Ischemia/Reperfusion: A Potential Cause for Tissue Necrosis

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2.1 Circulatory Anatomy

A patient with a nonhealing wound requires a comprehensive work-up including a focus on six primary points of interest. These points include the status of tissue perfusion, role of bacterial contamination, pressure applied to the tissue, the immune status of the host, comorbid medical conditions including the patient's psychosocial status, and lastly, the status of the wound itself. Even after reestablishing macrovascular flow, many wounds either fail to improve or paradoxically worsen. Potential mechanisms for these unexpected findings include reperfusion injury, no-reflow, the presence of stunned/hibernating tissue, and occasionally tissue necrosis.

The circulatory system can be divided into two distinctive vascular beds. The macro-circulation refers to all vessels large enough to be seen by the unaided eye. Much clinical attention has been

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focused on the macro-vasculature because of the large number of innovative therapeutic procedures that have been developed to treat these vessels (balloon angioplasty, atherectomy, laser, etc.). The microcirculation refers to a "web" of tiny vessels located throughout the body. A superficial (subpapillary) plexus and deep horizontal plexus of arterioles and venules are present within the dermis. Most of the microcirculation is found 1–2 μm below the epidermal surface in the upper, papillary dermis. The deep plexus is formed from the perforating vessels of the underlying muscle and subcutaneous fat. The outer layer of nonviable keratin known as the stratum corneum has a depth between 10 and 20 μm, while the epidermis has a depth between 40 and 150 μm. The microcirculatory net therefore is located 100 μm from the upper level. The second layer of skin, the dermis, has a depth between 1,000 and 4,000 μm. It is the superficial plexus that gives rise to the "capillary loop" into the papillary system, which represents the source of nutrition for the skin and the surface area for the exchange of gases and molecules between the skin tissue and blood. Each papilla contains one to three terminal capillary loops innervated by sympathetic, parasympathetic, and sensory nerve endings. There are specialized arteriovenous shunts (glomus bodies), which allow blood to bypass the capillary bed. These shunts represent the thermoregulatory function of the skin. This system is dominant representing 85 % of the total blood flow, while the nutritive bed represents only 15 %. Therefore, initial ischemia can be mitigated by shunting more blood to the nutritive capillaries providing a natural protection. The nutritive capillaries are responsible for tissue viability by providing oxygen, nutrients, and fluid exchange. The capillary density determines the diffusion distance for gases and nutrients to dissolve through the tissues. The distinction between nutritive and nonnutritive flow is difficult to assess with indirect techniques. It is therefore critical to know the depth of penetration for any instrument when assessing the skin microcirculation. Instruments that sample at 500 μm or less are measuring the nutritive cutaneous flow, whereas studies beyond this mark are analyzing shunted (nonnutritive) flow.

2.2 Diagnostics

Methods for analyzing the macro-circulation include Doppler waveforms, duplex scans, contrast angiography, CT angiography, and MR angiography. There are numerous methods to assess the microcirculation; however, limitations include cost, operator-dependent variability, and non-familiarity among clinicians. Intravital capillaroscopy is a noninvasive technique used to identify nutrient capillaries. It consists of an optical microscope with epi-illumination, which is applied to the nail fold capillaries. The capillaries lie parallel to the skin surface in the nail fold. This anatomical distribution of the loop creates an ideal place to measure capillary blood flow velocity. Further advances to this technique have been introduced with orthogonal polarization spectral (OPS) imaging, which works without the fluorescent dye and gives more flexibility to the analyses. With this technology, it is possible to measure the capillary blood cell velocity (CBV), capillary density (capillaries/mm^2), and the diameter of the erythrocyte column. Laser Doppler perfusion imaging (LDPI) is a technique which utilizes a low-intensity laser (helium-neon) light. The device measures the backscattering created by moving red blood cells over a specific rectangular area analyzing up to 4,096 individual points. The wavelength of this monochromatic light is 670 nanometers (nm) with a maximum accessible power of 1 mW. Doppler shift results are accumulated and translated into numeric values expressed in volts and in an image-colored map. The penetration of the laser beam reaches 500 μm when applied to intact skin; however; penetration can reach 2.5 times greater in other non-skin tissues like granulation tissue. Laser Doppler flowmetry (LDF) has been used since the 1970s and is the basis for LDPI. LDF consist of a Ne-He low-intensity laser of 638 nm transmitted by a fiber optic to a terminal provided with a heater and thermistor to maintain a temperature constant. Measurements are done at a single point after a set temperature is achieved. Measuring only one site results in inconsistent results due

