Part X

Factors influencing results

Reperfusion Injury following Surgical Treatment

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It is now well established that skeletal muscle hypoxia will result in irreversible damage after an ischaemic time of 4 to 5 hours (1). Historically it was believed that if ischemia (hypoxia) caused cellular injury, then reperfusion (re-oxygenation) would have a beneficial effect, preventing necrosis and regaining organ function. Over recent decades, however, studies have demonstrated that reperfusion may paradoxically augment tissue injury. This was first described by Haimovici in 1960 and coined the 'myonephropathic-metabolic syndrome' (2). Today cellular damage after the reperfusion of previously viable ischaemic tissue is defined as 'ischaemic-reperfusion injury' (3).

Ischaemic-reperfusion injury (IRI) is associated with a number of clinical scenarios including coronary thrombolysis, myocardial re-vascularisation, gut ischemia, organ transplantation and free tissue transfer (4). The severity of injury is primarily dependant upon the ischaemic time, although O_2 concentration, pH and temperature may influence damage. In vascular surgery IRI is most commonly encountered following the reperfusion of a large mass of skeletal muscle. This usually follows revascularisation of the acutely ischaemic leg or repair of the abdominal aortic aneurysm (AAA). Injury is characterised by the presence of an acute inflammatory reaction, and the clinical sequelae depend upon the severity of the original insult. Local complications include reperfusion oedema, acute compartment syndrome and the 'no-reflow' phenomenon. The intensity of the inflammatory reaction, however, may be so great as to produce systemic effects. These are manifest clinically as the systemic inflammatory response syndrome (SIRS), which may culminate in the multi-organ dysfunction syndrome (MODS).

The pathophysiology of skeletal muscle ischemia-reperfusion injury

Although the deleterious effects of cellular hypoxia have long been recognised, tissue reperfusion may initiate a complex inflammatory cascade that exacerbates tissue injury. This is characterised by the presence of leukocyte sequestration, enhanced reactive oxygen species generation, the release of inflammatory mediators, complement activation, impaired arteriolar vasodilatation and poor capillary perfusion. Endothelial intergrity may be disrupted leading to loss of fluid into the interstitial space, release of toxins and massive swelling. Animal studies have shown that cellular injury following skeletal muscle reperfusion is attributable to necrosis rather than apoptosis (5). It is now apparent that the reperfusion-induced inflammatory response may produce more necrosis than the original ischaemic insult alone. Consequently some authors have made the analogy that the reperfusion of ischaemic tissue is like 'pouring gasoline on to a smouldering fire' (6). To date a number of mechanisms have been implicated in the pathogenesis of IRI, and an understanding of these is essential to allow the development of novel therapeutic strategies.

(a) The cellular effects of ischemia

Under physiological conditions the degradation of adenosine 5' triphosphate (ATP) as an energy substrate (oxidative phosphorylation) results in the formation of hypoxanthine. This is oxidised by the enzyme xanthine dehydrogenase (XD), present in abundance, to form xanthine.

Cellular hypoxia depletes ATP energy stores, and metabolism switches to anaerobic glycolysis, with accumulation of lactate and a reduction in pH. If cellular energy requirements cannot be maintained then a failure in the cell membrane's Na^+/ K^+ ATPase results in a disturbance of the ionic gradient. This permits the influx of Na+, and H_20, and efflux of K^+. A rise in intra-cellular Ca^{2+} may activate proteases and cause lethal cell injury (7). Furthermore Ca^{2+} promotes the conversion of XD to xanthine oxidase (XO). This enzyme is unable to catabolise hypoxanthine, which accumulates in ischaemic muscle, and provides a source of reactive oxygen species (ROS) on reperfusion (see Figure 1.) Secondary autolysis follows and is characterised by lysosomal swelling, dilatation and vesiculation of endoplasmic reticulum, leakage of enzymes and proteins, and loss of cellular compartmentalisation. Ultimately membrane integrity cannot be maintained and cellular necrosis ensues (8).

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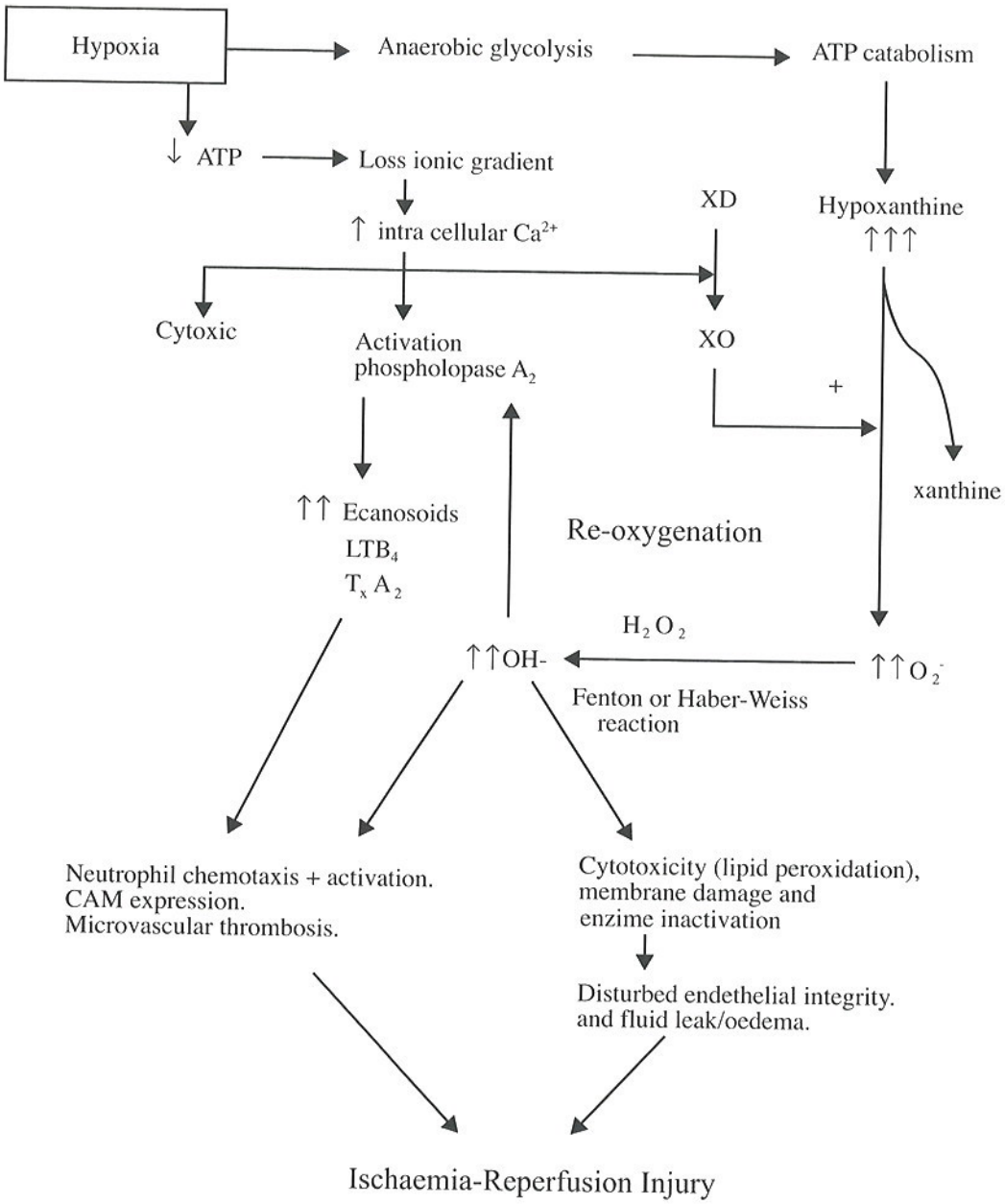

