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Secondary Injury After Musculoskeletal Trauma: A Review and Update

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Objectives: To revisit the secondary injury model, to incorporate several current pathophysiologic theories into the model, and to show the need for more direct research examining the model.

Data Sources: I searched MEDLINE and CINAHL from 1976 to 2001 for literature related to acute injury pathology and pathophysiology and selected classic articles and pathology, pathophysiology, and immunology texts.

Data Synthesis: Acute musculoskeletal injury management is based on a pathophysiologic model, often referred to as the *secondary injury model,* which was originally developed more than 25 years ago. In this model, acute trauma is referred to as primary injury, whereas secondary injury refers to damage to otherwise uninjured cells that was a direct consequence of the physiologic response to primary injury. In the original model, mechanisms for secondary injury were hypothesized based on then-contemporary understandings of immunology and cellular pathology. These mechanisms were broadly categorized as either enzymatic or hypoxic. Since this time, the pathologic paradigms for cell death from trauma have evolved, and the secondary injury model requires some updating. Some controversy now exists regarding the categorization of injury as primary or secondary, specifically whether posttraumatic damage is actually secondary injury in previously uninjured tissue or delayed death of primary injured cells. Similarly, the postulated mechanisms that lead to secondary injury now appear to be considerably more complex than originally anticipated.

Conclusions/Recommendations: The secondary injury model has been reconciled with our contemporary understanding of pathophysiology. Specifically, secondary hypoxic injury has been clarified to be secondary ischemic injury, and several specific mechanisms for ischemic injury have been identified. Similarly, secondary injury from mitochondrial failure and other potential mechanisms has been identified, and the role and interaction of these mechanisms in relation to total secondary injury have been expanded.

Key Words: pathophysiology, acute injury, cell pathology

Returning to competition after an injury requires adequate repair or regeneration of damaged tissues. The greater the quantity of damaged or necrotic tissue, the longer the time required for its removal and the more delayed the healing and return to competition. Therefore, with musculoskeletal injury, short-term management techniques that limit the quantity of damaged tissue are highly desirable. To this end, clinicians use numerous modalities for managing acute injury. Of these modalities, none is more commonly used for this purpose than cryotherapy.[1–8]

Although the acute and rehabilitative rationales for using cryotherapy differ and have changed throughout the years,[6] the clinical efficacy of this modality has not. One of the most widely accepted theories regarding the rehabilitative use of cryotherapy, that it produced therapeutic cold-induced vasodilation,[9] was discredited 20 years ago.[6,10] A few years earlier, the rationale that short-term cryotherapy was effective because it limited edema formation through vasoconstriction began to be replaced by the currently accepted theory involving retarding secondary injury.[6,11] This secondary injury model was a significant improvement over previous models because it strongly incorporated an understanding of immunology and cell pathology into acute injury management.

At the time of its introduction, the secondary injury model was a true landmark in terms of its ability to explain the sequelae of musculoskeletal injury. As is the case with all new theories, however, many aspects of the model were speculative when it was introduced. In the years since its introduction, numerous technologic advancements have provided avenues for examining the tenets of this model. Many of these tenets have been supported in subsequent literature.[5,12–15] On the other hand, some contemporary findings do not mesh well with the original model.[12,13,16,17] Contemporary understanding of the pathophysiologic events after acute trauma necessitates a few revisions to the secondary injury model. Therefore, the purpose of this review is to revisit the secondary injury model, to incorporate several current pathophysiologic theories into the model, and to show the need for more direct research examining the model.

MUSCULOSKELETAL TRAUMA

Trauma to tissues can be of large magnitude (macrotrauma), such as the trauma that exists with crush injuries and both moderate and severe sprains or strains. The trauma can also be of small magnitude (microtrauma), such as the trauma that typically exists with stress fractures and other overuse syndromes. Regardless of magnitude, trauma exists in several

forms, including physical, chemical, thermal, metabolic, and biological.[6,13,14,16–19] Injury from any of these sources induces an inflammatory response whose magnitude largely depends on the severity of the injury and the degree of vascularization of the tissue.[6,12–14,20–22]