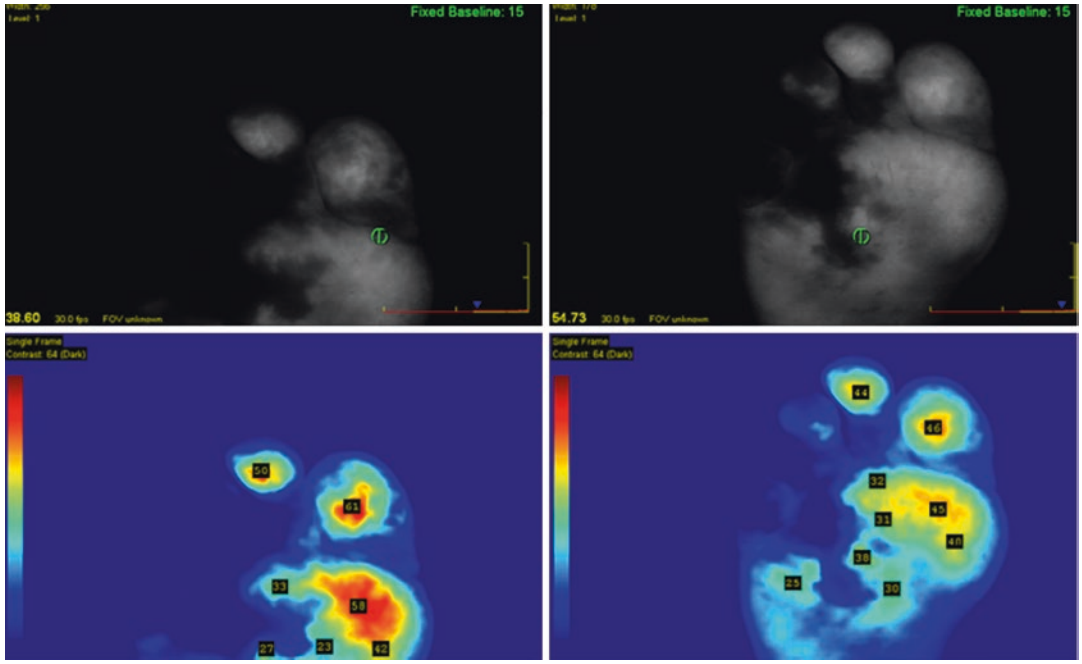

Fig. 2.1 First scans during surgery show reduced perfusion with manual graft compression at the medial portion covering three digits. Note the fourth digit and metatarsal head region which showed clinical signs of necrosis (Reprinted with permission from Perry et al. [1])

to the heterogeneity of microcirculation. TcPO_2, also called transcutaneous oximetry, is a technique widely used to detect the skin oxygen tension. A dime-sized Clarke-type solid-state polarographic electrode containing a platinum cathode with a reference electrode of silver chloride is housed in a probe tip along with a heater and thermistor. The reduction of oxygen at the cathode generates a current, which is then fed into the pO_2 channel of a monitor and converted into a voltage and digitized. The electrode is attached via a fixation device to the immediate periwound skin and heated to 43–45 °C, which induces hyperemia, and the dissolution of keratin lipids thereby increasing gas permeability. This procedure indirectly evaluates the microcirculation without an ability to differentiate nutritive from nonnutritive flow. More recently the use of indocyanine green dye has been employed to assess tissue perfusion at the microcirculatory level. This technique uses indocyanine green, a water soluble dye with a peak spectral absorption at 800–810 nm in blood. A laser light activates the dye and images are obtained from a charged coupled device camera. Unlike ultrasound images, these cutaneous angiographic images are intuitive to interpret, and due to rapid clearance, images can be repeated several times without accumulation of dye. This diagnostic technique might allow the bedside clinician to have a tool that allows for the assessment of tissue microcirculatory status and could be used in tandem with macrovascular studies for a complete picture of the perfusion of the skin. These images can be used to assess the efficacy of a revascularization procedure at the tissue level pre- and postoperatively [1] (Figs. 2.1 and 2.2).