FIGURE 1
Biochemical events in ischemic-reperfusion injury (IRI).

(b) Microvascular endothelial dysfunction in ischemia- reperfusion

Being susceptible to the effects of hypoxia and re-oxygenation the microvascular endothelium is likely to play a pivotal role in the pathogenesis of IRI (9). Prolonged hypoxia induces endothelial cell swelling, lifting and the adherence of activated leukocytes and platelets. This disturbance in endothelial integrity predisposes to fluid leak on reperfusion.

Endothelial dysfunction may, however, occur in the absence of morphological change and 'activated' endothelium secretes the soluble chemotactic factors interleukin-8 (IL-8) and platelet activating factor (PAF) (10,11). The expression or upregulation of cellular adhesion molecules (CAM's) facilitates interactions with leukocytes (12). Secretion of tissue factor and plasminogen activator inhibitor-1 (PAI-1) cause local activation of the coagulation cascade and an inhibition of fibrinolysis (13,14). Suppressed production of 'anti-thrombotic' fac-

tors including nitric oxide (NO), prostacyclin (PGI_2), protein S and tissue plasminogen activator (t-PA) is also seen (4). Thus the 'activated' endothelium regulates the sequestration of leukocytes in response to an injurious stimulus, resulting in a pro-inflammatory and thrombotic environment within the micro-vasculature.

Many of these responses are exacerbated by reperfusion (15) and microvascular dysfunction is manifest in a site specific manner (16). An impaired endothelial-dependant, NO-mediated response to vasodilatators is seen within the arterioles (17). In capillaries increased fluid filtration (18) and reduced perfusion occurs. The latter may relate to 'plugging' by activated leukocytes and/ or platelets (19), or hindrance to microvascular flow by detached endothelial cells and/or interstitial oedema (20). It is the post-capillary venules that bear the brunt of the inflammatory response and here leukocyte sequestration, platelet-leukocyte aggregation, reactive oxygen species (ROS) production, reduced NO bioavailability and increased endothelial permeability have all been demonstrated in post-ischaemic skeletal muscle.

(c) The role of polymorphonuclear cells (neutrophils)

Neutrophils are one of the key mediators of IRI and both local and systemic damage are associated with neutrophil sequestration. Neutrophils are often widely distributed throughout reperfused tissue (21). Although the mechanism is uncertain it would appear that adherence is a prerequisite for injury and this takes place in the post-capillary venules (22). Increased venular permeability (23) is now well documented after reperfusion and correlates with the number of adherent leukocytes (24).

'Activated' neutrophils express functionally active β2 integrins (ie CD11 / CD18 receptors) on their surface membrane which facilitate interactions with the endothelium, platelets and other neutrophils. A number of mediators are implicated in causing neutrophil 'activation' including IL-1β, IL-6, IL-8, tumour necrosis factor-α (TNF-α), PAF, leukotriene B_4 (LTB_4) and the complement product C5a. Within the interstitium 'activated' neutrophils undergo a 'respiratory burst' and cause tissue destruction by releasing ROS, proteolytic enzymes (collagenase, elastase, cathepsin G) and peroxidase (25). Furthermore the release chemoattractants including LTB_4, thromboxane and PAF may further amplify the inflammatory response (26,27). Injury in reperfused tissues is manifest as increased microvasculature permeabilty, oedema, thrombosis and cell death.

Leukocyte-endothelial interactions

Leukocyte-endothelial interactions are facilitated by the sequential binding of CAM's with their counter-receptors to form an 'adhesion cascade'. Interaction occurs in five distinct steps, as apparent on videomicroscopy, and are illustrated in Table I.

(i) *Margination*. At sites of inflammation leukocytes are pushed outwards from the central stream towards the endothelium, and this is likely arise from haemodynamic factors (28).
(ii) *Initial adhesion and rolling*. The selections are membrane bound glycoproteins and are the prime mediators of this stage. Only 'activated' endothelium expresses E and P selectins, which form transient bonds with leukocyte glycoprotein P-selectin glycoprotein ligand-1 (PSGL-1) (29). In vitro E-selectin expression is dependant upon cytokine stimulation (30) and causes neutrophils to decelerate and roll on the endothelial surface. Furthermore leukocyte bound L-selectin bonds to PSGL-1 of other leukocytes causing leukocytes to roll on adherent leukocytes

TABLE I
The stages of leukocyte-endothelial interaction and their molecular basis.

