

Reperfusion Injury After Hemorrhage

A Collective Review

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Objective: To review current knowledge of hemorrhagic shock and reperfusion injury.

Summary Background Data: Patients with hemorrhagic shock require optimal resuscitation and cessation of ongoing bleeding. Often our resuscitative measures, while necessary, cause a wide range of detrimental physiologic effects. Research continues to answer questions regarding measurable endpoints and optimal fluids used in resuscitation. Elucidation and understanding of the complex metabolic pathways involved in reperfusion injury are areas of intense current investigative effort.

Methods: A literature review was performed using MEDLINE and key words related to experimental and clinical studies concerning shock and reperfusion.

Results: Experimental studies have shown that resuscitation with colloid and crystalloid show no difference in outcomes in critically ill patients. Laboratory studies are showing promising results with immunomodulation of response to injury. However, no clinical trials have shown significance yet.

Conclusions: It is unlikely that a single treatment modality or "magic bullet" will be able to substantially block such a complex regulated process unless performed before feedback mechanisms known to be in place. Ongoing translational research will inevitably have a major impact on patient care.

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Shock is one of the primary causes of morbidity and mortality in the intensive care units of today's hospitals. The body's inflammatory response is profound and, if unchecked, can lead to multiple end organ failure and death. Although sepsis is the likely source prompting an inflammatory response in critically ill patients who become hypotensive and tachycardic, hypovolemic shock is also associated with severe systemic inflammatory processes based on components of innate immunity. Although the most common etiology of hypovolemic shock is hemorrhagic, there are other pathophysiologic processes that can result in this type

of shock state. The concept of shock has evolved since first being described in ancient traumatic wounds and hemorrhage. Initially, the correlation between blood loss and death was not made; treatment for shock during the time of Hippocrates (460–380 BC) was bloodletting. George James Guthrie (1785–1856) was the first to use the term "shock" to delineate physiologic instability.¹ Keith used dye dilution measurements to show that hypovolemia was a mechanism for shock in 1919.² However, in 1930, Alfred Blalock classic animal experiments proved, indisputably, that blood loss was the primary cause of traumatic shock.^{3,4}

The definition of shock has progressed considerably but in its most simple terms is defined as a physiologic state where delivery of oxygen does not meet metabolic demands. Traumatic blood loss is often easy to recognize; however, volume and location of bleeding can be extremely difficult to discern. To assist the surgeon with treatment of hemorrhage, classes of shock have been devised to delineate severity (Table 1). The first sign of hypovolemic shock is a decrease in pulse pressure. This phenomenon is due to an increase in diastolic blood pressure, secondary to a catecholamine surge. Compensatory responses to hypovolemia such as mild tachycardia, anxiousness, tachypnea, and decrease in urine output occur in class II shock. Sustained hypotension is seen with both class III and class IV shock.

TIMING OF TREATMENT FOR HEMORRHAGIC SHOCK

In addition to ensuring a stable airway and adequate ventilation, the primary treatment goal in hemorrhagic shock is to address the bleeding. Frequently, this requires surgical intervention. Replacement of blood loss is necessary to continue oxygen delivery, but timing and extent of resuscitation are still investigational issues. Different resuscitation strategies are used to maintain blood pressure in trauma patients until bleeding is controlled. However, while maintaining blood pressure may prevent shock, it often worsens bleeding. Several studies have investigated early versus delayed resuscitation in the hypovolemic patient.^{5–13} One of the largest randomized controlled trials by Turner et al showed no difference in mortality with early (10.4%) or delayed (9.8%) administration of fluids in 1309 trauma patients.⁸ Another randomized controlled trial by Bickell, Mattox, and colleagues reported no differences in mortality and coagulation times on a total of 598 hypotensive trauma patients with

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TABLE 1. Clinical Signs and Symptoms of Hemorrhagic Shock Based on Severity of Blood Loss