Primary and Secondary Injury Theory

In the mid-1970s, Knight[6,11] described the series of events that follow athletic injuries in what he referred to as the Sport Injury Model.[6] Because of its primary-secondary injury classification, the model has also been referred to as the *secondary injury model.*[4,5] This model has been widely accepted, although the body of literature directly examining this theory is limited. Currently, this model is one of the most (if not the most) commonly cited rationales for the short-term use of cryotherapy.[1–8]

In the secondary injury model, trauma from any of the aforementioned mechanisms causes immediate, pathologic, ultrastructural changes in the affected tissues.[6,20,23] These ultrastructural changes involve the direct disruption of the cell membrane and structural components of the cell, leading to a subsequent loss of homeostasis and, therefore, death and eventual necrosis of the affected cells.[6,12,20,22,23] The ultrastructural changes[20] and their immediate consequence of cellular death followed by necrosis[12,22] are referred to as primary injury.[6,11] Primary injury typically affects several types of tissue simultaneously, frequently including skeletal muscle and vascular, connective, osseous, and nervous tissues. At this time, we do not know when cell death from primary injury occurs.[13–15,22] We can reasonably speculate that some severely damaged primary injured cells die almost immediately, whereas other cells with less ultrastructural damage may die more slowly.

The physiologic response to primary injury can lead, in theory, to additional injury to otherwise uninjured cells. This resulting damage has been termed *secondary injury*[6,11] and can stem from several causes. In his sport injury model (Figure 1), Knight[6,11] suggested that secondary injury occurs from both enzymatic and hypoxic mechanisms, as discussed herein. Pathologic changes are thought to occur in the otherwise uninjured tissues surrounding the primary lesion, and these changes lead to secondary injury. Knight[6,11] proposed that secondary injury occurs in the cells on the periphery of the primary lesion.

Secondary Enzymatic Injury. In the first form of secondary injury, secondary enzymatic injury,[6,11] enzymes have been suggested to be released from the lysosomes of the dead and dying cells. Although they were not specifically identified in the original model, the enzymes would most likely be a variety of acid hydrolases, phospholipases, and various proteases[13,14,22,24] and perhaps might also include any of a number of human neutrophil proteins.[18,22] Several of the acid hydrolases and phospholipases lyse the membranes of nearby cells by cleaving hydrocarbon chains from the lipid portion of membrane phospholipids,[18,22] whereas the proteases cleave the peptide bonds of proteins, inactivating the proteins.[13,22] These changes in the structure of the membrane phospholipids lead to the loss of membrane polarity and therefore membrane fluidity and integrity. The loss of membrane integrity leads to increased hydropic swelling (oncosis), eventually causing cellular death.[13–15,16,22,25] The role of lysosomal damage and its intracellular partner, proteosomal damage, as a cause for cellular death is discussed again in the portion of this review devoted to the sequelae of musculoskeletal trauma.

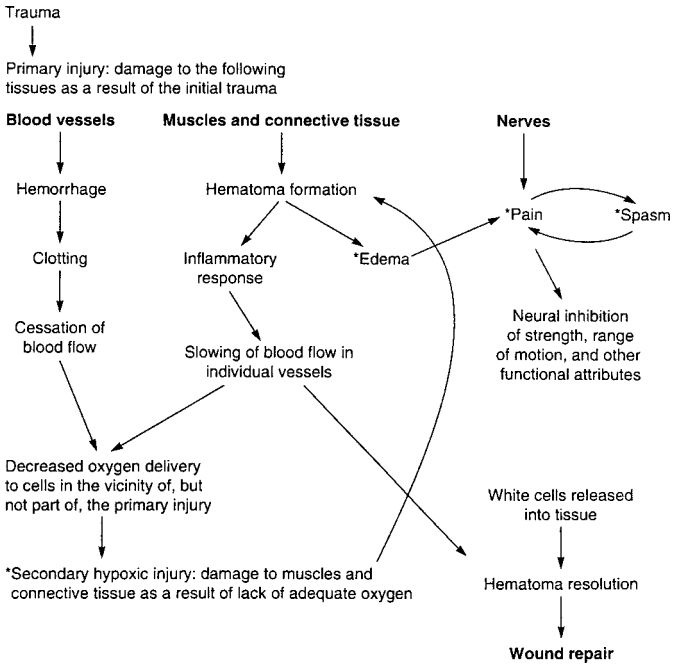

Figure 1. Knight's summary of the response to acute injury. Reprinted with permission from Knight KL. *Cryotherapy in Sports Injury Management.* Champaign, IL: Human Kinetics; 1995:33.

