclear cells, may be involved primarily in the repair process, while selective inhibition of the acute response, associated with PMNs, may protect the ischemic myocardium without compromising the healing phase. Further studies in this important area are mandated.

CLINICAL SIGNIFICANCE

There is a direct correlation between the extent of myocardial injury and both short- and long-term prognosis in patients with acute myocardial infarction (3, 188). The recognition of an inflammatory response as a contributing factor to ischemia-induced myocardial injury, and, in particular, reperfusion-induced damage, may provide a means of treatment even if the patient's hospital admission is delayed a few hours. Anti-inflammatory steroids have been demonstrated to be effective even when administered 6 hr after coronary occlusion (11), and BW755C is beneficial when given during reperfusion, after the one hour period of ischemia is over (12). Second, the thrombolytic agents such as streptokinase and tissue plasminogen activator represent a therapeutic breakthrough whereby blood flow to ischemic regions of the myocardium can be restored. The increasing application of thrombolytic therapy and/or coronary angioplasty has prompted awareness of reperfusion-induced injury and ventricular dysfunction (189–192). The use of free radical scavengers or anti-neutrophil agents coupled with procedures for the restoration of coronary artery blood flow, given either immediately before or during the time of reperfusion, may reduce complications associated with the extension of myocardial injury and cell death.

Finally, a contribution of leukocytes to the initiation of ischemic heart disease should also be considered, since there is a positive correlation between the circulating leukocytes count and the incidence of myocardial infarction (193–195), while the converse is also true (196). Moreover, smoking, which increases the leukocyte count, is also associated with an increased incidence of myocardial infarction (193, 194). Furthermore, cigarette smoke can activate

the alternative pathway of complement (197). A high proportion of patients with unstable angina who succumbed to sudden coronary death were found to have a clustered infiltration of inflammatory cells in the adventitia of the coronary artery, which may be related to coronary artery vasospasm (198).

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