Class	% Loss of Blood Volume	Pulse Rate	Systolic Pressure	Pulse Pressure	Capillary Refill	Respirations	Central Nervous System	Urine Output (mL/h)
I	<15	Normal	Normal	Normal	Normal	Normal	Normal	Normal
II	15–30	>100	Normal	Decreased	Delayed	Mild tachypnea	Anxious	20–30
III	30–40	>120	Decreased	Decreased	Delayed	Marked tachypnea	Confused	20
IV	>40	>140	Marked decrease	Marked decrease	Absent	Marked tachypnea	Lethargic	Negligible

penetrating torso injuries.⁷ Many studies have tried to answer this question of early versus late use of fluids for resuscitation. Currently, the best data available demonstrate that there is no difference in mortality outcomes if surgical intervention is immediately available. However, the Advanced Trauma Life Support protocol of the American College of Surgeons recommends the liberal use of isotonic crystalloid to correct hypotension in bleeding trauma patients.¹⁴

EXTENT OF RESUSCITATION

Extent of resuscitation is the target of several randomized control trials. Supraphysiologic, normotensive, and hypotensive resuscitation have all been evaluated.^{15–20} In one trial by Dutton et al, with a total of 110 hypotensive patients with blunt and penetrating injuries, mortality was equal in groups administered different volumes of fluid (1000 mL less than in the intervention group).²¹ In a study by Dunham et al, a significant difference in mortality was seen in patients administered smaller volumes of fluid via rapid infusion.²² Although vigorous fluid resuscitation may be lifesaving in some patients, results from clinical trials are inconclusive. Studies with adequate power analysis and appropriate controls are still needed to optimally discern the correct treatment protocols for patients in hemorrhagic shock. Because cellular oxygen utilization is the ultimate goal of resuscitation, several strategies have been proposed to measure cellular oxygen metabolism. Techniques such as gastric tonometry, tissue oxygen electrodes, and microdialysis have had limited use.²³ Complications due to the invasive nature of these techniques, limited monitoring intervals, and inaccurate values are reasons for their restricted clinical use. Near infrared spectroscopy is a portable bedside technique, similar to Doppler, which can be used to measure tissue oxygenation. Results from clinical trials have demonstrated that near infrared spectroscopy is promising.^{24–27} As technology advances, measurement for end points of resuscitation will continue to be a research topic of great interest to surgeons.

COLLOIDS VERSUS CRYSTALLOIDS

The type of fluids used for resuscitative efforts is still a topic of much debate. Although colloids are effective in expanding the circulation, there is no evidence that this improves outcomes in critically ill patients.²⁸ The U.S. Hospital Consortium Guidelines recommend that colloids be used in hemorrhagic shock before the availability of blood products, and in nonhemorrhagic shock after an initial crystalloid infusion. A 1995 survey of U.S. academic health centers,

however, found that the use of colloids far exceeded even the Hospital Consortium recommendations.²⁹ Volume replacement with colloids is considerably more expensive than with crystalloids. Clinical studies demonstrate that colloids and crystalloids have different effects on a range of important physiological parameters. Because of these differences, all-cause mortality is arguably the most clinically relevant outcome measure in randomized trials comparing the 2 fluid types. Previous meta-analyses of mortality in randomized trials comparing colloids and crystalloids do not satisfy criteria laid out for scientific overviews, and they predate most of the trials conducted using synthetic colloids or hypertonic crystalloid solutions.^{30,31} A recent prospective study, the Saline versus Albumin Fluid Evaluation trial, attempted to determine whether the use of albumin for fluid resuscitation had a detrimental effect on mortality in critically ill patients.³² There was no significant difference between albumin or saline in the number of days spent in the intensive care unit, days spent in the hospital, days of mechanical ventilation, days of renal replacement therapy, or all-cause 30-day mortality within this study. The most recent meta-analysis is a Cochrane review analyzing the use of colloids and crystalloids in resuscitation.³³ This review is a consortium of critically ill patients, however, not all subjects are purely victims of hemorrhagic shock. Nevertheless, these are the best data to date. This meta-analysis conveys several important implications. First, there is still no evidence that colloids are superior to crystalloids for intravascular volume resuscitation in critically ill patients. Importantly, the Saline versus Albumin Fluid Evaluation trial provides no evidence that the use of albumin is clinically advantageous. Second, albumin carries, at least, a theoretical risk of transmission of infections. Third, the economic cost of colloids for resuscitation is considerable and, therefore, its ongoing use in this context is unjustified.

