Metabolic Disturbances in Shock, and the Role of ATP-MgCl₂ and Sex Steroids

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Key Words

Cell metabolism · Low flow conditions · Hemorrhage · Oxygen · Hypoxia · Adenosine triphosphate · Estradiol · Gender · Trauma

Abstract

Hemorrhage following accidental injuries is a common cause of death in the industrialized world. Moreover, the impact of elective surgery and solid organ transplantation sometimes results in low flow conditions similar to those seen following hemorrhagic shock. A shortage in O₂ availability, or hypoxia, leads to sequential changes in cell metabolism and morphology, including inflammatory responses and the expression of hypoxia-inducible transcription factor-1, which controls the cellular adaptation to hypoxia. These endogenous adaptive responses show that O₂ deprivation is not an unforeseen event for cells. The purpose of this review article is to discuss the pathophysiologic principles of shock and the metabolic alterations that cells undergo during low flow conditions. Moreover, the rationale for therapeutic intervention by administering ATP-MgCl₂ and sex steroids following shock and trauma will also be discussed.

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Introduction

Metabolism of carbohydrates, proteins, and fat requires O₂ and produces the energy for life processes. Multicellular organisms have developed a system in which O₂ is bound to hemoglobin in red blood cells and delivered to the target tissues via the circulatory system. Systemic O₂ delivery is determined by cardiac output and arterial O₂ content [1]. Aerobic metabolism, i.e., complete oxidation of energy substrates, can only be accomplished if O₂ delivery and demand are matched [2, 3], as little O₂ can be stored.

Circulatory failure or shock is defined as a mismatch between O₂ demand and supply. Such a mismatch is aggravated as circulatory failure persists and endogenous catecholamines are released, leading to further vasoconstriction in less vital tissues [4–7]. Even when global circulation is restored, cell dysfunction may persist. Mitochondrial dysfunction is responsible for the inability to utilize O₂ following severe shock [2, 8].

Pharmacological intervention along with adequate fluid repletion is effective in restoring homeostasis [9–12]. In this article, the pathophysiology of shock and its effects on cell metabolism are described. Specific attention is paid to recent advances in cellular signaling and alterations at the cellular/protein/genomic level during low flow conditions.

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Pathophysiology of Hemorrhagic Shock

Several etiologies can lead to shock, i.e., insufficient O₂ delivery to maintain aerobic metabolism. They include depletion of intravascular volume, impaired myocardial performance, or decline in systemic vascular resistance. Hypovolemia is the major mechanism of shock following tissue trauma and hemorrhage. A decline in blood volume decreases venous return and adequate cardiac output cannot be maintained. Compensatory mechanisms such as an increase in heart rate and a fall in vascular resistance are activated. With moderate hemorrhage (5–15 ml/kg body weight), pulse pressure is reduced but mean arterial pressure is maintained. Catecholamines are released, increasing peripheral vascular resistance leading to a cool and pale skin and peripheral cyanosis. Respiratory rate is increased and patients display an intense thirst [13]. Additional tissue injury with stimulation of pain receptors obviously enhances further catecholamine release and the resulting adrenergic stimulation thereby enhances vasoconstriction and shunting in the peripheral circulation. Inadequate perfusion of the tissues leads to anaerobic glycolysis with production of lactic acid.

Sympathetic output is increased when vascular mechanoreceptors (arterial baroreceptors) are less stretched during hypovolemia, resulting in reflex tachycardia and vasoconstriction. Vasoconstriction is most marked in the skin, followed by the kidneys and viscera. In parallel, reflex sympathetic venoconstriction helps to maintain cardiac filling pressures. The adrenal glands are stimulated following hemorrhage and they release epinephrine, aldosterone, and glucocorticoids. Simultaneously, the renin-angiotensin system is activated to maintain mean arterial pressure and to preserve body water and sodium [14–16]. If the blood loss, however, exceeds 40% of the intravascular volume, these compensatory mechanisms are exceeded and life-threatening shock occurs. Patients in extremis exhibit an impaired level of consciousness, and die if volume is not immediately restored [6, 17]. Prior to describing metabolic alterations in shock, it is pertinent to describe O₂ transport, O₂ delivery, and O₂ consumption under normal as well as pathophysiologic conditions.