2.3 Ischemia and Ischemia/Reperfusion Injury

Since the macro- and microvascular beds are connected in series, a reduction in macrovascular flow will lead to a decrease in microvascular flow unless compensatory mechanisms are stimulated. The

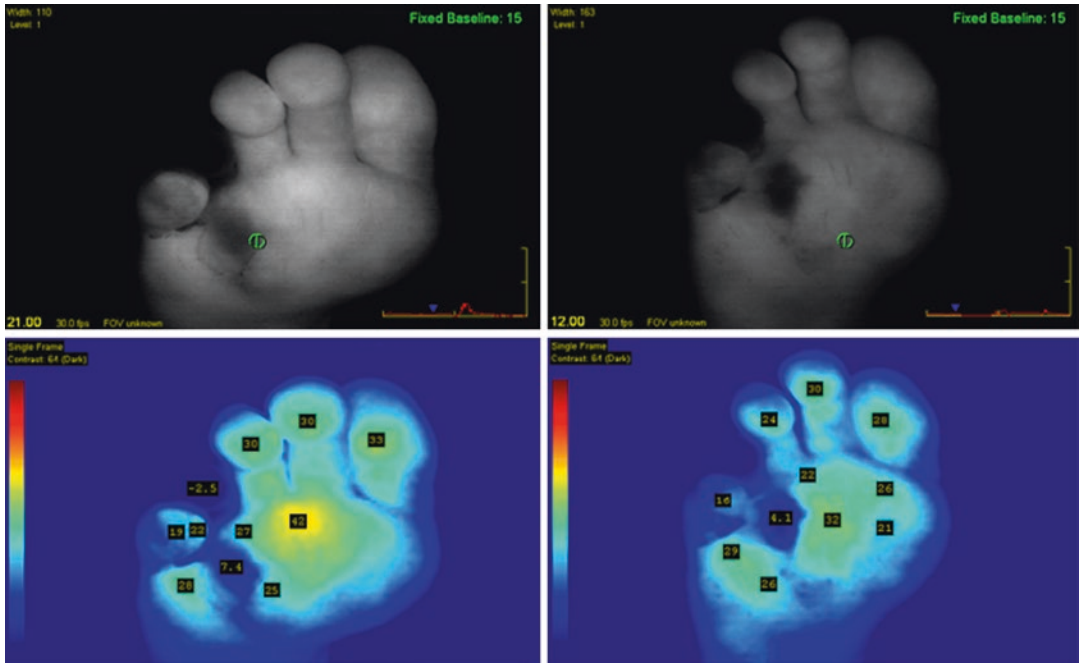

Fig. 2.2 Two follow-up SPY sequences following bypass procedure. Note that the fourth digit continues to demonstrate signs of necrosis and was eventually amputated (Reprinted with permission from Perry et al. [1])