Stage	Cellular Event	Molecular mechanism
I Margination	RBC's 'pushed' from centre stream at sites of inflammation	Haemodynamic factors
II Initial adhesion and rolling	Leukocytes decelerate and roll on endothelium.	Selectin expression on 'activated' endothelium. Transient bond formation with leukocyte PSGL-1.
III Stable arrest and adhesion	Formation of stable leukocyte-endothelial adhesion bonds	'Activation' / upregulation of leukocyte β2 integrins. Stable bond formation with endothelial ICAM-1.
IV Transepithelial migration/ diapedesis	Leukocytes flatten and extend pseudopods through inter-cellular endothelial junctions	β2 integrins – ICAM-1/ PECAM-1interactions and chemoattractants.
V Interstitial migration	Leukocyes migrate against concentartion gradient. Effector function.	Chemoattractants

and aggregate (31). This may be a means of enhancing leukocyte recruitment, although neutrophil plugging has been implicated in capillary malperfusion.

(iii) *Stable adhesion*. This stage is primarily mediated by leukocyte β_2 integrins, cell surface glycoproteins that are constitutively expressed. Although upregulation of specific β_2 integrins (eg Mac-1) occurs, conformational change is likely to be necessary for ligand binding (32). β_2 integrin-activation may be induced by inflammatory mediators and chemotactic agents and renders them competent to form stable bonds with the endothelial counter-receptor intercellular adhesion molecule-1 (ICAM-1) (33, 34). ICAM-1 is a member of the immunoglobulin superfamily, abundant cell surface glycoproteins. Expression is upregulated on 'activated' vascular endothelium by the inflammatory cytokines (35). The soluble form (sICAM-1) is shed by activated endothelial cells and this may be functionally active within the circulation (36). The physiological significance of this is not yet known, although elevated plasma levels have been demonstrated in inflammatory disorders.

(iv) *Transendothelial migration/diapedesis*. The sequential formation and breakage of β_2 integrin-ICAM-1 bonds allows leukocytes to 'crawl' towards inter-cellular endothelial junctions and in part facilitates diapedesis. Platelet-endothelial cell adhesion molecule-1 (PECAM-1) is constitutively expressed and mediates adhesion at endothelial cell junctions. PECAM-1 adhesive interactions and chemoattractants are required for diapedesis (37).

(iv) *Interstitial migration and effector function*. Neutrophils migrate along a concentration gradient of chemoattractants secreted by macrophages and somatic cells within the interstitium (38). These include the 'classical' chemoattractants (eg anaphylotoxin C5a, PAF, LTB4) and the chemokines (eg IL-8, PF4).

Role of the neutrophil is skeletal muscle IRI

Animal models have shown the sequestration of neutrophils in post-ischaemic skeletal muscle and these are likely to play a vital role in inducing tissue damage (20,39). Both CAM and chemoattractants have been shown to mediate this recruitment (40). Monoclonal antibodies directed against Mac-1, ICAM-1and PECAM-1 may attenuate muscle injury (see section 4).

There are few clinical studies but a significant increase in plasma elastase and urinary albumin: creatinine ratio has been demonstrated following elective femoro-distal bypass grafting for critical limb ischemia (CLI) (41). This suggests that neutrophils are activated during elective bypass surgery resulting in an increase in microvascular permeability. Interestingly this was accompanied by a fall in plasma sICAM-1, peripheral neutrophil CD11b expression and in-vitro neutrophil adhesion. It was postulated that this represents a physiological response whereby shed endothelial ICAM-1 binds to the neutrophil CD11b receptor, producing reduced adhesion and an apparent fall in levels. Subsequently increased in-vitro neutrophil adherence has been demonstrated after femoro-distal bypass. This was not related to an increase in CD11b expression (42), suggesting that a qualitative change in the CD11b receptor may be required. Furthermore adherence was blocked by increasing doses of sICAM-1, which suggests that elevated sICAM may play a role in the regulation of neutrophil trafficking in inflammatory disorders.

(d) Reactive oxygen species (ROS) in ischemia-reperfusion

The reperfusion of ischaemic tissue results in the enhanced production of ROS, initially by the endothelium (primarily venular) and secondarily by 'activated' neutrophils (43).

With re-oxygenation hypoxanthine is catabolised by XO, resulting in a burst of superoxide anion (O_2^-) production. Under physiological conditions O_2^- is converted to hydrogen peroxide (H_2O_2) by superoxide dismutase, and H_2O_2 to water by catalase. But during ischemia-reperfusion endogenous scavengers are overwhelmed and O_2^- generation predominates. O_2^- can react with H_2O_2 (via the Haber-Weiss or Fenton reactions) and NO to form the highly reactive hydroxyl radicle (OH^-). This has been illustrated in Figure 1. OH^- is a potent oxidizing agent that causes lipid peroxidation, enzyme inactivation and damage to membrane carrier proteins. Lipid peroxidation may have devastating effects on endothelial integrity leading to interstitial fluid leak (44). They may potentiate the inflammatory response by the direct destruction of cellular structures, inducing leukocyte chemotaxis and activation, enhancing ICAM-1 and E-selectin expression (45) and reducing NO bioavailability (46). O_2^- promotes the formation of PAF (47) and LTB4 (34).

O_2^- has been demonstrated in skeletal muscle (48) and the venous effluent of reperfused skeletal muscle in animal models (49) Pattwell et al. demonstrated a large increase in OH^- activity within the interstitial space of post-ischaemic skeletal muscle, and suggested that therapy should be directed against this rather than the O_2^- (50). Furthermore Serra et al. demonstrated that the peroxynitrite anion causes oxidative skeletal muscle injury in a rabbit model (51).

Few clinical models exist but ROS have been shown to be generated after femoro-popliteal bypass and are responsible for lipid peroxidation (52) In a small clinical

follow-up study patients undergoing distal bypass for CLI have been shown to have a significantly reduced total anti-oxidant capacity (TAC), increased lipid peroxidation and increased vascular permeability (urinary ACR) pre-operatively as compared to control subjects (53). After reperfusion patients who developed SIRS had a greater reduction in TAC, increased lipid peroxidation and vascular permeability as compared to those with an uneventful post-operative recovery. This suggests that low pre-operative TAC may predispose patients to the deletrious effects of ROS damage and their adverse consequences.