Secondary Hypoxic Injury. Knight[6] suggested that, in secondary hypoxic injury, hemorrhaging from damaged blood vessels, hemostasis from the clotting cascade, reduced blood flow from the inflammation-induced increase in blood viscosity, and the increased extravascular pressure from an expanding hematoma and muscle spasm can lead to a period of ischemia. Additionally, although not discussed in the original model, hydropic swelling of cells after membrane damage has been shown to be capable of occluding vasculature,[14,22,26] providing another possible mechanism for postinjury ischemia.

The ischemia produces a resulting hypoxic period, which Knight[6] identified as causing a metabolic imbalance and resulting in oncosis, acidosis, and lysosomal digestion. Hypoxia results in an inability to use oxygen as the terminal oxidizer in oxidative phosphorylation and leads to a dependence on the glycolytic pathway for adenosine triphosphate (ATP) production.[13–15,19,22,27] The relative inefficiency of this anaerobic pathway compared with the aerobic tricarboxylic acid cycle–oxidative phosphorylation pathway allows adequate energy production for only a limited time.[19,27–29] This period may last anywhere from minutes to 6 hours, depending on the tissue involved.[22,27,28] When the glycolytic pathway can no longer provide adequate ATP, membrane ion pumps (Knight spoke specifically of the sodium-potassium-adenosine triphosphatase [ATPase] pump)[6,11,13] and other homeostatic mechanisms fail, resulting in oncosis and eventually cell death, followed by necrosis.[6,13,14,16,22]

Knight[6] proposed that secondary injury, particularly secondary hypoxic injury, is a significant problem after musculoskeletal trauma. This premise has been widely accepted in the sports medicine community during the last 25 years. In fact, the initial management of musculoskeletal trauma has commonly been based on the premise that cold reduces the metabolic rate of these hypoxic tissues, allowing them to better

survive the period of hypoxia.[1–8] The hypothesis appears reasonable, considering the fact that the rate of chemical reaction in vitro or in vivo is temperature dependent via the Q_{10} effect.[29] Reducing the temperature would lower the rate of chemical reactions and, therefore, the demand for ATP. Decreased cellular ATP demand would translate into less demand for oxygen at the terminal step of the oxidation phase of oxidative phosphorylation. This would, in turn, lead to potentially longer survival during hypoxia.[11,30] An example of this would be the increased potential for resuscitation of persons who have drowned in very cold water compared with their less fortunate counterparts who drowned in warm water.

UPDATING THE SECONDARY INJURY MODEL

As suggested, the secondary injury model has been well accepted during the past quarter century and not without reason. This model has proven to be very insightful, and the tenets of the model continue to be confirmed over time.[5,12–15] However, as is the case with all theories, new discoveries and changing pathologic paradigms have brought us to the point where several aspects of the model would benefit from updating, and some new questions about the sequelae of musculoskeletal trauma need to be asked. These updates and questions are not refutations of the original secondary injury model; instead, they should be considered additions and refinements.

Primary or Secondary Injury?

The first update, and one of the most important new questions from a research standpoint, is the importance of the ability to distinguish primary injured tissues from secondary injured tissues. This is relatively easy from a conceptual standpoint but is not nearly so easy in laboratory practice. To adequately examine the secondary injury theory from a scientific standpoint, it is important to make this distinction, which allows researchers to examine the efficacy of our therapeutic interventions on the progression of secondary injury. If we can specifically identify secondary injury in cells and tissues, then we can monitor this damage and use it as a dependent variable in short-term intervention studies. Some recent evidence[5] suggests that cryotherapy retards some of the sequelae of musculoskeletal injury. Specifically, cold retards the loss of oxidative function that follows crush injury, as indicated by activity of cytochrome-c oxidase.[5] However, it is unclear whether the retardation of this loss occurs in primary injured tissues or in secondary injured tissues.