term ischemia is used to denote reduced blood supply due to obstruction of the arterial inflow and was first used in the early nineteenth century. During prolonged ischemic periods, adenosine triphosphate (ATP) levels along with intracellular pH decrease as a result of anaerobic metabolism and lactate accumulation. Tissue injury and cell death are related to the duration and magnitude of the ischemia. The downstream biochemical effects include cellular swelling, increases in intracellular calcium levels, the generation of reactive oxygen species, and mitochondrial dysfunction [2] (Fig. 2.3). There are organ-specific differences that influence the extent, severity, and reversibility of organ damage after an ischemic event. Single-organ ischemia and reperfusion injury can occur in the heart, kidney, intestine, and brain. The brain, for example, is the most sensitive organ to reductions in blood supply due to its underlying high metabolic rate per unit weight. The brain also has an absolute requirement for glucose as an energy substrate and lower levels of protective antioxidants. As the largest organ in the body, the skin has not been studied as extensively as other organs in relation to ischemia and reperfusion injury. Ischemia reperfusion injury can have an effect on remote organs as well as the ischemic local organ. Multiple organs are injured in clinical conditions such as circulatory arrest, sickle cell anemia, sleep apnea, and during trauma and resuscitation. The mechanism of action for remote organ injury has been identified as the same factors implicated for local organ dysfunction such as reactive oxygen species formation, leukocyte activation, and inflammatory mediators. These circulating factors are responsible for the distant organ effects. The overall total organ injury sustained during prolonged ischemia followed by reperfusion is therefore attributable to an ischemic component, followed by a second component after reestablishing blood flow [2] (Fig. 2.4). Tissue-specific tolerances and confounding clinical conditions account for the variable responses noted in individual patients.

The skin and chronic wounds are dependent on the microcirculation for oxygen, nutrients, and the elimination of metabolic wastes. Decreased quantities of oxygen lead to decreased bacterial killing

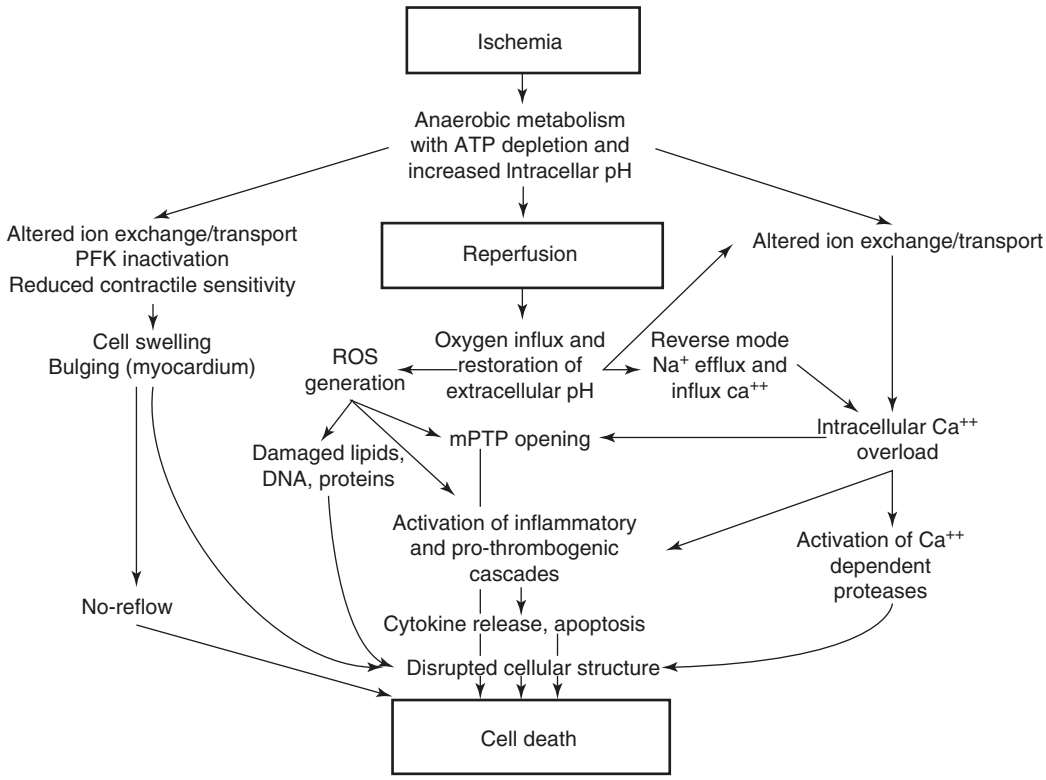

Fig. 2.3 Major pathologic events contributing to ischemic (*upper panel*) and reperfusion (*middle panel*) components of tissue injury, with overall integrated responses to I/R injury summarized in the *bottom panel* (Reprinted with permission from Kalogeris et al. [2])