In groundbreaking work by Rhee and colleagues, hypertonic saline has been advocated as a viable option for resuscitative fluid for hemorrhagic shock.³⁴ In this study, looking at differing resuscitative fluids and neutrophil function, oxidative burst was suppressed when exposed to hypertonic saline. Changing the isomer of lactate in Lactated Ringer's solution from racemic (mixture) to a specific (L) isomer has a similar effect on neutrophil activity. Using only (L) isomer lactated Ringer solution in resuscitation of rodents, leukocyte genes known to be involved in inflammation, cell migration, and apoptosis were down-regulated. Immunomodulation of type-specific fluids is now a burgeoning topic in trauma resuscitation research.

EXPERIMENTAL OXYGEN TRANSPORTERS

Initially, hemoglobin-based oxygen carriers (HBOC) were a project of the United States Army after the Vietnam War. The impetus was for a blood substitute that could be given in forward areas until soldiers were evacuated to definitive medical care. Tetrameric hemoglobin has been the front runner but is plagued with adverse organ toxicities, such as renal, hepatic, cardiovascular, and pancreatic.^{35,36} Preparations that are more contemporary have been designed by cross-linking the tetramer with either dapsirin or raffinose. Unfortunately, a recent trial involving cross-linked hemoglobin had to be closed due to increased mortality in the investigational arm.³⁷ Another product undergoing phase III trials is a polymerized human hemoglobin product known as PolyHeme (Northfield Laboratory).^{38,39} Interim analysis has shown no toxicity or increase in mortality, however, Northfield is currently preparing for review by the Food and Drug Administration. Animal studies have demonstrated that the HBOC PolyHeme improves recovery from postshock oxygen debt and reduces acute lung injury.⁴⁰ Further animal studies using swine have established that bovine polymerized hemoglobin, HBOC-201, is superior to Dextran with regard to resuscitation and survival.⁴¹ Oxygen carrying fluids continue to evolve and develop for the treatment of shock.

Resuscitation with trans-sodium crocetinate (TSC), a vitamin A-analog, has been used in animal models for improved oxygen delivery after hemorrhage.^{42,43} Increase in oxygen delivery is due to TSC-mediated diffusivity of oxygen through plasma. TSC has also shown increases in whole body oxygen consumption. No clinical trials have been performed to date; however, infusion with TSC in animal models has shown promising results.^{42,43} Although oxygen-carrying fluids will continue to be studied, it must be noted that the return of oxygen to ischemic tissue heralds the beginning of reperfusion injury.

ENDOTHELIUM, ITS ROLE IN REPERFUSION INJURY

Reperfusion injury starts with the simple reoxygenation of tissues after ischemic insult causes hypoxia. Regardless of the cause of hypoxia, endothelium seems to be the primary starting ground for reperfusion injury. Endothelial cells are known to be sensitive to hypoxia, manifesting damage via an increase in cellular volumes, loss of cytoskeletal organization, and decreased membrane fluidity. Energy stores are depleted and certain bioactive agents including prostacyclin and nitric oxide (NO) are used to maintain anticoagulation.⁴⁴ Early in reperfusion, endothelial cells exhibit morphologic damage including cellular swelling, loss of adherence to the basement membrane, and adherence of activated leukocytes.⁴⁵

The location of endothelial damage is found throughout the vascular system specifically at the level of arterioles, capillaries, and venules. The postcapillary venule is the most common site of inflammatory response due to reperfusion.⁴⁶ Injury is heralded by several venule responses after ischemia, which includes the following: leukocyte-endothelial cell adhesion, transendothelial migration, platelet-leukocyte aggregation, and enhanced oxidant production. Intravital micros-

copy has been a key tool in verification of endothelial damage occurring in venules during reperfusion after ischemia. Several studies have shown endothelial leakage using fluorescein isothiocyanate conjugated albumin as a marker.⁴⁷⁻⁴⁹ This "leaky" vasculature has been linked to adhesion of leukocytes as well. It seems that the volume of adherent leukocytes is directly correlated with the amount of extravasated albumin.⁵⁰