In the original model, Knight[6] described primary injury in terms of both its cause (physical or mechanical trauma in the case of sports injuries) and loosely in terms of the time frame when it occurred. He wrote, ''When an injury occurs, whether a contusion caused by direct compression or a sprain or strain caused by a stretching force, immediate ultrastructural changes take place in muscle, connective tissue, or both; nerves and blood vessels may also be broken. All this damage is called primary traumatic damage.''[6] Knight then went on to describe secondary injury in terms of 2 hypothesized causes but not in specific terms of its timeframe in relation to primary injury. Failure to describe the timing of secondary injury in detail was not an oversight, however; there was (and still is) simply a lack of data to characterize the timing of this phenomenon.

It makes sense that secondary injury occurs at some time subsequent to primary injury, yet there are currently no data to specifically characterize the timeframe for secondary injury after musculoskeletal injury. Because we do not yet have a clear picture of when primary injury ends or when secondary injury begins, it is unclear whether death of tissues at some time after the initial trauma is secondary injury or just the delayed death of cells that were primarily injured but not completely destroyed during the initial trauma. Most likely, there is a fair amount of overlap between these two.

Although the difference between primary and secondary injury may appear to be easy to clarify in terms of the causes of cell death, clarifying the difference temporally will most likely prove to be difficult. On examination of dead and dying tissue, the difference between primary and secondary injury is not easily discernible except in cells with obvious primary injury (complete obliteration). In cells that die more slowly, the cause or severity of the injury is difficult if not impossible to discern.[13,14,22,31]

This leads to one of the biggest research questions regarding the secondary injury theory: Is the efficacy of short-term cryotherapy and other short-term interventions explained by a reduction or prevention of secondary injury in cells not initially damaged by primary trauma, or is the efficacy explained by rescuing or delaying the death of the cells that were primarily injured but not initially destroyed? This question currently is unanswered.

Hypoxic Injury or Ischemic Injury?

A second aspect of the original model that requires an update concerns the hypoxic injury designation. Many practitioners cite secondary hypoxic injury as a rationale for cryotherapy, ignoring the secondary enzymatic form. Although this practice is troublesome, the use of the term *secondary hypoxic injury* itself is also somewhat problematic. Although we have collectively used the term *secondary hypoxic injury* for some time, we would be more correct to refer to this phenomenon as *secondary ischemic injury.* Ischemia is the loss of perfusion, whereas hypoxia refers to less-than-normal oxygen tension.[19,22] Technically, it is possible to have hypoxia in a normally perfused tissue (eg, breathing 10% oxygen gas instead of room air). The distinction between hypoxia and ischemia is important because hypoxia presents a single physiologic challenge (inadequate oxygen), whereas ischemia presents 3 separate challenges: inadequate oxygen, inadequate fuel substrates (eg, glucose), and inadequate waste removal (eg, lactate).[19,22]

All 3 of these challenges could potentially contribute to secondary injury or hasten the progression of primary injury. Inadequate oxygen, as discussed previously, leads to a dependence on anaerobic metabolism. Inadequate fuel substrates lead to dependence on limited intracellular fuel stores to produce ATP. Because these intracellular fuel reserves are limited,[29] they are able to provide for the tissue's energy needs for only a brief time.[13,14,22,29] Inadequate waste removal leads to accumulation of cellular waste products, many of which are toxic and some of which are inhibitory to metabolic pathways (eg, lactate inhibits phosphofructokinase in glycolysis).[14,16,29] The accumulation of these wastes and the resulting cellular acidosis and subsequent inhibition of bioenergetic pathways limit the cells' ability to produce ATP.[22,29] Although these findings are not directly applicable to musculoskeletal trauma, there is evidence that with ischemic injury in some organs (liver and kidney), cell death occurs primarily in the first 2.5

to 3 hours.[22] Similarly, complete ischemia in skeletal muscle produced with a tourniquet leads to significant loss of mitochondrial function after 3 hours[28] (shorter periods have not been adequately studied). In nervous tissue, ischemic injury occurs much more quickly, in as few as 4 minutes.[13]

Injured or Dead?