by leukocytes, decreased collagen production, and decreased epithelialization. A patient may have compromised macroflow but, due to compensatory mechanisms such as the development of collateral flow, may be able to heal a wound. In a study of 111 patients with non-reconstructable vascular disease, the microcirculatory assessment was predictive of ultimate limb salvage [3]. The clinician can treat the microcirculation through the use of various energy-based modalities (i.e., ultrasound, electrical stimulation) that can increase angiogenesis and local blood flow to the wound bed [4]. A concept known as the push-pull theory has been presented by the authors as a working theoretical construct [5]. The push is achieved by the macrovascular-based arterial reconstruction. Other forms of "push" include increasing cardiac output, volume resuscitation, and the use of medications in the treatment of shock. Regardless of any potential negative side effects from revascularization, the first treatment option is to rapidly restore flow. The "pull" is essentially created by decreasing peripheral resistance and increasing the quantity of available capillaries, a process known as capillary recruitment. After the initial increase in microcirculatory flow, mediated by nitric oxide release from the endothelium within the microcirculation, a second phase of increased microcirculatory flow is achieved through the process of angiogenesis. Local microcirculatory perfusion can also be influenced by both vasoconstriction and adequate volume status. Noxious stimuli such as hypothermia, stress, pain, and depression can all lead to increased sympathetic tone and subsequent decreased tissue perfusion. Smoking, through the action of nicotine, can also result in decreased microcirculatory flow.

After a successful vascular intervention, the wound team needs to monitor the healing

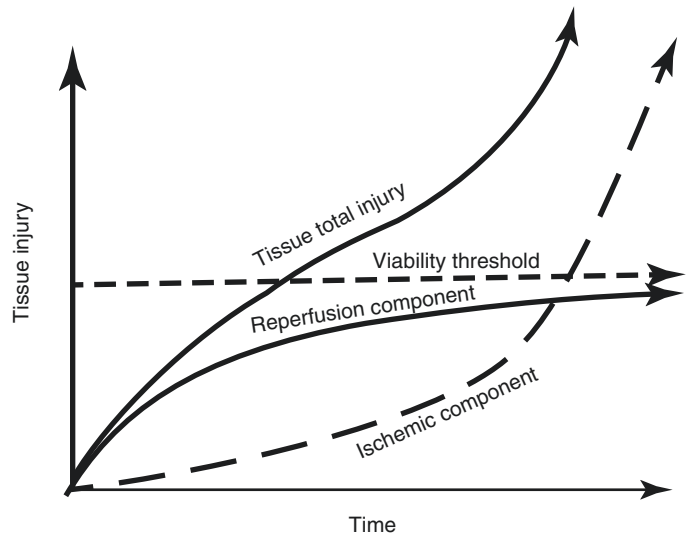

Fig. 2.4 Total injury sustained by a tissue subjected to prolonged ischemia followed by reperfusion (I/R) is attributable to an ischemic component and a component that is due to reestablishing the bold supply. At the onset of prolonged ischemia, two separate pathologic processes are initiated. The first are processes of tissue injury that are due to ischemia per se. The second are biochemical changes during ischemia that contribute to the surge in generation of reactive oxygen species and infiltration of proinflammatory neutrophils when molecular oxygen is reintroduced to the tissues during reperfusion particularly the initial phases. For a treatment to be effective when administered at the onset of reperfusion, reestablishing the blood supply must occur before damage attributable to ischemia per se represents a major component of total tissue injury. Therapeutic approaches that target pathologic events contributing to both the ischemic and reperfusion components of total tissue injury, such as ischemic or pharmacologic preconditioning, should be more effective than therapies administered when the blood supply is reestablished, which limit only the progression of reperfusion injury (Reprinted with permission from Kalogeris et al. [2])

