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ISCHAEMIA-REPERFUSION INJURY: PATHOPHYSIOLOGY AND TREATMENT

BY

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20. ABSTRACT (Continue on reverse side if necessary and identify by block number) Ischaemia is a common clinical event leading to local and remote injury. Evidence indicates that tissue damage is largely caused by activated neutrophils which accumulate when the tissue is perfused. If the area of ischaemic tissue is large, neutrophils also sequester in the lungs, inducing non-cardiogenic pulmonary oedema. Ischaemia-reperfusion injury is initiated by production of reactive oxygen species which initially appear responsible for the generation of chemotactic activity for neutrophils. Later, once adherent to endothelium, neutrophils mediate damage by secretion of additional reactive		

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oxygen species as well as proteolytic enzymes, in particular elastase. Therapeutic options for limiting ischaemia-reperfusion injury include inhibition of oxygen radical formation, pharmacological prevention of neutrophil activation and chemotaxis, and also use of monoclonal antibodies which prevent neutrophil-endothelial adhesion, a prerequisite for injury.

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Ischaemia is a common clinical event with potentially serious consequences. Evidence suggests that the major part of tissue injury occurs upon reperfusion and is mediated by activated neutrophils. The ensuing damage, termed ischaemia-reperfusion injury, is manifested by oedema and increased microvascular permeability to protein. This resulting injury is thought to be similar in most states of tissue hypoperfusion, including limb ischaemia, transplantation, stroke and hypovolaemic shock. In situations such as myocardial infarction, reperfusion of the occluded coronary artery may not occur and part of the myocardium will inevitably die. Adjacent myocardium will often have its blood supply critically diminished, but will still be viable. It is this poorly perfused area which is subjected to "reperfusion" injury and the extent of this injury which determines eventual infarct size. If the mass of ischaemic tissue is large, such as the legs or the gastro-intestinal tract, reperfusion results not only in local damage, but also in lung injury, manifest by non-cardiogenic pulmonary oedema (1,2). In this article we discuss the cellular basis of ischaemia-reperfusion injury, review the inflammatory mediators involved and consider potential strategies for treatment.

During the last decade enormous advances have been made in the understanding of the reaction of tissue subjected to ischaemia-reperfusion or hypotension. Central to our improved understanding have been two observations. First, reperfusion per se causes tissue injury considerably in excess of that induced by ischaemia alone. This was shown dramatically by the fact that the histological changes of injury after 3 hours of feline intestinal ischaemia followed by 1 hour

of reperfusion were far worse than the changes seen after 4 hours of ischaemia without reperfusion (3). Secondly, the neutrophil has been demonstrated to be the prime mediator of the additional injury. It has been known for many years that neutrophils infiltrate the myocardium after infarction, and their presence was initially thought to be a beneficial part of the healing process (4). However, in 1983, it was observed that the eventual size of a myocardial infarct produced by temporary coronary artery clamping in the dog could be substantially reduced by depleting the animal of circulating neutrophils beforehand (5). This led to the suggestion that neutrophils actively injure tissue during ischaemia-reperfusion and in fact largely determine the size of the infarct. Evidence from various experimental models supports this hypothesis, as described below. More recently, knowledge has rapidly accumulated regarding the mechanism of the neutrophil-mediated injury, including a clearer understanding of the factors responsible for neutrophil recruitment into reperfused tissue, and the means by which neutrophils then cause injury. This level of understanding has permitted the development of antagonists with the ability to reduce injury in animal models of ischaemia.

Biochemistry of reperfusion

Reperfusion injury is initiated by biochemical events occurring during ischaemia which result in the generation of reactive oxygen metabolites such as superoxide anion, hypochlorous acid and hydrogen peroxide (6). The first step is the depletion of ATP which is degraded to hypoxanthine. Normally, hypoxanthine is oxidized by the enzyme xanthine dehydrogenase to xanthine using

nicotinamide adenine dinucleotide (NAD) in a reaction converting NAD to NADH. However, during ischaemia xanthine dehydrogenase, which is usually present in large quantities, is converted to xanthine oxidase. This enzymatic ("D-to-O") conversion is central to the hypothesis of oxygen radical-mediated reperfusion injury. Xanthine oxidase has recently been shown to build up during periods of as little as 60 minutes of skeletal muscle tourniquet ischaemia in humans (7).