(e) Role of arachiodonic acid metabolites

The ecanosoids are metabolites of arachiodonic acid that are produced by a variety of cells including endothelium, leukocytes, platelets and somatic cells. Metabolism by the lipo-oxygenase pathway yields the leukotrienes, including leukotriene B_4 (LTB_4). This is chemotactic, causes neutrophil 'activation' and induces CAM expression. The cyclo-oxygenase pathway yields the prostaglandins (eg PGE_1, PGE_2), prostacyclin (PGI_2) and thromboxane (T_xB_2). T_xB_2 is a powerful vasoconstrictor and induces platelet aggregation, whereas endothelially-derived PGI_2 antagonises these effects. PGE_1 is also a vasodilator, inhibits neutrophil and platelet aggregation, and suppresses TNF production.

The breakdown products of arachiodonic acid are found in high concentrations in plasma after the reperfusion of ischaemic tissue. ROS would appear to be a prerequisite for their generation (54) and there is evidence from animal models to suggest that free radicle inhibition prevents the release of ecanosoids during skeletal muscle reperfusion (55). ROS increase intra-cellular Ca^{2+} which activates membrane-bound phopholipase A2 causing lipid peroxidation, releasing arachiodonic acid and lipid peroxyl free radicles. Imbalance of ecanosoid production may result in increased capillary permeability, oedema and cell aggregation (56). Ischemia-reperfusion may result in altered ecanosoid production with a net increase in vasoconstrictors and decrease in vasodilators and consequent 'low-reflow' (57,58). This may include enhanced thromboxane (T_xB_2) secretion and endothelial PGI2 suppression (59). Evidence suggests that LTB_4 is generated in sufficient quantities to induce diapedesis (60). T_xB_2 has been shown to increase vascular permeability in post-ischaemic muscle (61). Finally arachiodonic acid metabolites have been shown to induce neutrophil activation and enhanced free radicle generation (62).

(f) Complement

Skeletal muscle ischemia and reperfusion causes activation of the complement plasma protein cascade via the alternative pathway (63,64). This results in the generation of the bioactive peptides including the anaphylotoxins (C3a and C5a) (65). These are potent inflammatory mediators which may induce cytokine production, alter vascular tone and increase capillary permeability. Their effect on neutrophils includes chemotaxis, 'activation' and enhanced FOS production (64). C5b-9 may also inhibit endothelium-dependant relaxation (65).

(g) The role of the inflammatory cytokines

The inflammatory cytokines have an important role in regulating the acute inflammatory response. Injurious stimuli, including hypoxia, may cause interstitial mast cell degranulation and the release of inflammatory cytokines including IL-1β and TNF-α. These cytokines are powerful chemoattractants and may induce neutrophil, endothelial and platelet 'activation' (66,67). These also promote the production of chemokines (eg IL-8) by somatic cells (68) and enhance endothelial PAF and IL-6 production (69). IL-6 primes neutrophils for their respiratory burst and release of free radicles.

Both IL-1 and IL-6 have been shown in high concentrations in the plasma after the reperfusion of skeletal muscle in animal models (70). The role of TNF-α in directly causing skeletal muscle injury is unclear. Using a rat hind-limb acute ischemia model Seekamp et al. has shown that TNF-α blockade reduced skeletal muscle injury (71). Other authors have, however, have failed to demonstrate this relationship (72). More recently Gaines et al. found that acute hind limb ischemia in a rat model produced a systemic TNF-α response. This response was partly responsible for the associated skeletal muscle injury and injury was attenuated using TNF antibodies (73).

(h) Nitric oxide-superoxide imbalance in ischemia-reperfusion

Under physiological conditions the endothelium produces NO in abundance and this is 'protective' towards the microvasculature by scavenging ROS, reducing arteriolar tone and preventing interactions with platelets and leukocytes. Reperfusion causes a burst of superoxide (O_2^-) production, which inactivates NO (74). Bioavailability may be further reduced by suppression of NO synthase (NOS) under hypoxic conditions (4). Thus reperfusion adversely affects the NO: O_2^- ratio, and this is manifest as an impaired arteriolar response to vasodilators (eg acetyl choline), platelet aggregation and venular inflammatory response (17). It seems likely that O_2^- is responsible for impaired arteriolar NO-mediated vasodilatation because superoxide dismutase can restore this response (17). Furthermore mice that are genetically deficient in leukocyte (CD11/CD18) or endothelial

CAM's (P-selectin, ICAM-1) do not exhibit impaired vasodilatation, suggesting that activated neutrophils provide an important source of O_2^- (75).

The clinical sequelae of skeletal muscle ischemia-reperfusion

(a) Local complications

(i) Post-reperfusion oedema

Perhaps the most common 'complication' following lower limb re-vascularisation is leg oedema. This complicates between 40% and 100% of elective procedures (76,77) and is characterised by the presence of oedema within the subcutaneous tissues (78,79). Oedema may be considerable, accounting for a 9% mean difference in lower limb volume. (80). The control of oedema has been shown to be important in minimising wound complications after pedal bypass (81).

Reperfusion oedema is likely to be of a multi-factorial aetiology. The disruption of lymphatic channels may play a role (82,83), although oedema may complicate percutaneous angioplasty, where lymphatic disruption does not take place (84). As such it seems likely that other mechanisms are involved. The post-reperfusion hyperaemic response may contribute to increased capillary leak (85), although this does not explain the delayed increase in limb volume.

Ischemia-reperfusion causes the release of activated neutrophils, ROS and inflammatory mediators, which may damage endothelial integrity and cause fluid leak. Certainly free radical generation with lipid peroxidation has been demonstrated after elective femoro-popliteal bypass and may contribute to lower limb oedema (52, 86). This might explain why the lipid-rich subcutaneous tissues are primarily prone to oedema formation.