trajectory and measure the microcirculatory status. The improvement in macro-level perfusion can be the result of bypass surgery, an interventional vascular procedure, medical management of fluid status or blood pressure, or improvement in cardiac function. The presence of chronic ischemia results in an adaptive peripheral arteriolar vasodilation in various organ systems and the lower extremity. In fact it has been demonstrated both in human and animal modeling that small episodes of ischemia can actually improve tissue tolerance to reperfusion, a concept known as ischemic preconditioning. The microcirculation can become both structurally and physiologically altered during the ischemic state. Reestablishing macroflow results in a large volume of blood entering a dysfunctional microcirculation with resulting problems such as "revascularization edema," which can further compromise tissue perfusion. Caselli demonstrated, for example, that transcutaneous oxygen levels increase over a 4-week period after successful revascularization but do not demonstrate consistent elevations except for a brief initial rise, when revascularization is unsuccessful [6].

The soft tissue and skin can suffer similar effects of reperfusion injury noted in other organ systems [7] (Fig. 2.5). How the skin responds to reperfusion will require further study. We need to be able to predict outcomes and therefore require surrogate markers that can be studied preoperatively to maximize outcomes. The production of oxygen free radicals and intracellular calcium overloading are two mechanisms of action reported for ischemic-reperfusion (IR) injury. Single episodes of IR can result in myocardial stunning. Stunning refers to the reversible mismatch of perfusion-contraction that occurs despite adequate macro-flow. Extended periods of ischemia can lead to an adaptive condition known as tissue hibernation. It is now appreciated that over time, cells exposed to chronic

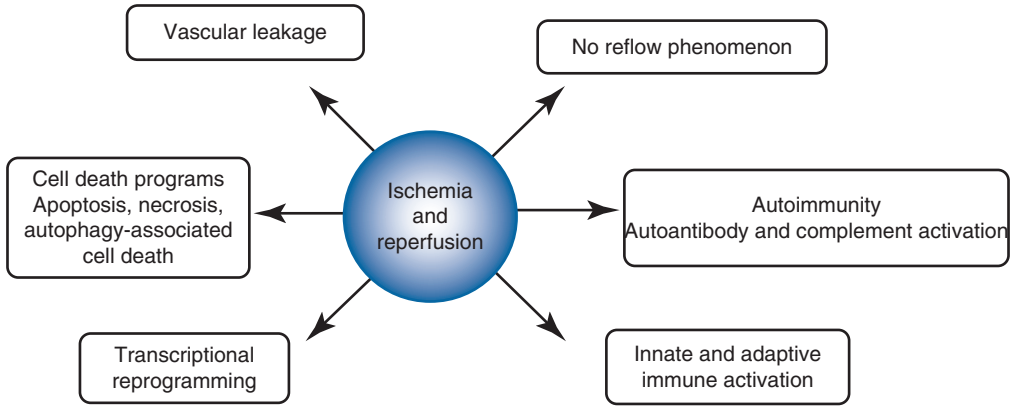

Fig. 2.5 Biological processes implicated in ischemia/reperfusion injury (Reprinted with permission from Eltzschig and Eckle [7])

ischemia will adapt by increasing the production of molecules known to produce an antiapoptotic effect. Another post-revascularization process reported in the cardiac literature is the concept of "no-reflow." The endothelium becomes dysfunctional during the revascularization period leading to subsequent cell swelling, leukocyte-induced inflammatory responses, in situ thrombosis, decreased nitric oxide production, vasoconstriction, and possibly distal embolization [8]. The reversibility of no-reflow is dependent on patient risk factors and the type of device used in the revascularization procedure. In lower extremity revascularization procedures with nonhealing wounds and, in particular, with open guillotine amputations, our team has observed progressive tissue necrosis and a heterogeneous pattern of granulation tissue formation that might be a result of the no-reflow process despite adequate revascularization. Even if tissue necrosis does not occur as a result of ischemia-reperfusion injury, we are uncertain that the quality of healing will be affected by the resulting biochemical changes. For example, changes might occur in tensile strength or the amount of scar formation, both of which ultimately may affect wound recidivism rates. The mechanism of cell death after IRI has many potential pathways [2] (Fig. 2.6). Extrinsic factors, such as depleted cellular energy stores, and the release of inflammatory mediators were thought to be responsible for the majority of tissue necrosis in IRI; however, it is now known that several additional mechanisms add to the overall process. Apoptosis (programmed cell death) is a regulated pathway that leads to cell shrinkage and condensation of the nucleus and cytosol. Autophagy is the cellular process of removing obsolete or dysfunctional cells. This process can generate much needed amino acids and fatty acids which can actually provide nutrition for the cell during times of sublethal stress situations. Uncontrolled autophagy, however, will ultimately lead to tissue necrosis. Programmed necrosis, also known as necroptosis, is thought to occur during IRI. This specific biochemical pathway is thought to occur in addition to the more random process of generalized tissue necrosis.