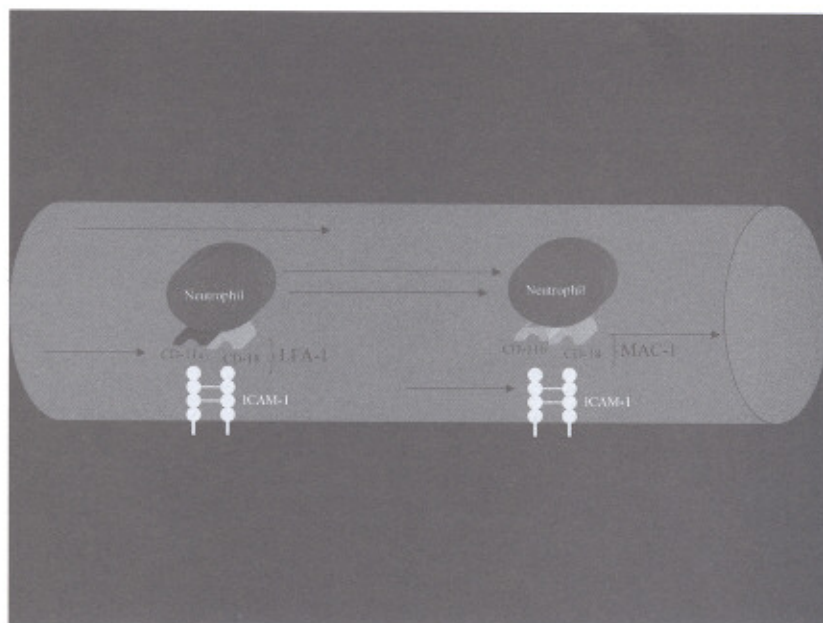
LEUKOCYTES AND THEIR ADHESION MOLECULES

Leukocytes use binding proteins to attach themselves to endothelium and facilitate extravasation. A review of selectins, integrins, and other adhesion molecules is beyond the scope of this review, however, several are relevant to reperfusion injury (Fig. 1). All 3 selectins (endothelial-E, platelet-P, and leukocyte-L) are involved in various aspects of reperfusion injury. Intercellular adhesion molecule-1 (ICAM-1) is expressed by endothelial cells after damage and increased by tumor necrosis factor (TNF)- α and interleukin (IL)-1. This protein binds to a beta integrin dimer that is present on neutrophils. This dimer is made of the 2 monomers CD18 and CD11. CD11 has 3 subtypes including CD11a, CD11b, and CD11c. One of these 3 monomers is expressed together with CD18 to create a binding complex with ICAM-1. This CD11/CD18 complex is integral to 5 activities that promote neutrophil damage during reperfusion. Initially, CD11/CD18 allows neutrophil adhesion to endothelium by the binding of ICAM-1 as described above⁵⁰⁻⁵³ and is required for transendothelial migration.⁵² This dimer is also necessary for H₂O₂ production by neutrophils that are adherent.⁵⁴ CD18 itself is the mediator of neutrophil-neutrophil adherence and clumping.⁵⁵ Finally, CD11/CD18 is the receptor complex for the complement fragment C3bi, which mediates neutrophil and macrophage phagocytosis of C3bi bound particles.⁵⁶ CD18 blockade by monoclonal antibodies has proven effective at decreasing neutrophil extravasation in animal models. CD18 is the one constant monomer that comprises the dimer CD11/CD18 complex and, as such, is the theoretical optimal target for therapy. However, blockade of this target has shown no mortality benefit in several clinical trials.^{57,58}