The second important event in ischaemia is that excess levels of hypoxanthine accumulate in tissue. This occurs for two reasons: 1) xanthine dehydrogenase is no longer present, and 2) xanthine oxidase uses oxygen as its substrate instead of NAD and is therefore unable to catalyse the conversion of hypoxanthine to xanthine because oxygen is unavailable. Nothing untoward happens as a result of the high levels of xanthine oxidase and hypoxanthine until reperfusion. When oxygen is re-introduced, xanthine oxidase converts hypoxanthine to xanthine, with the generation of large amounts of superoxide anion (Fig. 1). This burst of superoxide production starts a cascade of reactions which release other oxygen radicals and H_2O_2 within endothelial cells (8,9).

Oxygen radicals were shown to be of importance in ischaemia-reperfusion injury when injection of superoxide dismutase, a highly specific scavenger of superoxide anion, prevented the increased capillary permeability occurring upon reperfusion in a feline model of intestinal ischaemia (10). Others have observed similarly in myocardial ischaemia (11,12). However, the intracellular onslaught of reactive oxygen species does not by itself appear to be sufficient to account

for the extent of injury, because profound protection is afforded in most settings of ischaemia-reperfusion injury by prior depletion of circulating neutrophils.

Initially, we believe the main pathological effect of oxygen radical release to be the generation of chemotactic activity leading to the directed migration of activated neutrophils into the reperfused tissue, with consequent injury (8,13).

Essential role for arachidonic acid products

The precise steps linking xanthine oxidase and reactive oxygen metabolites to chemotactic activity are not well understood. Moreover, the exact nature of the chemotactic agents operative in different settings of ischaemia are unknown, although arachidonic acid products and complement fragments, the latter particularly in myocardial ischaemia, appear to be of key importance (14). Break-down products of arachidonic acid are found in high concentrations in plasma soon after reperfusion of ischaemic tissue. One consequence of oxygen radical release is that intracellular free Ca^{++} rises dramatically, and this is thought to be a crucial step in the activation of plasma membrane phospholipase A_2 and subsequent generation of products of arachidonic acid (15). Certainly, inhibition of oxygen radicals with scavenging enzymes prevents the release of arachidonic acid metabolites after reperfusion (16), and the superoxide ion seems to be a prerequisite for the generation of neutrophil chemotactic activity (17). The main pathways of arachidonic acid breakdown are shown (Fig. 2).

Evidence is lacking that arachidonic acid metabolites induce endothelial injury in ischaemia and reperfusion independently of neutrophils. There are,

however, three mechanisms by which arachidonic acid products might influence neutrophils in reperfusion injury. First, they could act as chemoattractants and induce neutrophil adhesion to endothelium. The lipoxygenase product leukotriene B₄ and the cyclooxygenase product thromboxane A₂ are potent chemoattractants known to do this (18,19). Evidence suggests that leukotriene B₄ is generated in sufficient quantity in ischaemia-reperfusion to induce neutrophil diapedesis (20). Other observations indicate that lipoxygenase products are of central importance in determining reperfusion injury. For example, their inhibition reduces neutrophil accumulation and injury in the myocardium (21) and following hindlimb tourniquet ischaemia (22).

Secondly, arachidonic acid products may activate neutrophils to produce more oxygen radicals and also proteolytic enzymes. Leukotriene B₄ is a potent stimulus for neutrophil generation of hydrogen peroxide and elastase (23), and has been shown to activate neutrophils to induce endothelial permeability *in vitro* and *in vivo* (24). Thromboxane A₂ also activates neutrophils and mediates their H₂O₂ production following ischaemia (25).

Thirdly, leukotrienes and thromboxane affect blood flow and therefore tissue perfusion by direct action on the microvasculature. For example, thromboxane-mediated vasoconstriction exacerbates poor capillary flow after reperfusion (26). A demonstration of this phenomenon is in ischaemia and reperfusion of the kidney where there is debate as to whether reperfusion injury is in fact neutrophil-dependent (27,28). It is thought that the major mechanism of this injury is the development of an imbalance between vasodilating and con-

stricting agents. The importance of thromboxane is indicated by the ability of vasodilating prostaglandins to attenuate damage, but only if thromboxane is inhibited at the same time (29,30). The kidney's need for vasodilating prostaglandins is shown clinically by the potential for renal damage following administration of cyclooxygenase inhibitors, such as ibuprofen or aspirin, even though these drugs inhibit thromboxane synthesis, (31). The renal injury is due to inhibition of prostaglandin synthesis, which limits the ability of the renal vasculature to vasodilate. It is not known whether leukotrienes C₄ and D₄ are important in ischaemia, although they are potent vasoconstrictors (32). Leukotriene B₄ does not directly affect blood flow.