A third aspect of the secondary injury model to be updated involves the mechanisms by which secondary injured tissues die. In Knight's[6,11] original model, he postulated these mechanisms as being either hypoxic or enzymatic, as described herein. His theory reflected a solid understanding of the pathologic paradigms for cell death that were common in the mid-1970s. During the past 25 years, however, these paradigms have evolved[13–16,22] to reflect additional research findings, and the secondary injury model would benefit from incorporation of some of this contemporary information.

Although a great deal is known about cellular injury and cellular death, the actual progression from injury to death is still somewhat unclear.[5,12–16,20,22] We know that injured cells display a number of characteristics, including hydropic swelling[13–16,22,25] and fluid-filled or fatty intracellular accumulations.[12–16,22] Yet it is not easy or even possible in most cases to distinguish between injured cells that will recover and those with irreversible injury.[13–15,22] In fact, Kane[14] stated, ''Identification of the precise biochemical and morphologic events that determine the transition or point of no return from reversible to irreversible injury remains elusive.'' Similarly, defining the exact moment at which an injured cell dies is also not yet possible.[13–16,22] Other than when a cell is literally torn apart, we are unable to distinguish injured cells from newly dead cells until these dead cells undergo the postmortem secondary morphologic changes that are summed up by the term *necrosis*.[13,22,32]

Although the terms *necrosis* and *death* are often used synonymously, they actually have slightly different meanings. All necrotic tissues are dead, but not all dead tissues are necrotic, at least not initially anyway. Necrosis refers to a set of postmortem morphologic changes in a cell or tissue that can be summed up as a loss of organized cellular structure.[13–16,22] Necrosis has been classified into at least 4 subtypes[13–16,22]: coagulative, liquefactive, fatty, and caseous. Necrotic tissues have a series of common and very identifiable features, including shrunken (pyknotic) and disorganized (karyolytic) nuclei,[13,14,16] accumulation of calcium salts,[13,14,22] liberation or crystallization of membrane cholesterol,[14,16,22] myelination and disruption of membrane phospholipids,[13,14,16,22] and hydropic swelling with dispersed ribosomes.[13,16,22,25] These necrotic changes are not visible immediately after death and may not be seen until as long as 12 to 24 hours postmortem.[13–16,22,32] Unfortunately, this delay between death and observable necrosis renders these easily recognizable changes relatively useless as a tool for distinguishing living but seriously injured cells from cells that have died within the previous few minutes or hours.[13,22] An ability to make such a distinction would be useful in examining secondary injury because it would allow us to gauge the efficacy of our short-term management techniques in preventing secondary tissue death.

Mechanisms of Traumatic Death

Cells die by a variety of means.[13–16,22] For the sake of organization and discussion, it is useful to loosely classify these as suicide, murder, or accident. In cellular suicide (apoptosis), cells die as the result of a programmed cascade of enzymatic reactions,[13,14,33,34] which occur in nearly all mammalian cells.[12–14,22,34] These reactions produce a characteristic shrinking of the cell and fragmentation of the DNA.[13,14,16,22,33,34] Apoptosis is the normal means by which cells die at the end of their lifespan.[13,22,34] Apoptosis can also be a form of cellular murder when it is induced as a part of an immune response, as is the case with immune-mediated destruction by cytotoxic lymphocytes.[14,34] Additionally, there is evidence that apoptosis is also induced after burn trauma.[35] This is a promising area for future research.

In cellular murder, immune cells (primarily neutrophils) and other specific and nonspecific defense mechanisms attack cells, leading to cell death.[12–14,16,18,22,36] The lysing of cell membranes, typically through enzymatic cleavage of the phospholipids[13,14,16,18,22] or oxidation of the membrane fatty acids,[12,18,21] is one of the most common mechanisms for cell killing. Cellular murder plays a critical role in our immune defenses against foreign cells and proteins. The defense mechanisms are numerous and include T cells, B cells, natural killer cells, a variety of proteolytic and lipolytic enzymes, antibodies, and the complement system.[12] Murder can also be caused by infection with a variety of pathogens,[12,22] although infection is not generally the primary problem in athletic trauma.