MITOCHONDRIAL OXIDATIVE METABOLISM AND REPERFUSION INJURY

Associated with high-energy phosphate production and oxygen consumption, mitochondria have been defined as the "workhorse" of the cell. Mitochondria consume greater than 90% of cellular oxygen.⁵⁹ In patients undergoing resuscitation, it has been noted that optimal delivery of oxygen does not result in increased utilization of that oxygen. Inability of the mitochondria to use oxygen, due to electron chain transport uncoupling, is a focus of ongoing investigation. This phenomenon of uncoupling has been termed "cytopathic hypoxia."⁶⁰ Reperfusion injury diminishes the ability of mitochondria, utilizing the electron transport chain, to formulate adenosine triphosphate via oxidative phosphorylation. Ninety percent of oxygen consumption, along the electron transport chain, occurs at cytochrome *a*, *a*₃ (also known as cytochrome *c* oxidase). Cytochrome *c* oxidase contributes to reperfusion

FIGURE 1. Leukocyte and endothelial cell adhesion molecules. The leukocyte adhesion molecule is a dimer composed of 2 entities, CD-11a/CD-11b and CD-18. CD-18 is the constant member of this association. LFA-1 or MAC-1 bind to the endothelial receptor ICAM-1 causing activation and extravasation of the leukocyte. ICAM-1 indicates intercellular adhesion molecule-1; LFA-1, lymphocyte function antigen-1; Mac-1 macrophage antigen-1.



injury through production of reactive oxygen species in the mitochondria.^{25,61} Changes in the cellular redox state may modulate the cell's vulnerability to the inflammatory response in shock states. Nuclear factor kappa-B (NF- κ B) is known to be a redox sensitive transcription factor with regards to the production of TNF- α . Mitochondrial respiration is important in activation of NF- κ B and subsequent proinflammatory mediator production.^{62,63}

ENZYMATIC MEDIATORS OF REPERFUSION INJURY (XANTHINE OXIDASE AND NITRIC OXIDE SYNTHASE)

Endothelium is also damaged in reperfusion injury secondary to oxidative radicals. Not only leukocytes, but also endothelial cells create oxygen radicals. Endothelial cells have an abundance of xanthine oxidoreductase, a metalloflavoprotein, which exists in 2 functional states.^{64,65} Constitutively expressed as a dehydrogenase, it normally reduces nicotinamide adenine dinucleotide. Post-translational modification converts this enzyme to xanthine oxidase, which starts to reduce O_2 instead of nicotinamide adenine dinucleotide during times of hypoxia. When oxygen is reintroduced into the hypoxic tissues during reperfusion, an accumulation of hypoxanthine allows for a burst of superoxide and hydrogen peroxide production. Several recent studies have looked at red wine constituents,⁶⁶ chimeric superoxide dismutases,⁶⁷ and more traditional antioxidants including allopurinol and deferoxime⁶⁸ for treatment of reperfusion with promising results.

NO and its effects on the endothelium have been prolifically investigated since the first classic studies by Pohl and Busse.⁷⁰ NO is the metabolic by-product of the conversion of L-arginine to L-citrulline by a class of enzymes dubbed nitric oxide synthases (NOS). To date, 3 isoforms of NOS

have been identified. Neuronal NOS (nNOS or NOS1) is expressed constitutively by neurons in the brain and enteric nervous system, whereas endothelial NOS (eNOS or NOS3) exhibits constitutive expression that is confined to the endothelial cells lining the vasculature.^{69,70} The third isoform of NOS is inducible NOS (iNOS or NOS2) and, as the name implies, is expressed only in response to certain inflammatory stimuli. Such inflammatory stimuli include bacterial products, cytokines, and lipid mediators.^{71,72} Vasodilatory effects of endotoxin were shown to have an NO-derived component in studies in our laboratory.^{73,74} NO induces vascular relaxation at mild hypoxic levels. However, when severe hypoxia ($pO_2 < 65$ mm Hg) is induced, NO-dependent vascular response is lost.⁷⁵ During severe ischemia ($pO_2 < 35$ mm Hg), relaxation of smooth muscle is associated with a potassium-adenosine triphosphate pump hyperpolarization.⁷⁶ Several studies have evaluated the effects of NO on adhesion molecules. In cultured endothelial cells NO down-regulates the production of endothelial adhesion molecules (ECAMs).⁷⁷⁻⁷⁹ Similar results were also produced in animal models; increased ECAM expression was observed in NOS-deficient mice.⁸⁰ Formation of NO adducts with hemoglobin iron, protein thiols, and protein nitrites/nitrates have demonstrated early formation in red blood cells after hemorrhage. Results from these studies indicate this phenomenon is independent of iNOS up-regulation.⁸¹ Bioreactive NO species could be transported by red blood cells systemically after hemorrhage or trauma. There has been extensive research into blockade of NO production with conflicting results. Inhibition of NO production, using L-N⁶-iminoethyl-lysine, demonstrates that iNOS is not responsible for up-regulation of the ECAMs.⁸² This method reveals that iNOS is not responsible for extravasation of neutrophils in an animal model.⁸³ Although much has been