Neutrophil adherence is a prerequisite for endothelial injury

Evidence suggests that neutrophils entering tissue which has just been reperfused become activated to increase synthesis of oxygen metabolites and proteolytic enzymes, and to become more adhesive for endothelium. These neutrophils can then induce injury by adhering to endothelium in two sites: the pre-capillary sphincter and the post-capillary venule. As activated neutrophils become more viscous, they may not be malleable enough to pass through the pre-capillary sphincter. The result is that capillaries become plugged (the no-reflow phenomenon) upon reperfusion, with possible exacerbation of anoxic injury (33). Most invading neutrophils, however, adhere to endothelium in the post-capillary venule. The mechanism by which neutrophils then induce injury is via the secretion of proteolytic enzymes such as elastase (34), probably in

conjunction with neutrophil-generated oxidation products such as HOCl and H₂O₂. These result in lysis of essential structural matrix proteins such as elastin, leading to increase microvascular permeability.

Neutrophils must not only be present but their adherence to endothelium appears to be a prerequisite for microvascular injury (Fig. 3). Neutrophil-endothelial adhesion is thought to create a microenvironment which permits high concentrations of injurious agents. Initial evidence supporting the importance of adhesion in reperfusion injury was the finding that after intestinal ischaemia mucosal damage could be prevented by pretreating the animals with a monoclonal antibody directed against the neutrophil adhesive glycoprotein, the so called CD 18 complex (35). This antibody inhibited neutrophil accumulation into the reperfused intestinal mucosa, by preventing the process of neutrophil adhesion to endothelium. Others have extended these observations of the importance of the CD 18 adhesion molecules to include reduction of injury and neutrophil influx into the heart (36), skeletal muscle (37) and lungs (38) following ischaemia-reperfusion of these organs. Furthermore, it has been demonstrated that the endothelial ligand for CD 18, the intercellular adhesion molecule-1 (ICAM-1, CD 54), is also involved in reperfusion injury because an antibody directed against this antigen effectively reduces infarct size, and does so to the same extent as a CD 18 antibody (39). Moreover, the binding action of neutrophil CD 18 to endothelium appears to be the signal required for the neutrophil to release H₂O₂ and possibly proteases into the extracellular environment

(40). These paired adhesion molecules, CD 18/ICAM-1, are the only antigenic determinants of neutrophil-endothelial adhesion for which clear roles have been demonstrated in ischaemia-reperfusion injury.

Treatment with a CD 18 monoclonal antibody also has profound effects in experimental hypovolaemic shock and resuscitation, which might be considered as a form of whole body continuous ischaemia and reperfusion (41,42). In these studies, not only did treatment with the CD 18 antibody prevent mortality after rabbits were subjected to shock, but it also significantly reduced their requirements for continuing fluid replacement during and after the period of resuscitation. This important finding indicated indirectly that neutrophil activation contributes to the increased microvascular permeability characteristic of shock.

Because microvascular aggregates could exacerbate ischaemia-reperfusion injury, and platelets are known to aggregate in response to thromboxane, it has been suggested that this cell might be important in ischaemia-reperfusion injury. However, platelets are not thought to be directly involved, for several reasons. First, they do not accumulate in ischaemic tissue during reperfusion, as demonstrated by intra-vital microscopy of skeletal muscle (43) and by using ^{111}In -labelled platelets as a tracer (1). This contrasts with the consistent presence of neutrophils. Secondly, platelets do not possess CD 18 antigens. Therefore, the protection afforded to reperfused tissue by CD 18 antibodies cannot be explained by an effect on platelets. Thirdly, although stimulated platelets are known to be a prominent source of thromboxane, neutrophils are more likely to be the cell responsible for the generation of thromboxane in ischaemia-

reperfusion, as prior depletion of circulating neutrophils almost completely inhibits the rise in thromboxane, without affecting the platelet count (22). These considerations do not deny the possible indirect role of platelets whereby their secretion of thromboxane and other agents might trigger neutrophil activation.