Prophylaxis and treatment of reperfusion oedem

All patients undergoing lower limb revascularisation should be subject to simple preventative measures including gentle elevation, calf pump muscle exercises, early ambulation and deep vein thrombosis (DVT) prophylaxis. The use of anti-oxidants and allopurinol may be of benefit and are outlined in section 4. In the presence of significant post-operative limb swelling DVT should always be excluded. It is of interest to note that Hamer phlebographically demonstrated DVT in all patients with circumferential leg swelling of more than 4.5cm (87).

(ii) Acute post-ischaemic compartment syndrome

The compartment syndrome was first described by Volkmann in 1881 (88). It may be defined as a condition in which high pressure within a closed fascial space reduces capillary blood perfusion below a level necessary for tissue viability (89). 'Primary' compartment syndrome develops prior to reperfusion (90) and this may relate to spontaneous thrombolysis, collateral supply or incomplete ischemia (89,90). 'Post-ischaemic' compartment syndrome occurs after the reperfusion of an ischaemic extremity. This commonly follows acute ischaemic insults including thrombo-embolism, graft thrombosis, trauma or repair of the ruptured aortic aneurysm (91). Here reperfusion after prolonged ischaemic times may cause enhanced transcapillary fluid leak and a consequent rise in intra-compartmental pressure (92).

Although raised compartment pressures have been demonstrated following elective revascularisation these are not usually sufficient to cause a compartment syndrome (93,94). The incidence of fasciotomy after elective surgery is low, and has been estimated at between 0.15% and 0.45% of cases (95). In Jensen et al.'s series, although only four patients required fasciotomy after elective vascular surgery, all were critically ischaemic and this may suggest that the degree of pre-operative ischemia correlates with the risk of compartment syndrome.

Pathophysiology

Under physiological conditions resting compartmental pressures range from 0 to 12mmHg. Reperfusion-induced capillary leak causes a rise in interstitial pressure. Thin-walled veins collapse, obstruct venous outflow, and exacerbate oedema. A reduction in the arterio-venous pressure gradient and increased interstitial pressure results in reduced transcapillary diffusion. When interstitial pressure equals that of the capillaries tissue perfusion ceases. A vicious cycle is thus established with progressive swelling, rising compartmental pressure and cellular ischemia (89). Unless rapidly decompressed irreversible hypoxic neuro-muscular damage follows, with Volkmann's ischaemic contracture and ischaemic neuropathy (89).

Prophylaxis and treatment of post-reperfusion compartment syndrome

There are two therapeutic strategies currently employed to prevention the development of post-reperfusion compartment syndrome. These are particularly salient when revascularising the viable acutely ischaemic limb. Firstly to reduce tissue swelling by minimising the ischaemic time (i.e. prompt revascularisation) or the use of novel therapeutic agents including controlled reperfusates, free radicle scavengers, anti-oxidants and vasodilators (see section 4). Secondly to allow swelling but to release the fascial compartment prophylactically, which is required in between 7% and 22% of patients with

acute ischaemic (96). There is, however, no agreement on criteria for prophylactic fasciotomy. Indications may include prolonged ischaemic times (typically > 6 hours), hypotension, massive soft tissue injury, tissue swelling, failure of arterial repair (97).

Post-operatively the diagnosis of compartmental hypertension is often difficult and it is always important to maintain an index of suspicion. Clinical features may include pain (often disproportionate to physical signs), pain on passive stretch of the muscle group, paresis, hypoaesthesia in the distribution of peripheral nerves running through the compartment and swelling of the fascial boundaries. In the terminal phase compartmental pressures may exceed systolic pressure, peripheral pulses are lost and limb loss is the likely outcome. Intra-compartmental pressure measurements can be made by needle pressure transducer, and are often a useful diagnostic adjunct. There is, however, little agreement as to the pressure level above which fasciotomy should be performed. Pressure levels above 30mmHg (98), 45mmHg (99) and within 10-30mmHg of the diastolic pressure (100) have been advocated. Within our own institution we have a low threshold and will perform fasciotomies above 25-30mmHg. The aim of fasciotomy is to reduce intra-compartmental pressure thus allowing tissue perfusion. The 'open' decompression of all four fascial compartments is usually advocated as it avoids the risk of inadequate decompression associated with a subcutaneous procedure (97,101).

(iii) The 'no-reflow' phenomenon

Over recent years it has become apparent that prompt revascularisation is not always enough to salvage the acutely ischaemic limb. Despite a technically successful bypass micro-circulatory thrombosis may result in perfusion failure and this is known as the 'no-reflow' phenomenon. 'No reflow' is well recognised in other surgical specialities especially after myocardial revascularisation, free tissue transfer and organ transplantation. In 1995 Weinzweg and Gonzalez postulated that free-flap failure was not invariably an 'all-or-none' phenomenon with sudden anastomotic thrombosis resulting in tissue necrosis. Instead they noted that some flaps die a slow progressive and partial death due to secondary thrombosis of the micro-circulation (102).

Similarly the 'no reflow' phenomenon may occur in post-ischaemic skeletal muscle due to progressive micro-circulatory occlusion (103) and becomes more severe as the ischaemic-time increases (104). Proposed pathogenic mechanisms include neutrophil or platelet plugging (105), hypoxic vasoconstriction (106) and widespread endothelial injury (103). Using a rat model Hardy et al. demonstrated a triphasic pattern of skeletal muscle blood flow after 6 hours of hindlimb ischemia (107). Immediately after the release of the tourniquet there was extremely low blood flow ('low reflow'). This improved slightly after 2 hours (relative reperfusion) before an ultimate decline (reperfusion injury). This clearly mirrors the clinical scenario in that limb viability is tenuous after the procedure, but then may temporarily improve before the patient finally requires an amputation. They postulated that initial 'low reflow' was due to transient vasoconstriction, allowing a transient improvement in blood flow before reperfusion injury culminated in microvascular occlusion.