2.4 Clinical Case Example

Two patients with critical limb ischemia, diabetes, and hypertension were recently treated in our unit with very different outcomes. Patient number 1 was an African-American male, 68 years of age with distal foot necrosis and infra-popliteal arterial occlusions leading to a reconstituted posterior tibial vessel. A reverse saphenous graft bypass graft was performed from the popliteal artery to the posterior tibial artery, and an open guillotine transmetatarsal amputation was performed several days later. The patient was transferred to our subacute wound unit for postoperative management. Treatment included non-contact kilohertz

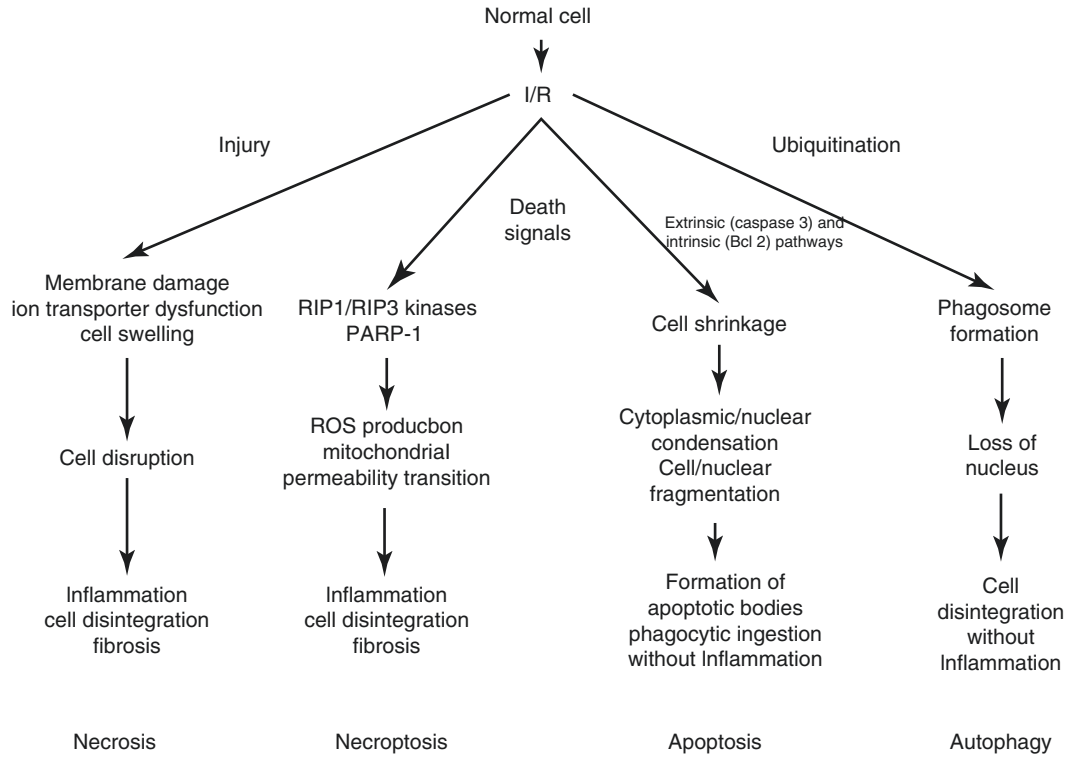

Fig. 2.6 Mechanisms of cell death in ischemia/reperfusion (I/R). I/R-induced necrosis generally occurs as a result of dysfunctional ion transport mechanisms, which causes cells to swell and eventually burst, effects that are exacerbated by plasma membrane damage. Release of proinflammatory mediators and damaged biomolecules initiates the influx of inflammatory cells such as neutrophils, which disrupt the extracellular matrix and cause damage to parenchymal cells by release of cytotoxic oxidants and hydrolytic enzymes. Apoptosis is a regulated form of cell death that causes cell shrinkage and condensation of the cytosol and nucleus (Reprinted with permission from Kalogeris et al. [2])

ultrasound therapy to enhance angiogenesis and capillary recruitment. Ultraviolet light was used to control wound bio-burden and dressings maintained a moist environment. Over two weeks, a homogeneous granular wound bed began to form, and negative-pressure wound therapy was implemented at 75 mmHg intermittent for an additional two weeks. Nasal oxygen was used to enhance tissue oxygenation, and edema was minimized through gentle compression and leg elevation. At the end of 4 weeks, an autologous split-thickness skin graft was used which resulted in 100 % take. An offloading orthotic shoe was created and the patient was discharged. Patient number 2 had identical vascular anatomy and tissue loss. The same bypass and open amputation was performed, and the patient was also discharged to the subacute unit. Within 2 days of arrival, the peripheral margins of the tissue began to show signs of necrosis. Only two to three small buds of granulation tissue developed, while implemented the same energy-based treatments as those used in patient # 1. Progressive peripheral necrosis expanded, and the fatty tissue became dusky and malodorous with a heavy exudate despite elevation and IV antibiotics. Due to continued soft tissue necrosis, the use of negative-pressure therapy was not started. The patient ultimately was readmitted and underwent a below-the-knee amputation. While there are always patient-specific difference that could account for such variation in clinical outcomes, the macrovascular pattern of disease and initial soft tissue loss were identical. We currently have no diagnostic methods to