Lung injury following lower torso ischaemia

The remote consequence of ischaemia-reperfusion is non-cardiogenic pulmonary oedema, secondary to neutrophil-mediated abnormal permeability of the lung microvasculature to protein (1,44). It is assumed that the larger the mass of ischaemic tissue, and the longer the ischaemic time, the more probable it is that remote lung injury will occur. Indeed, in a series of 20 consecutive patients undergoing abdominal aortic aneurysm repair, clinical and radiological evidence of pulmonary oedema developed in each case (45). Experimental models suggest that this injury is also caused by the sequestration of activated neutrophils within the pulmonary microvasculature, because both neutrophil depletion and use of inhibitors of neutrophil activation effectively prevent the resulting pulmonary oedema (46,47). The mechanism whereby neutrophils selectively accumulate in the lung to cause injury following ischaemia of the lower torso is not fully understood. It is likely that additional mediators such as cytokines are involved since in other experimental settings these agents have been found to stimulate neutrophils to adhere to pulmonary endothelium (48). Cytokines are also involved in the processes determining neutrophil adhesion, by directly activating endothelium to become adhesive for neutrophils (49).

Treatment of ischaemia-reperfusion injury

Clearly the avoidance of prolonged periods of ischaemia limits the likelihood of large quantities of hypoxanthine and xanthine oxidase accumulating within ischaemic tissue. However, in clinical situations such as occlusive arterial trauma, transplantation, myocutaneous flaps, and in operations using tourniquets and arterial clamps, the opportunities for ischaemia-reperfusion injury are many. How might this injury be limited? First, inhibitors of oxygen radicals may be effective in limiting the generation of arachidonic acid products following ischaemia. Indeed, mannitol, which is a hydroxyl radical scavenger, reduces the lung oedema and permeability following aneurysm surgery by a mechanism independent of its ability to promote osmotic diuresis (50). Allopurinol, a xanthine oxidase inhibitor, has limited ischaemic injury in several animal models (6). Secondly, inhibitors of lipoxygenase or thromboxane synthetase might reduce generation of chemoattractants and thereby prevent neutrophil migration into reperfused tissue, and improve capillary blood flow. Thirdly, a monoclonal antibody directed towards the neutrophil CD 18 complex or endothelial ICAM-1 could be used. The *in vivo* studies reported suggest that antibody therapy is an effective means of reducing neutrophil accumulation into reperfused tissue.

The use of mannitol 0.2 g/kg may be of benefit in limiting reperfusion injury in other situations besides aneurysm surgery. These might include operations to release a strangulated hernia or testis, during femoral embolectomy or prior to revascularization of a traumatically ischaemic limb. Unfortunately,

cyclooxygenase inhibitors such as ibuprofen or aspirin are potentially hazardous in these clinical settings because they might hinder renal function through their inhibition of prostaglandins.

Summary

Ischaemia is a common clinical event leading to local and remote injury. Evidence indicates that tissue damage is largely caused by activated neutrophils which accumulate when the tissue is reperfused. If the area of ischaemic tissue is large, neutrophils also sequester in the lungs, inducing non-cardiogenic pulmonary oedema. Ischaemia-reperfusion injury is initiated by production of reactive oxygen species which initially appear responsible for the generation of chemotactic activity for neutrophils. Later, once adherent to endothelium, neutrophils mediate damage by secretion of additional reactive oxygen species as well as proteolytic enzymes, in particular elastase. Therapeutic options for limiting ischaemia-reperfusion injury include inhibition of oxygen radical formation, pharmacological prevention of neutrophil activation and chemotaxis, and also use of monoclonal antibodies which prevent neutrophil-endothelial adhesion, a prerequisite for injury.

Figure 1. Ischaemia leads to build up within endothelial cells of hypoxanthine and xanthine oxidase. The re-introduction of O_2 with reperfusion leads to generation of superoxide anion ($O_2^{\cdot-}$) and other reactive oxygen metabolites within endothelial cells. The hydroxyl radical (OH^{\cdot}) may be produced by the reaction of superoxide and H_2O_2 in the presence of Fe^{++} ions or Cu^{++} (the Haber-Weiss reaction) or H_2O_2 alone in the presence of Fe^{++} (the Fenton reaction). Following neutrophil activation, myeloperoxidase (MPO) in the neutrophil generates HOCl. SOD denotes superoxide dismutase. (Diagram adapted from Granger, 1988 [6]).