(b) Remote Effects - the Systemic Inflammatory Response Syndrome and Multiple Organ Dysfucntion Syndrome

One devastating consequence of IRI is the damage of remote organs by non-specific activation of the 'inflammatory cascade'. This is manifest clinically as the systemic inflammatory response syndrome (SIRS) and may culminate multi-organ dysfunction syndrome (MODS). In an attempt to stratify septic-related conditions a consensus conference was held in 1991 to produce a series of universal definitions for these conditions (108). SIRS is defined as a clinical response to a non-specific insult comprising of at least two variables illustrated in Table II. If compensatory mechanisms are unable to maintain homeostasis organ failure may ensue and this is known as MODS.

SIRS has been demonstrated in 40% of patients undergoing elective femoro-distal bypass grafting for critical limb ischemia (53). Progression to end-organ failure in this small clinical study, however, was not documented. MODS has been documented following revascularisation of the acutely ischaemic limb (109), but is more commonly encountered following aortic cross-clamping. Here the whole lower torso (and potentially the left colon) is subject to ischemia-reperfusion (110). This insult may be compounded during emergency aortic aneurysm repair by hypotension and massive blood transfusion (111). MODS is now the leading cause of death after elective aortic aneurysm repair (112).

Pathogenesis of SIRS and MODS

In 1996 Bone proposed that there were three stages to the development of the SIRS (113). In stage I there is the local production of cytokines to promote an acute inflammatory response and wound healing. In stage II there is systemic release of cytokines, which induce an acute phase response to amplify the inflammatory process. This is usually tightly controlled by the down-regulation of pro-inflammatory mediators and upregulation of endogenous antagonists. A failure in homeostasis, however, allows the development of stage III –SIRS. Clinical outcome (ie resolution versus MODS and/or death) is dependant upon the balance between SIRS and

TABLE II

Criteria for the systemic inflammatory response syndrome (SIRS), sepsis and multi-organ dysfunction syndrome (MODS).

(from the American College of Chest Physicians and Society of Critical Care Medicine consensus conference, 1991)

	Definition
SIRS	Clinical response manifested by two or more of the following criteria: Temperature >38oC or <36 °C. Heart rate > 90 beats/minute. Respiratory rate > 20 breaths/minute. Leukocyte count > 12,000 or < 4,000 cells/mm^3.
Sepsis	SIRS with documented infection.
Severe sepsis	Sepsis with haemodynamic compromise.
MODS	A state of physiological derangement where organ function is not capable of maintaining homeostasis.

the compensatory anti-inflammatory response (CARS) (114). If SIRS predominates then this leads to a generalised state of microvascular dysfunction, characterised by vasodilatation and capillary leak. The 'activation' of endothelium and leukocytes result in micro-vascular thrombosis, oedema and neutrophil/ inflammatory mediator-induced end-organ damage. Ultimately an inability to maintain end-organ oxygen requirements culminate in the MODS (see Figure 2).

Mediators of MODS in skeletal-muscle ischemia and reperfusion

Early work has shown that the reperfusion of ischaemic limbs releases toxic metabolites including K^+, lactate (H^+) and myoglobin into the circulation causing renal, cardiac and pulmonary dysfunction (115). Profound IRI also causes the systemic release of ROS, 'activated' leukocytes, inflammatory mediators and complement fragments. These may precipitate a generalised inflammatory response and induce remote organ injury.

Activated leukocytes may re-enter the circulation on reperfusion and are implicated in causing end-organ damage (116). Up to 60% of the circulating neutrophil population are stored in the low pressure pulmonary vasculature (117). The lungs are thus prone to the sequestration of neutrophils and acute lung injury (118). Both neutrophil elastase and ROS have been shown to participate in PMN-induced lung injury in acute ischemia animal models (119). The enhanced expression of CAM may facilitate neutrophil-endothelial interactions. The plasma obtained after aortic cross-clamping induces the expression of ICAM-1 and this may facilitate pulmonary injury (120).

Enhanced free radicle production may contribute to remote organ injury and enhanced plasma xanthine oxidase activity has been documented after aortic cross-clamping (121).

The inflammatory cytokines are also released from reperfused tissues and are known to be the prime mediators of the SIRS and MODS (122). Animal models have shown that TNF-α causes pulmonary injury after skeletal muscle ischemia-reperfusion (123,124). Both TNF-α and thromboxane are released after aortic cross-clamping and relate to the development of remote organ injury (120). Increased thromboxane levels have been demonstrated after skeletal muscle ischemia (62). Finally the reperfusion of skeletal muscle results in the release of LTB_4 (125), PAF and activated complement fragments (126), which all may play a role in inducing remote organ damage.

Overwhelming evidence implicates the gut in the pathogenesis of MODS and many now believe that the gut may be both an instigator and victim of MODS (127). Splanchnic vasoconstriction, an adaptive response to maintain blood supply to vital organs, may result in ischaemic-reperfusion injury and the system release of inflammatory mediators (128). Loss of gut-mucosal barrier integrity may allow bacterial and endotoxin translocation, causing portal mast cell degranulation and the release of inflammatory cytokines. Clinical studies have shown endotoxin in the plasma of patients undergoing aortic aneurysm repair, a procedure that may involve ligation of the inferior mesenteric artery (129). In a rat model of bilateral hind-limb ischemia Yassin et al. showed that isolated skeletal muscle reperfusion also led to a significant increase in plasma endotoxin levels compared to controls (130). This work indicates that enhanced gut permeability may play an important role in the deve-