delineated, a clear role for NO and each of the NOS enzymes in reperfusion is not fully understood. Evaluating the most current data, it seems that constitutively expressed NOS is involved with adhesion of neutrophils and endothelium, whereas inducible forms of NOS are involved in other activities, such as activation of NF- κ B.⁸⁴

NF- κ B AND REPERFUSION INJURY

NF- κ B, a DNA binding protein, belongs to a family of transcription factors that allow for the production of many proinflammatory molecules. NF- κ B exerts its action by binding to a specific decameric DNA sequence motif in the promoter regions of several genes. After hemorrhagic shock NF- κ B is involved in apoptosis and the inflammatory cascade. NF- κ B is a heterodimer consisting of 2 proteins, either p50 or p52 and p65 (corresponding to their molecular weights). The subunit p50 is also known as nfkb1, p52 is nfkb2, and p65 is Rel-A. They are sequestered in the cytoplasm of nonstimulated cells, through noncovalent interactions, with inhibitor proteins commonly referred to as I κ B. Degradation of I κ B via phosphorylation, ubiquitination, and proteolysis results in the release of NF- κ B dimers and unmasking of a nuclear localization sequence. This results in the nuclear translocation of NF- κ B and the transcription of specific target genes (Fig. 2). Phosphorylation occurs via several kinases including the alpha and beta inhibitors of kappa light polypeptide gene enhancer in B-cells (IKK α and IKK β).^{85,86} A decrease in NF- κ B activity is demonstrated by the inhibition of these kinases.^{87,88} Biochemical and genetic data suggest that the IKK complex contains 2 catalytic subunits (IKK α and IKK β) and a regulatory subunit (NEMO/IKK γ). IKK is the master regulator of NF- κ B-mediated inflammation. NF- κ B can be activated by 2 pathways. Interestingly,

only the IKK β subunit is essential for NF- κ B activation in response to proinflammatory stimuli.⁸⁵ This signal-induced phosphorylation targets IKK β for polyubiquitination and subsequent degradation by the proteasome releasing NF- κ B that consists of 2 subunits from a family of proteins, such as p65 (also called Rel-A) and p50. However, the NF- κ B pathway is complex and can be influenced at many steps. Activation of NF- κ B can occur in what appears to be multiple signaling pathways. Serial activation of phosphatidylinositol 3 kinase and protein kinase B (AKT) lead to activation of NF- κ B.⁸⁹ Another pathway of NF- κ B activation, called the alternative pathway, has been elucidated using tumor necrosis factor-associated factors (TRAFs) and NF- κ B kinase (NIK).⁹⁰ This occurs principally in B cells in response to stimulation of receptors for lymphotoxin β and CD40 ligand among others. Stimulation of these receptors activates the protein kinase, NIK, which activates IKK α . NIK is a member of the MEKK (mitogen-activated protein kinase/extracellular signal-regulated kinase family). IKK α then phosphorylates another NF- κ B family member, p100. Phosphorylation of p100 converts p100 to p52. The p52 subunit and its binding partner RelB, like p65-p50, translocate into the nucleus to regulate gene expression. The 2 NF- κ B pathways, the classic and alternative, can be activated by overlapping sets of stimuli.