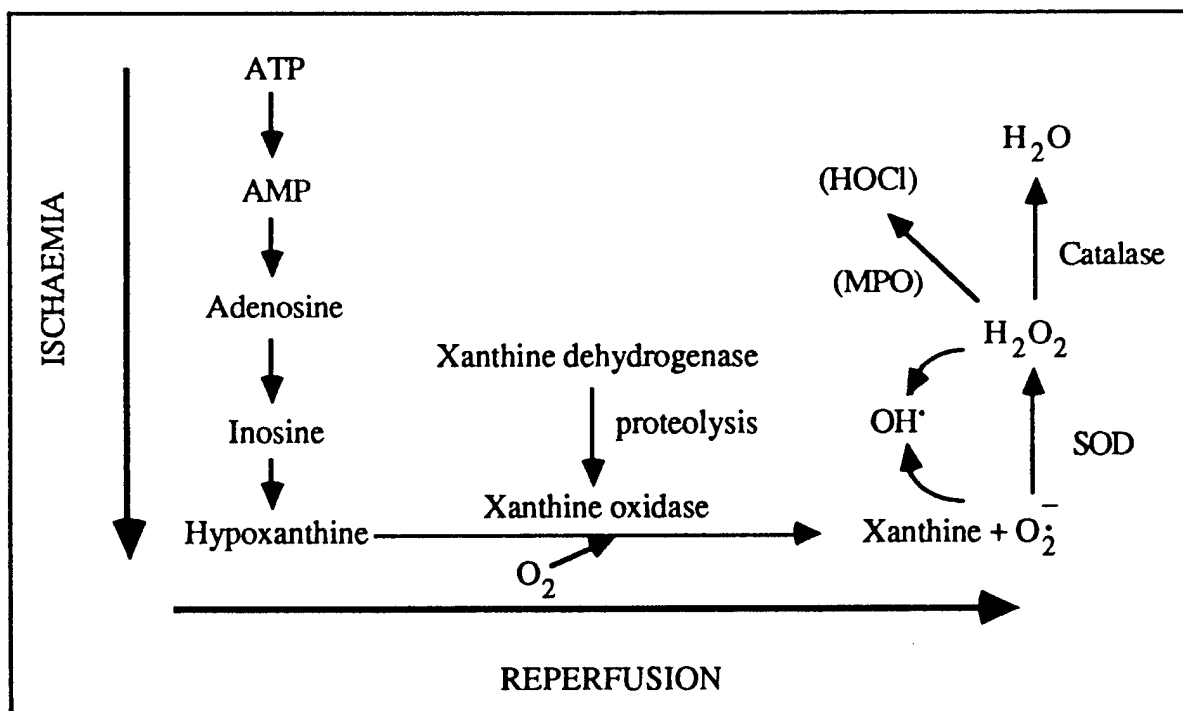


Figure 2. The arachidonic acid cascade is illustrated showing essential products and intermediates. LT, Tx and PG denote leukotriene, thromboxane and prostaglandin respectively.