In accidental cell death, as seen with primary injury from athletic injuries, cells die as the result of trauma or environmental stresses that exceed the ability of the cell to cope.[13–16,22] The progression of accidental cell death is presumed to be variable and dependent on several factors, including perfusion, immune response, and the severity of cellular injury.[13,22] Severe ultrastructural damage, in which a cell is literally torn apart, obviously leads to rapid cell death, whereas other processes such as ischemia are thought to lead to a slower death.[22,37]

In most of the articles that describe the mechanisms of cellular death, researchers have primarily focused on organs and organ preservation for transplantation[22,37–40] rather than on musculoskeletal tissues. Unfortunately, this requires us to make a small leap of faith and assume that the mechanisms are similar in musculoskeletal tissues, although some differences probably exist. From this literature, a number of mechanisms and theories regarding the final cause of cellular death have been developed.[13–16,18,21,22,31,39–41]

For the sake of discussion, we will organize the postulated mechanisms under several broad headings, but these are certainly not the only possible classification systems. These classifications are lysosomal mechanisms,[13–16,22,24,39] protein denaturation mechanisms,[13–16,22,41,42] membrane permeability mechanisms,[13–16,22,31,43,44] and mitochondrial mechanisms.[13–16,22,31,40] In isolation, none of these categories is adequate to explain all of the mechanisms by which cells die. In fact, there is a large degree of overlap among these theories, with multiple factors simultaneously contributing to cell death.

Lysosomal Mechanisms. In the lysosomal mechanisms category,[13,18,22,24,36,39] lysosomes act as agents of cell death by releasing their highly destructive enzymes (eg, phospholipase A, cathepsin B), which in turn destroy cells. These enzymes are extremely damaging to cell membranes and cellular proteins and typically require activation by an environment with a low pH.[13,14] The most common mechanism of destruction is via disturbing the lipid organization of the membrane by

cleaving the phosphates from the fatty acid subunits from membrane phospholipids[22,36,39] and oxidizing the sulfhydryl groups or peptide bonds of various membrane-bound proteins (eg, ATPases), inactivating them.[14,22,24] Under normal circumstances, these destructive enzymes are stored in specialized membrane-enclosed vacuoles to prevent them from damaging cells accidentally. Also, lysosomal enzymes are largely used in phagocytosis as a part of normal immune function.[18,12,13,22] Fisher et al[24] offered some recent support for the role of lysosomal proteolytic enzymes after acute trauma. They observed that, after experimentally induced crush injury, the use of inhibitors of lysosomal proteolytic pathways reduced postinjury muscle atrophy by 44%.

Elements of the lysosomal theory are clearly seen in Knight's[6,11] secondary injury model, in which the enzymatic form of secondary injury is largely explained as the accidental release of these enzymes from dead and dying cells, leading to unwanted collateral damage. In the lysosomal explanation, the role of the lysosomes is paramount and considered to be a prominent means for cell killing.[13,14,22,39] A limitation to this early suggestion of lysosomal mechanisms for secondary injury is that it largely ignores the role of the proteasome.[45] Like lysosomes, proteasomes are single membrane-bound organelles that degrade proteins and other molecules. However, proteasomes are primarily responsible for degradation of intracellular proteins (eg, transcription factors and damaged proteins), whereas lysosomes are primarily responsible for degradation of extracellular proteins that are taken into the cell by endocytosis.[12,28,29] It has been suggested that proteasomes are the primary means of breaking down muscle proteins in most forms of atrophy.[24,45,46]

A second limitation to the lysosomal explanation for secondary injury is that it involves the accidental release of lysosomal enzymes from dead and dying cells. Given the role of lysosomes in degrading endocytosed extracellular proteins, we know that killing through the action of lysosomal enzymes is an important mechanism for phagocytes.[12–14,16,18,21,22,36] However, inadvertent cell death that results from the accidental release of these enzymes from dead cells into the extracellular space has not been well examined. It has been suggested[13–16,22] that in the latter stages of lethal cell injury, lysosomes release their contents within their own cells, leading to autolysis and much of the structural destruction that eventually appears as necrosis. It is plausible but not clear whether these enzymes released intracellularly in dying cells eventually reach the extracellular space and degrade surrounding cells. Similarly, because most lysosomic enzymes work best in acidic environments (pH $\leq$5),[45] the normal extracellular pH of approximately 7.2 would hamper their action.[45] It is possible, however, that after exercise or injury, the tissue would become acidotic and these enzymes would be active.