Blockade of NF- κ B was undertaken by our laboratory in previous studies related to septic shock.^{91,92} These studies revealed that blockade of NF- κ B abrogated the vasodilation of arterioles exposed to endotoxin. NF- κ B is a popular target for blockade and multiple studies have been undertaken to prove its role in reperfusion injury. Several clinical trials have evaluated the blockade of NF- κ B end-products, such as TNF- α and IL-6, with limited results.^{93,94} Although these may be promising targets, it is our belief that due to the

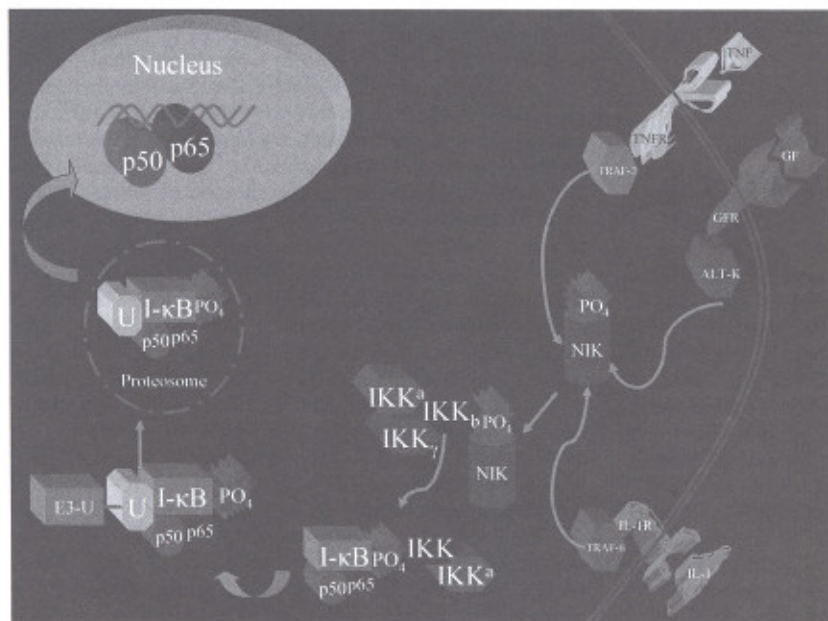
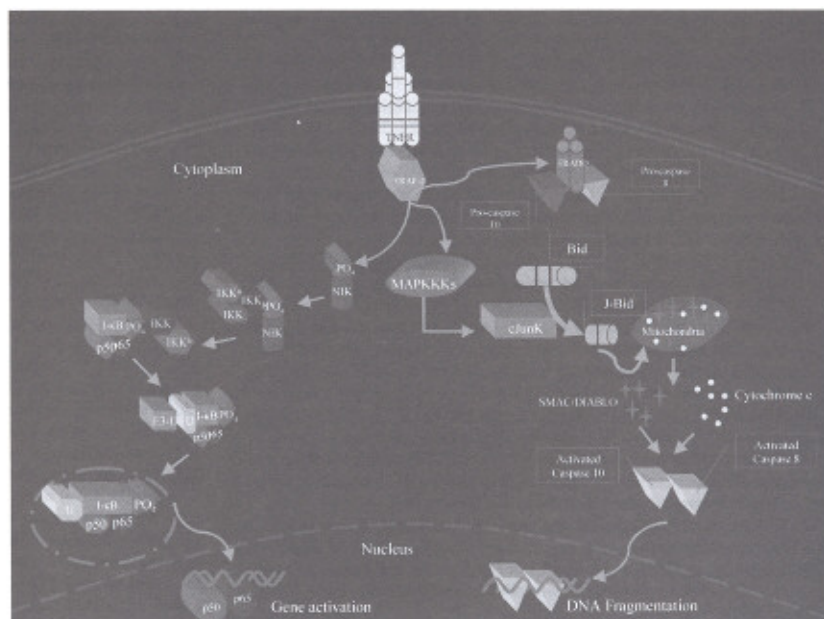


FIGURE 2. NF- κ B activation. Multiple cytokines can activate their respective receptors such as IL-1, TNF- α , and growth factor. Secondary messengers TRAF-2, TRAF-6, and ALK-1 activate the kinase NIK. NIK activates the IKK complex consisting of IKK α , IKK β , and IKK γ the regulatory subunit. IKK phosphorylates the NF- κ B complex. This allows ubiquitination via E-3 ubiquitinase. This phosphorylation and ubiquitination tags the NF- κ B complex to be taken up by a proteasome, where the inhibitor I κ B is degraded. NF- κ B moves into the nucleus where it acts as a transcription factor for cytokines.