Oxygen Delivery and Consumption

The O₂ delivery system in the body consists of the lungs and cardiovascular systems and depends on the amount of O₂ entering the lungs, pulmonary gas exchange, blood flow, and the capacity of the blood to carry O₂. Oxy-

gen, which has a partial pressure of 159 mm Hg in dry air at sea level, is transported along a partial pressure gradient from the air to the lung alveoli, to the blood, and ultimately to the various tissues. Most (99%) of the O₂ which dissolves in the blood combines with the O₂-carrying protein hemoglobin, which increases the capacity of blood for O₂ by 70-fold. The heme moiety of hemoglobin contains iron and binds O₂ reversibly, a process described by the O₂ hemoglobin dissociation curve. Increased affinity results in a shift to the left and, therefore, inhibits unloading of O₂ in the periphery. Myoglobin in skeletal muscle facilitates oxygenation of target tissues, since it has less affinity for O₂ than hemoglobin [18, 19].

The total amount of O₂ delivered to the tissues per minute (DO₂), regardless of blood flow distribution, is defined as the product of cardiac output and arterial O₂ content. Under resting conditions, DO₂ is about 1,000 ml O₂/min in humans [19–21]. However, due to the reserve in O₂ extraction, VO₂ remains unchanged over a wide range of DO₂. When DO₂ falls below a critical value, VO₂ becomes dependent on DO₂ and starts to decrease. This marks the onset of shock, with O₂ delivery being insufficient to meet the demand of the tissues. Anaerobic glycolysis becomes more dominant and lactic acidosis arises.

Mitochondrial Dysfunction in Shock

Under normal conditions, the cytochrome c oxidase redox state closely reflects cellular O₂ delivery. With decreasing tissue O₂ delivery, the redox state becomes reduced and, following restoration of adequate O₂ delivery, the cellular redox state oxidizes. Under normal conditions, O₂ availability dictates the redox state of the cell, meaning that both O₂ delivery and O₂ utilization processes are coupled. A hallmark of hemorrhagic shock with impaired O₂ availability is decoupling of the cell redox state from transfer of electrons to O₂ [2]. Decoupling may result in the production of reactive O₂ species. Reactive O₂ species then drive the inflammatory response by activation of nuclear factor- κ B and subsequent activation of proinflammatory cytokines such as tumor necrosis factor- α (TNF- α), interleukin (IL)-1 β , and IL-6. Moreover, it appears that once circulatory failure has progressed beyond a certain point, mitochondrial dysfunction persists, even when the O₂ supply is restored due to intrinsic mechanisms such as a decreased supply of pyruvate to the mitochondrial tricarboxylic acid cycle, collapse of the mitochondrial proton gradient and inhibition of mitochondrial enzymes. For example, nitric oxide (NO) blocks

cytochrome c oxidase and, thereby, inhibits the electron transport chain [14, 22, 23]. Transient hypoxia at the cellular level also generates peroxynitrate, which impairs mitochondrial respiration. These and other observations collectively suggest that restoration of O₂ delivery may be inadequate to restore and maintain cell functions once mitochondrial respiration has been impaired.

Hypoxia and Reperfusion Injury

Ischemia is a condition in which there is total lack of blood flow (such as complete occlusion of a blood vessel or cardiac arrest) whereas hypoxia refers to decreased oxygen availability and is therefore the more precise term. Nonetheless, it is recognized that with severe shock certain areas such as the skin and nonvital tissues may indeed be ischemic [24–33]. Part of the hypoxia-reperfusion injury is attributable to the phenomenon of ‘slow reflow’ or ‘no reflow’, which is characterized by reduced blood flow despite the restoration of adequate perfusion pressure. Although this phenomenon is still poorly understood, it appears that leukocytes, at least partially, mediate postischemic microvascular compromise [29–38]. Following ischemia or hypoxia/reperfusion injury, leukocyte adhesion to the endothelium is significantly enhanced and is mediated by several adhesion molecules on the surface of leukocytes and/or endothelial cells such as intercellular adhesion molecule-1 (ICAM-1), platelet endothelial cell adhesion molecule-1 (PECAM-1), vascular cell adhesion molecule-1 (VCAM-1), integrins (CD11/CD18), and selectins (E-, P-, L-selectin) [34, 39–48]. Post-ischemic/hypoxic endothelial edema and perfusion dysfunction occur when soluble mediators such as proinflammatory cytokines (TNF- α , interleukins, platelet-activating factor) and leukotrienes are released following reperfusion or low flow conditions [25, 49–51]. Free radicals generated during reperfusion with O₂-rich blood cause endothelial cell damage and microvascular dysfunction.