Some recent work[24] strongly suggests that lysosomes do play a role in the postinjury atrophy of skeletal muscle. However, the lysosomal digestion in this enzymatic form of secondary injury is proposed to be from the lysosomic enzymes of immune cells and not from the dead and dying cells. If the lysosomal enzymes had been accidentally released from the dead and dying cells, we would also expect to have seen significant activity of the proteasomes from these cells, which would have also been accidentally released. In fact, although proteasomes have been theorized[24,45,46] to play a significant role in the secondary atrophy that follows muscle injury, Fisher et al[24] observed that this may not be the case. They inhibited proteasomic activity after contusion injury and observed no influence on posttraumatic protein catabolism, suggesting that proteasomes do not play a meaningful role in secondary posttraumatic atrophy. This finding casts some doubt about the role of proteasomes and lysosomes from dead and dying cells in secondary injury and strengthens the notion that chemical attack from the immune system through inflammation may be more important.

The lysosomal explanation for death of uninjured cells is less commonly cited in the cell trauma literature today than it was during the 1970s,[13,14,22] and other theories, principally those related to the mitochondria, have been cited more commonly.[13,14,16,22,40]

Denaturation Mechanisms. Denaturation mechanisms[13,22,41] for cellular death were first espoused in the 1960s and still have many valid components. The most important of these is that before a cell dies, its proteins begin to denature,[41,42] and this denaturation results in a loss of cellular function and eventually cellular death. Much of the contemporary literature in this area focuses on cytoskeletal protein denaturation[13,16,22,42] causing a loss of cellular structure and organization, whereas some of the early literature[22,41] focused on denaturation of key enzymes in energy metabolism. Denaturing of cellular proteins certainly does occur,[13–16,22] and the resulting loss of some cellular functions almost certainly contributes to cell death, but denaturation in isolation does not entirely explain cell death. In fact, protein denaturation leading to cell death is more likely a by-product of another lethal process instead of an isolated explanation for cell death.[22] Cellular proteins do not denature spontaneously without some cause. For example, there is certainly evidence that the pH of the cell drops with metabolic failure[13,22] and that this change in pH leads to the denaturing of a number of proteins.[22,41,42] This denaturing can easily alter or eliminate some cell functions. Similarly, the drop in pH leads to the activation of a number of proteolytic enzymes (eg, acid hydrolases).[13,14,22,45] These enzymes can degrade cellular structures (as in the lysosomal theory) and result in cell death. In such a case, the loss of cellular functions and the enzymatic degradation of cellular components would both contribute to the death of the cell. This is an example of overlap among theories.

Membrane-Permeability Mechanisms. In membrane-permeability mechanisms,[13,14,22,25,43,44] changes in membrane permeability are thought to lead to oncosis (cellular swelling) that eventually causes the cell to burst or alters a cell's homeostatic mechanisms.[13–16,22,26] Membrane-permeability changes associated with a loss of function of membrane ion pumps and voltage-gated ion channels would pose a very severe challenge to the cell, and the resulting uncontrolled influx of ions could lead to cellular death. Originally, sodium and potassium were thought to be the primary villains,[13,22,43] and this view is reflected heavily in Knight's description of secondary hypoxic injury.[6,11] The importance of the sodium-potassium-ATPase pump in cellular survival cannot be overstated; in fact, most cells spend more than 30% of their energy on fueling this pump.[47] However, more contemporary thought suggests that increased calcium permeability also plays a major role in cell death.[13–16,22,24,31,43] Increased calcium permeability leads to activation of phospholipases that cause phospholipid membrane disruption and cellular death.[13–16,22,43] Similarly, after trauma, inhibition of calcium-dependent proteolysis reduced the overall increase in muscle atrophy by 18%.[24]

Of course, the failure of membrane pumps, the concomitant increase in intracellular concentrations of sodium and calcium, and their consequence of cellular oncosis do not happen in isolation. The membrane ion pumps and cell membranes fail for a reason, because of a lack of fuel,[11,13,14,22] a loss of the transmembrane sodium gradient that typically drives calcium transport out of the cell,[13,43,48] or degradation of the ion-transporting proteins through denaturing,[22,41,42] enzymatic cleavage,[13,14,22,39] or other mechanisms. Again, as was the case with the denaturation theory, these varied causes are evidence that this theory does not typically describe events in isolation but instead that there is some overlap among the theories.