predict these outcomes until they present themselves clinically. We are therefore always in a reactive mode and not a proactive or preventive mind set. There are a number of recently described potential therapeutic approaches that might improve our limb salvage rates in the future.

2.5 Treatment Options

Investigators are evaluating methods to enhance tissue tolerance to ischemia through preconditioning. Exposure to short, nonlethal episodes in ischemia can result in an attenuated tissue injury response following revascularization [7]. Through the use of animal modeling, investigators are trying to determine the biochemical steps responsible for the positive impact of preconditioning so that biological targeted therapy might be developed. Patients with long-standing lower limb ischemia and robust collateral formation have potentially already benefitted from these phenomena. Other investigators are approaching the problem via post-conditioning, remote conditioning, controlled reperfusion, and pharmacological manipulation of the perfusate [7]. Pharmacological therapy prior to revascularization has been evaluated with HMG-coA reductase inhibitors, immune suppressive therapy, and stem cells [9].

There is an opportunity for improving limb salvage rates, quality of life, and cost through an improved understanding of ischemia and reperfusion in the wound care community. Ischemia and reperfusion injury is considered important in venous ulcer pathology, sickle cell and vasculitic ulcers, and pressure ulcers. Variations in muscle and skin flow, for example, are critical when deciding on sitting protocols for paraplegic patients to prevent pressure ulcer formation [10]. Improved diagnostic and therapeutic options for this condition will allow us to strategize our treatment plan. Some patients may benefit for energy-based modalities and/or stem therapy in order to increase a low-resistance outflow bed prior to a revascularization. Others may benefit from controlled reperfusion and pharmacological interventions. Limb salvage rates continue to improve in many centers throughout the world, but with an aging population and an increased prevalence of diabetes, more and more patients will have concomitant soft tissue loss in combination with ischemia. Personalizing their therapy will be critical to achieve functional, successful, and cost-effective outcomes.

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