Depletion of ATP diminishes the ability of endothelial cells to maintain transmembrane gradients of cations and anions, leads to cell swelling, impairs endothelial cell integrity, causing extravasation of macromolecules and edema [10, 11, 52–55]. Under these conditions there is also a marked reduction in the production of endothelium-derived NO, which regulates the vascular tone and has antiadhesive properties [3, 24, 56–63]. Thus, localized hypoxia caused by conditions such as hemorrhagic shock and vascular trauma followed by reperfusion injury

of the tissues with oxygenated blood can compromise microvascular integrity [24–26].

The proinflammatory cytokines and the generation of O₂ free radicals trigger the activation of intracellular signaling pathways, leading to translocation of nuclear transcription factors, induction of stress genes and de novo protein synthesis [64–66].

Transcription Factors

Cellular hypoxia does not appear to be an unforeseen event for cells. Evidence for this suggestion is the fact that a hypoxia-inducible transcription factor (HIF-1) has been described as a central regulator of hypoxic gene expression [67, 68]. It controls cellular adaptations to hypoxia including erythropoiesis, angiogenesis, vasodilatation, glycolytic enzymes of anaerobic metabolism, and a variety of genes such as those for inflammatory mediators and for signaling molecules such as NO, all of which have a HIF-1 binding element [3, 69–72]. Hypoxia stimulates the induction of vascular growth factor and also that of acute phase genes, which helps explain why low flow conditions perpetuate the inflammatory response. Cells also have the ability to maintain their ATP levels, decreasing the ATP utilization rate by reducing the expression of ATP requiring enzymes such as the Na⁺ K-ATPase [3, 73–80].

Pharmacological Modulations

A large number of studies have shown that following severe shock, crystalloid resuscitation and even transfusion of packed red blood cells may not be sufficient to restore homeostasis. Thus, despite the fact that global O₂ delivery has been restored, local ischemia may persist. This has been also described as the ‘no-reflow’ phenomenon [33]. Microcirculation is compromised because endothelial swelling and slugging of blood impairs capillary blood flow despite adequate precapillary perfusion pressure.

Since the hallmark of circulatory failure is a decrease in energy availability, i.e., a decrease in ATP levels, pharmacologic adjuncts centered on the administration of ATP have been described. Studies have shown that infusion of ATP-MgCl₂ following hemorrhagic shock and other low flow conditions significantly improve survival of animals [81–87]. At the cellular level it was noted that administration of ATP-MgCl₂ corrects abnormalities in cell function

replenishing intracellular ATP levels, restoring altered cell membrane permeability, decreasing lactate production, and normalizing mitochondrial calcium levels [88–105]. At the level of the circulatory system, administration of ATP-MgCl₂ restored organ blood flow in liver and prevented renal ischemia/reperfusion injury. Since infusions of ATP or of MgCl₂ alone do not improve organ and cell function, it can be concluded that a synergistic effect of ATP and MgCl₂ improves organ function following trauma and hemorrhage. This can be due to the fact that following hemorrhagic shock, not only cellular levels of ATP but also of Mg are depleted. Thus, it appears that provision of ATP-MgCl₂ following adverse circulatory conditions is a useful adjunct to conventional fluid resuscitation.

Recent studies have indicated that female animals in the proestrus stages have normal immune function after trauma and hemorrhagic shock as opposed to a markedly depressed immune response present in male animals [106, 107]. Moreover, castration of male animals before the induction of trauma and hemorrhage prevents the occurrence of immune depression [108], suggesting that testosterone is involved in producing the immune depression under such conditions. Similarly, castration also prevents the depression of myocardial function in male animals after trauma and hemorrhagic shock [109]. This would suggest that 5 α -dihydrotestosterone receptor blockade in males might be an effective approach for maintaining cell and organ functions following adverse circulatory conditions, such as trauma and shock. In this regard, flutamide, a nonsteroidal receptor antagonist, has been shown to attenuate vascular endothelial cell dysfunction as well as immune depression and to improve survival following trauma-hemorrhage and subsequent sepsis [110, 111]. Studies have also showed that flutamide can directly induce vascular relaxation in large and small blood vessels [112] and maintains vascular endothelial function following trauma-hemorrhage [113].