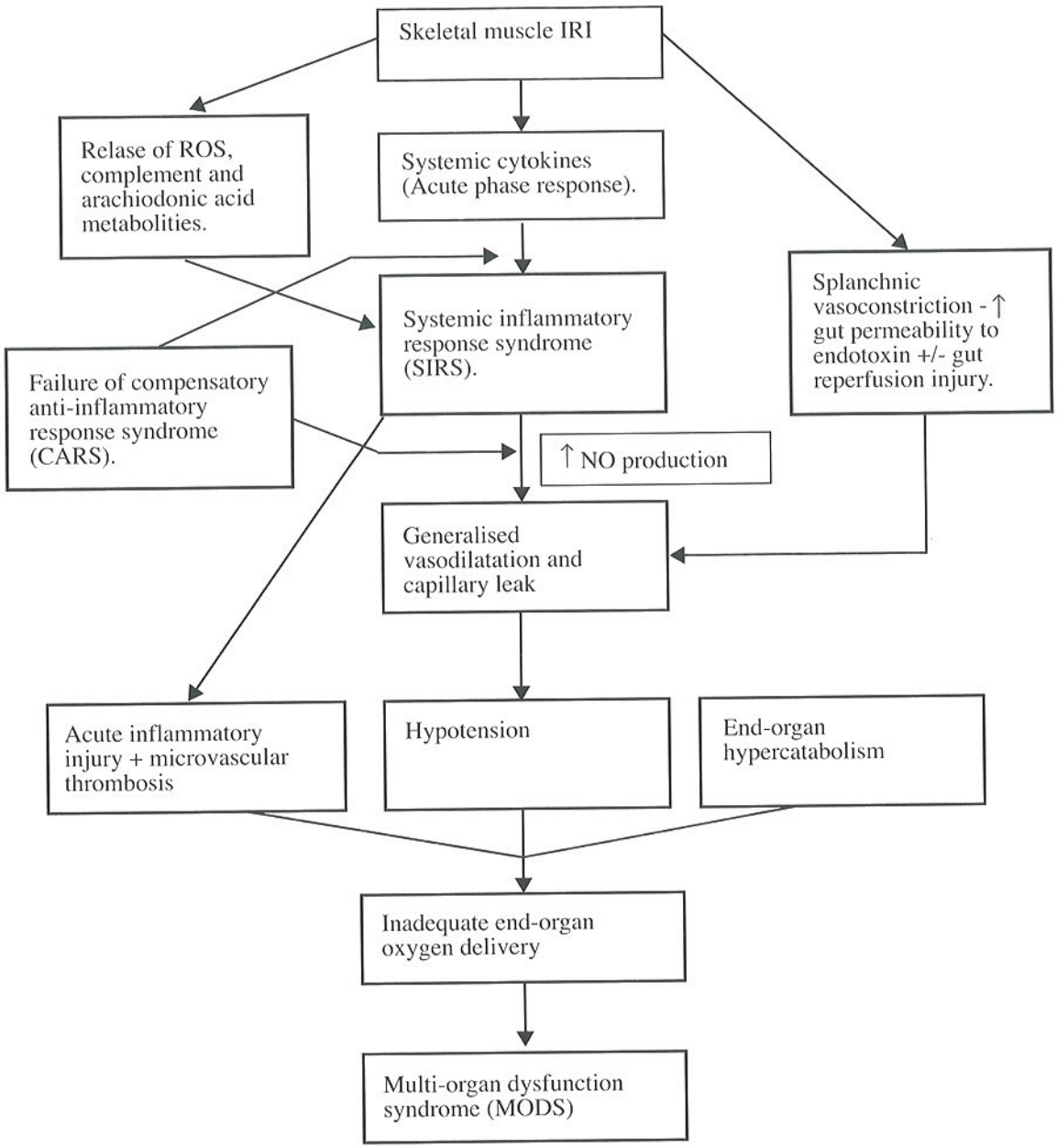

FIGURE 2

The pathogenesis of remote onjury after ischemia-reperfusion injury (IRI)

lopment of MODS after ischemia-reperfusion.

Clinical manifestations of MODS

MODS is now the leading cause of death on the intensive care unit, accounting for 30% to 40% of deaths (131). Mortality correlates with the number of organs that have failed with rates of approximately 30-40%, 50-60% and 80-100% when one, two or three organ systems have failed respectively.

Pulmonary injury is the most common presentation and is characterised by neutrophil sequestration, alveolar oedema, microvascular thrombosis, fibrosis, alveolar collapse and surfactant depletion on post-mortem studies. Clinically this results in progressive respiratory failure, ranging from 'acute lung injury' to the 'acute respiratory distress syndrome' (ARDS). The onset of ARDS is insiduous and occurs 24-72 hours after the initial insult. Progressive dyspnoea with profound hypoxaemia, reduced pulmonary compliance and diffuse bilateral infiltrates on chest X-ray are typical. Pulmonary artery pressures of < 16mmHg exclude cardiogenic pulmonary oedema. Hypoxaemia results from a combination of oedema, reduced compliance, microvascular thrombosis, small airway collapse and regional NO inhibition.

Septic-like states induce the production of NO with

consequent generalised vasoplegia, hypotension and increased cardiac output. Furthermore oedema, microvascular thrombosis and a cytokine-induced hypermetabolic state result in inadequate oxygen delivery to tissues. This potentiates the onset of organ dysfunction and renal failure, myocardial depression, hepatic dysfunction and gut failure (ileus and stress ulceration) all follow. The enhanced secretion of pro-thrombotic factors and fibrinolytic factors may result in disseminated intra-vascular coagulation.

Prevention and Treatment of MODS

The key to the prevention of acute lung injury and MODS is to minimise the factors that evoke a strong inflammatory reaction. In clinical practice this largely involves minimising cross-clamp times and the prevention of massive haemorrhage, shock and the need for massive blood transfusion. Gut-mucosal integrity can be maintained by early enteral feeding and stress ulcer prophylaxis (sucralfate) should be given. A number of novel therapeutic agents have been implicated in reducing end-organ damage and these are outlined in section 4. The treatment of established MODS is undertaken on the intensive care unit and is largely supportive. It is not within the scope of this chapter to discuss this in further detail.

Novel therapeutic strategies in skeletal muscle ischemia-reperfusion

A number of novel therapeutic strategies have been evaluated to attenuate skeletal muscle ischemia-reperfsuion injury. As many of these approaches are directed against the inflammatory cascade, modulation carries the theoretical risks of immunosuppression, impaired wound healing and post-operative infection. These statergies are outlined below.