FIGURE 3. Apoptosis in reperfusion injury. The secondary messenger TRAF-2 associated with both the inflammatory cascade and apoptosis, activates the proapoptotic protein c-Jun Kinase via MAPKKK. This in turn causes intrinsic apoptosis via the mitochondria activating caspases 8 and 10. These in turn are sent to the nucleus to fragment DNA for programmed cell death.



complex nature of NF- κ B feedback and regulation, blocking these products may be difficult. NF- κ B has been the subject of intense investigation for many years; however, its complex role in the inflammatory cascade is far from elucidated and, therefore, ensures continued research efforts on this topic.

APOPTOSIS AND REPERFUSION INJURY

Programmed cell death, or apoptosis, is a natural and integral part of cellular growth and development. Apoptosis is also a known protective reaction to multiple types of cellular damage such as infection, inflammation, or cancer. NF- κ B is known to protect against apoptosis via transcription of specific inhibitory proteins.^{95,96} There is some evidence that NF- κ B is an integral part of apoptosis via cross talk with known apoptotic genes of the mitogen activated protein kinase (MAPK) family: c-Jun Kinase (JNK) and p38-MAPK.⁹⁷ There are 3 known isoforms of JNK: JNK1, JNK2, and JNK3.⁹⁸ Although p-38 MAPK is activated during ischemia, and stays activated during reperfusion, JNK proteins are activated only with reperfusion. JNKs can be activated by TNF- α via a secondary messenger system, which also activates NF- κ B (Fig. 3). Tissue culture studies suggest that JNK is involved in fibroblast and neural cells apoptosis.^{99–103} However, gene knockout studies utilizing mice demonstrate that JNK may be involved in protection against apoptosis, confusing the issue.^{102,103} The mechanism by which JNK is involved in apoptosis remains unknown. Further research is underway to determine whether JNK initiates apoptosis or simply modulates it.

Mitochondria, already involved in reperfusion injury as discussed above, are central to apoptotic pathways as well. Members of the B cell lymphoma-2 family activate the mitochondrial apoptotic pathway after cleavage by JNK (Fig. 3). The mitochondrial permeability transition pore is acti-

vated by interaction with proapoptotic factors such as BAX, BAD, and eNOS.^{104–106} Activation of this protein allows for egress of cytochrome *c* and other proapoptotic proteins.¹⁰⁵ Apoptotic factors, released from the mitochondria, cause activation of the enzymes caspase 3 and caspase 9.^{107,108} These caspases are instrumental in DNA fragmentation and subsequent cell death. Caspase inactivation is another method in which NF- κ B protects against apoptosis.^{99,103} Specifically, NF- κ B activation through the phosphatidylinositol 3 kinase-AKT pathway leads to phosphorylation and inactivation of caspases 3 and 9.

CONCLUSIONS

Patients with hemorrhagic shock require optimal resuscitation and cessation of ongoing bleeding. Often our resuscitative measures, although necessary, cause a wide range of detrimental physiologic effects. Research continues to answer questions regarding measurable endpoints and optimal fluids used in resuscitation. Elucidation and understanding of the complex metabolic pathways involved in reperfusion injury are areas of intense current investigative effort. It is unlikely that a single treatment modality or "magic bullet" will be able to substantially block such a complex regulated process unless performed before feedback mechanisms known to be in place. Over the decades, there has been an exponential increase in our knowledge base regarding molecular and cellular mechanisms responsible for reperfusion injury. Ongoing translational research will inevitably have a major impact on patient care.

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