Another sex steroid, 17 β -estradiol, has also been shown to alleviate cell and organ dysfunction following adverse circulatory conditions [114–127]. Studies have also demonstrated that cardiovascular and hepatocellular organ functions were significantly depressed in male animals after trauma and hemorrhagic shock and were restored in animals receiving 17 β -estradiol following trauma and hemorrhagic shock [114–118, 120–122, 124–127]. However, simultaneous administration of an estradiol-specific receptor antagonist abolished the beneficial effect of estradiol treatment despite high circulating levels of this sex steroid [121]. This would suggest that adminis-

tration of 17 β -estradiol should be considered as a novel and safe adjunct for improving cell and organ functions following hemorrhagic shock in ovariectomized and postmenopausal women because of their low estradiol levels in the circulation [128].

Mechanism by Which Sex Steroids/Receptor Antagonists Improve Cell and Organ Function

Testosterone is a major circulating sex steroid, however, it must be converted to 5 α -dihydrotestosterone for high affinity binding to androgen receptor and thus for its maximal activity. The pathway for androgen metabolism is: testosterone $\rightarrow$ 5 α -dihydrotestosterone $\leftrightarrow$ 5 α -androstane-3 α , 17 β -diol $\rightarrow$ androsterone and involve the enzymes 5 α -reductase, 3 α - and 17 β -hydroxysteroid dehydrogenases. Recent studies demonstrate the presence of these enzymes in splenic T lymphocytes, indicating active steroid metabolism in the lymphoid cells [129]. Studies also indicate decreased activity and expression of the enzymes for 5 α -dihydrotestosterone metabolism following trauma-hemorrhage and thus less conversion of 5 α -dihydrotestosterone into androsterone, an inactive metabolite, in T lymphocytes from males [130]. However, castration of males before trauma-hemorrhage abolished 5 α -reductase expression and increased the expression of 17 β -hydroxysteroid dehydrogenase oxidative isomers, hence preventing 5 α -dihydrotestosterone conversions to androsterone [129]. Thus increased synthesis and decreased catabolism of 5 α -dihydrotestosterone are the likely cause for the depressed T lymphocyte functions, which is reflected by lowered splenocyte proliferation and release of IL-2 and IL-6 in males following trauma-hemorrhage [129, 130]. The presence of the androgen receptor in the lymphocyte [131] permits 5 α -dihydrotestosterone to interact and regulate the immune system in the spleen, thus altering its function following trauma-hemorrhage. However, the precise mechanism remains to be determined. Nonetheless, studies have shown that flutamide administration following trauma-hemorrhage upregulates estrogen receptors in T cells from males and thus may be one of the mechanisms responsible for the salutary effects of flutamide in males [131].

Additional studies indicated increased 17 β -estradiol synthesis and low conversion into estrone in T lymphocytes of proestrus but not of ovariectomized mice [132]. Since 17 β -estradiol is able to regulate cytokine genes and the splenic T lymphocyte cytokine release is altered following trauma-hemorrhage, continued synthesis of 17 β -

estradiol in proestrus females is probably responsible for the maintenance of T lymphocyte cytokine releases associated with the protection of immune functions following trauma-hemorrhage. Furthermore, studies suggest that estradiol acts as a facilitator of the intestinal blood flow via the increased production of NO, decreased production of vasoconstrictors, attenuated neutrophil adhesion, and decreased formation of oxygen free radicals [133]. Since estradiol effectively protects the organs from circulatory failure following various adverse circulatory conditions [133], numerous studies have been performed to clarify the molecular mechanism of estradiol action with regard to tissue circulation. It is clear that estradiol improves the macro- and microcirculation of the splanchnic organs by the multiple mechanisms [133]. Nonetheless, it remains unclear which mechanism plays the most important role

in the treatment trauma-hemorrhage. Further studies are required to elucidate the precise mechanism of estradiol action and to determine the usefulness of estradiol treatment in patients with severe hemorrhage. Nonetheless, it is clear that in addition to fluid resuscitation, the use of pharmacological agents, such as ATP-MgCl₂, flutamide, or 17 β -estradiol, as adjuncts can help improve cell and organ function and decrease septic complications and mortality following severe adverse circulatory conditions.

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