(a) Controlled reperfusion

Efforts have been made to reduce local cellular injury and tissue swelling by manipulating the initial re-perfusate solution. Controlled reperfusion may reduce skeletal muscle injury by the gradual restoration of blood flow, preven accumulation of the tone anaerobic by-products or the washout of these products. Acutely ischaemic canine hindlimbs reperfused with hypothermic solutions show less muscle oedema, a higher interstitial pH and reduced permeability than controls (132). In a porcine model, using neutrophil-free reperfusate, a reduction in both local and systemic complications was obseved (133). In this study other agents (free radicle scavengers, calcium chelators and energy substrates) were added to the reperfusate and it is difficult to ascertain whether this beneficial effect was due to neutrophil depletion or other additives. Oredsson et al. demonstrated that reperfusion with neutrophils, superoxide dismutase and catalase produced less injury than with neutrophils alone (134). However, both of these produced more injury than reperfusion with a cell free buffer. In a non-controlled clinical study of acute limb ischemia, limb salvage was achieved in 11/14 patients using a reperfusate of oxygenated blood diluted 6:1 with a tightly controlled reperfusate solution (calcium, glucose, pH, free radicle scavengers) (135).

(b) Free radicle scavengers (FRS)

Efforts have been made to reduce ROS generation using scavengers including superoxide dismutase, catalase and mannitol. Animal models of acute limb ischemia have shown that the use of FRS may be of benefit in preventing skeletal muscle injury (136) and the development of compartment syndrome after prolonged ischemia (137).

Mannitol is perhaps the most widely studied of the free radicle scavengers and has been shown in animal models to increase blood flow after reperfusion and reduce the release of creatinine kinase (138).

Furthermore it causes a significant reduction in compartmental pressures through a combination of it's osmotic and free radicle scavenging properties (139). Clinical studies examining the efficacy of mannitol in reducing lower limb swelling after infra-inguinal bypass surgery have contradictory results (140,141). Others have shown that mannitol reduces the risk of renal injury (142) and acute lung injury (143) after AAA repair.

(c) Allopurinol

Allopurinol is an inhibitor of XO and thus reduces ROS generation. Animal models have shown that allopurinol reduces skeletal muscle IRI and compartment pressures in animal models (144). In a pig model Smeets et al. showed ischaemic-reperfusion injury after aortic repair in those animals with pre-operative shock, this was significantly reduced by the administration of allopurinol (145). In a small double-blind controlled clinical trial of patients undergoing femoro-popliteal bypass, the pre-operative administration of allopurinol has been shown to reduce lipid peroxidation and lower limb oedema compared to control subjects (146).

(d) Anti-oxidants

Anti-oxidants anatagonise lipid peroxidation and limit damage from ischemia-reperfusion. Clinical studies on patients undergoing aortic cross-clamping that Vitamin E reduces neutrophil accumulation in quadricep muscle

biopsies and may have a protective role against injury (147). A cocktail of anti-oxidants (containing alpha-tocopherol, ascorbic acid, and retinol) has been shown to reduce both lipid peroxidation and post-operative leg oedema in a heterogenous group patients undergoing bypass for acute and chronic leg ischemia (148).

(e) Prostaglandins

The infusion of prostaglandins that are 'protective' towards the microcirculation, including PGE_1 and iloprost (a PGI_2 analogue) may help ameliorate local reperfusion injury. Animal models of acute limb ischemia have shown the administration of PGE1 and iloprost improve blood flow at 4 hours post-reperfusion, and abolish reperfusion injury (149). The addition of FRS (superoxide dismutase and catalase) abolished initial 'low-reflow', suggesting that free radicles may mediate initial vasoconstriction. One small clinical trial demonstrated that iloprost significantly reduced the degree of capillary damage and endothelial swelling following femoro-distal reconstruction (150).

(f) Neutrophil interventions

Neutrophil depletion during cardiopulmonary bypass has been used successfully in animal models to reduce myocardial infarct size and post-operatively to improve lung function. The effect of leukocyte filters in preventing skeletal muscle IRI, however, is less clear. In animal models leukocyte filters have been shown to prevent the increased permeability and vascular resistance seen in the control group (151). Clinical studies have failed to demonstrate any clinical benefit in patients with critical limb ischemia undergoing elective bypass.

The extravascular migration of neutrophils is dependant upon neutrophil-endothelial interactions and this has also been a therapeutic target. Monoclonal antibodies directed against CD11b (40), ICAM-1 (152) and PECAM-1 (153) have all been shown to attenuate muscle injury in animal models. There are no reports regarding the safety or efficacy of these agents in clinical trials.

(g) Ischaemic preconditioning (IPC)

It has been observed that subjecting tissue to short episodes of ischemia confers protection against more prolonged ischemia and this is known as 'ischaemic pre-conditioning' (IPC). Although the exact mechanism remains obscure IPC may be associated with an intra-cellular rise in adenosine levels. IPC in skeletal muscle is based on intermittent vascular clamp application before a period of prolonged ischemia. Animal models have shown that both IPC and the administration of adenosine (a natural occurring pre-cursor) ameliorates skeletal muscle reperfusion injury compared to controls (154). A significant increase in NO metabolites and reduction in the systemic TNF-α response was also seen. Although these results are encouraging it is difficult to see the absolute value of IPC during acute limb ischemia (rather than elective bypass), where the complications of IRI are most commonly encountered.

(h) Heparin

Heparin has been shown to reduce muscle injury in animal models, possibly by reducing intra-vascular sludging via it's anti-coagulant effect (155).

Conclusions

Over recent years our understanding of the complex pathophysiological processes governing IRI has much improved and, as a result, a number of potential therapeutic agents have been identified. Evaluation using animal models is suggestive that many of these ameliorate both local muscle and remote organ injury. Nevertheless the safety and efficacy of many of these agents has not yet been evaluated clinically. Clinical studies published to date are often small and of poor methological quality, and this may be a reflection of the relative rarity of acute limb ischemia. Others have assessed therapeutic benefit after elective revascularsation, where the risk of 'significant' IRI is clearly much smaller. Consequently none of the above strategies have gained widespread acceptance in surgical practice. The identification of therapeutic interventions, that are safe and effective, is likely to prove a formidable challenge and require adequately powered multi-centre randomised controlled clinical trials. Furthermore the complexity of the inflammatory cascade may mean that single 'magic-bullet' therapy is perhaps unrealistic and 'combination therapy' may need to be utilised.

Until these issues have been addressed then the prompt reperfusion of ischaemic skeletal muscle remains the cornerstone of clinical practice.

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