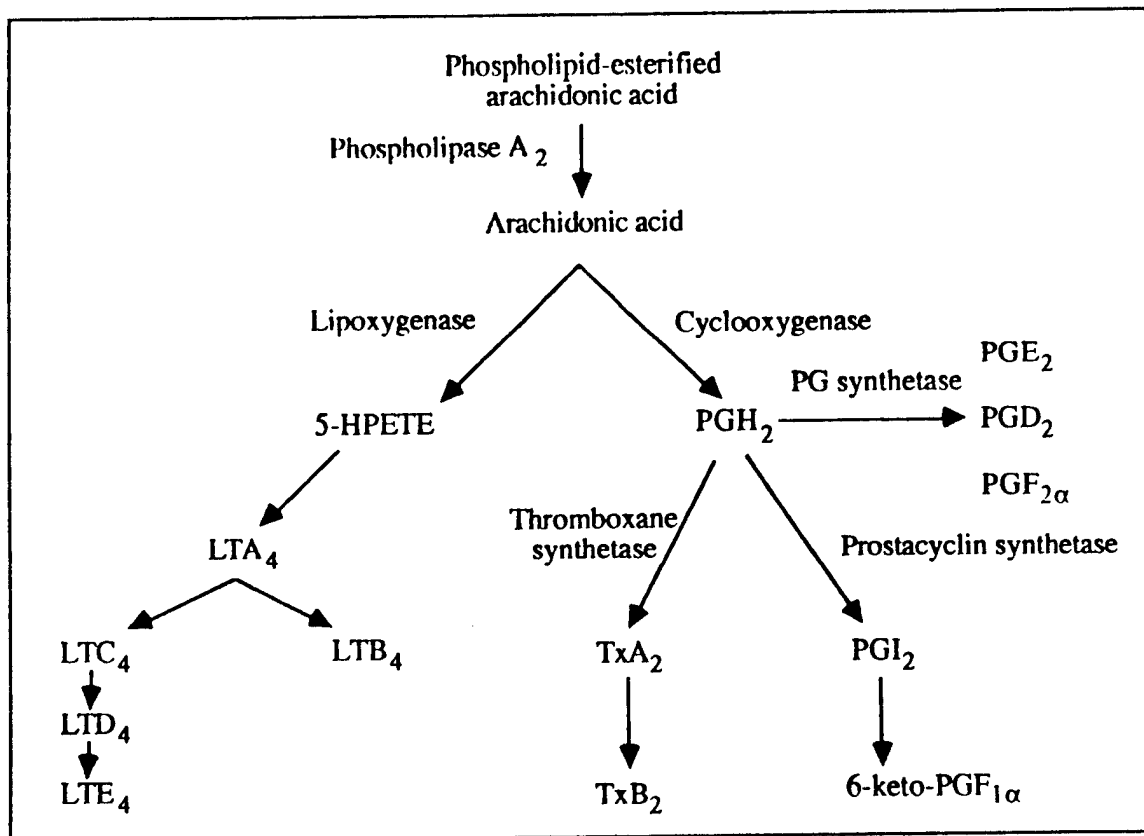
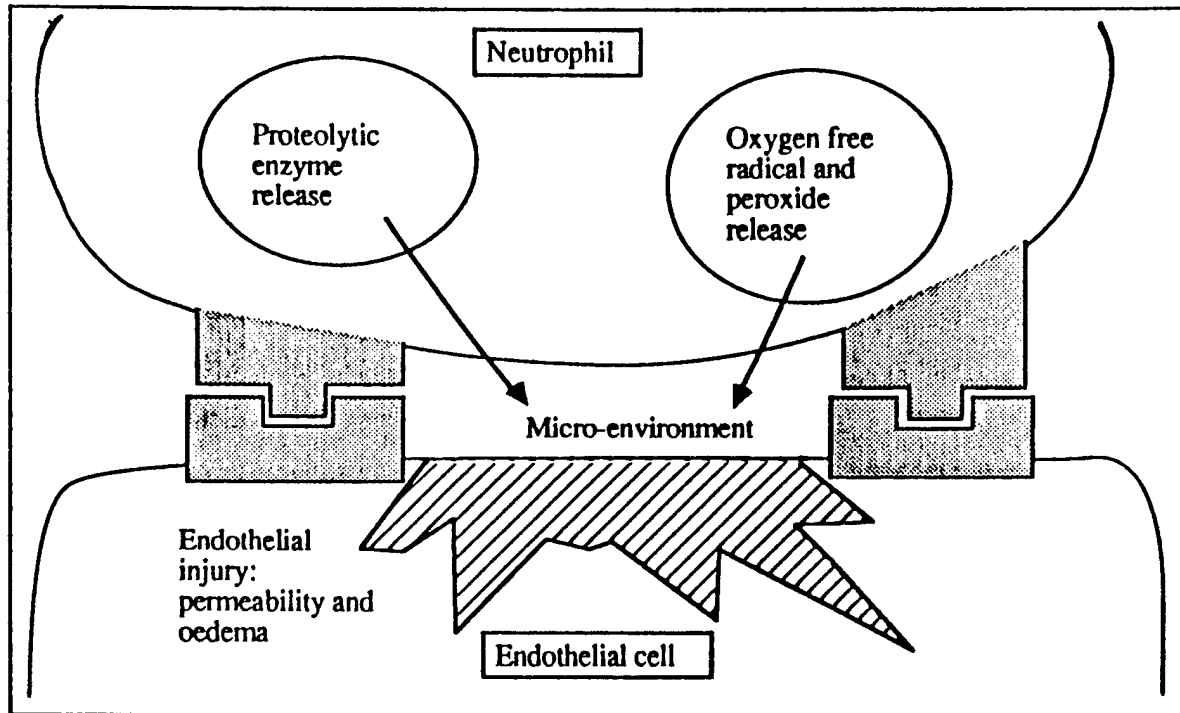


Figure 3. Schematic diagram of neutrophil-mediated endothelial injury. Following binding of its adhesion receptors (the CD 18 complex) to endothelial ligands, the activated neutrophil releases proteolytic enzymes and reactive oxygen metabolites into the extracellular space. This results in tissue permeability and oedema.



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