Mitochondrial Mechanisms. Lane et al[49] suggested that mitochondria have become some of the most heavily studied organelles since they were identified as the power plant of the cell in 1940. Mitochondrial explanations for cell mortality* are perhaps the most commonly cited mechanisms today, holding that progressive mitochondrial injury leads to metabolic inadequacy in the cell and that this loss of mitochondrial function is one of the leading initiators of cell death. It is very likely that metabolic failure through mitochondrial damage is one of the most important causes of the other lethal pathophysiologic processes that we have identified. This is evidenced by researchers[22,28,37,38,40] who, using electron microscopy, have identified specific changes in the appearance of the mitochondria, including mitochondrial swelling and blebbing of the mitochondrial membrane. These morphologic changes have been classified into 6 grades[22,40] and are thought to correspond to mitochondrial damage.[14,22] These mitochondrial changes are among the earliest indicators of lethal cell injury.[13,14,16,22,31,40,50]

The timing of these mitochondrial changes is the most probable reason why this theory is commonly cited. Because mitochondrial changes appear to happen before cellular oncosis, loss of cytoskeletal structure, or loss of cell membrane fluidity,[14,22,50] it is very likely that the mitochondrial changes initiate or contribute to these other changes.[13,14,16,22,31] As mentioned herein, none of the theories about the mechanisms for cellular death are adequate in isolation, and they all appear to have some degree of overlap. It appears that insufficient ATP, resulting specifically from mitochondrial failure, may be the trigger for many of the other mechanisms contributing to cell death.[22,40]

Knight[6,11] spoke to the link between inadequate ATP and cell death in the secondary injury model. In his model, however, he primarily attributed the lack of adequate ATP to the hypoxia-induced shift from aerobic to anaerobic metabolism. Although this is most certainly true, as mentioned herein, the more recent view is that the lack of ATP is one of several concurrent lethal mechanisms and that hypoxia is only one of several causes for the lack of ATP. It appears that the lack of ATP also probably results from the failure of the mitochondria itself[13,14,22,31,50] and not just from the shift from aerobic to anaerobic metabolism.

Unfortunately, there is much we do not know about mitochondrial injury that occurs specifically in skeletal muscle or connective tissues. First, most of the studies of mitochondrial injury have been performed on organ tissues. Second, many of the investigators have used a complete ischemia model. Third, many of these researchers have not directly examined flux through metabolic pathways but have instead centered on morphologic appearance[40] or dye exclusion tests[52] (dead cells cannot prevent dye from diffusing through their cell membranes).

*15, 19, 22, 27, 28, 31, 40, 48, 50, 51.

Causes of Mitochondrial Injury

The progressive failure of the mitochondria appears to be a consequence of several different mechanisms.[13,14,22] Three of the most commonly accepted of these are hypoxic or ischemic,[22,28,37,38,40] oxidative or reperfusive,[13,14,22,28,50,53] and calcium-initiated mechanisms.[14,22,43,48,54]

Hypoxic or Ischemic Injury. Several authors[13–16,19,22,27] suggested that mitochondrial failure is commonly caused by hypoxia and ischemia, and much of what is known about this model has been learned through ischemia studies.[22,28,37,38,40] During ischemia, the mitochondria no longer have oxygen available to serve as the terminal receptor for electrons in the first phase of the oxidative phosphorylation pathway. As a result, flux through this pathway is severely limited or ends altogether, and the oxidative production of ATP ceases to be adequate for mitochondrial or cellular homeostasis.

The mitochondrion itself appears to be among the first organelles adversely affected by the hypoxia-induced lack of ATP.[13,14,22,50] Mitochondria are thought perhaps to be the descendants of archaic bacteria that infected primal cells, and they retain many of their own basic cellular mechanisms, including their own DNA, their own mechanisms for transcription and translation, and their own membrane ion pumps.[22,55,56] Failure of these mitochondrial ion pumps during hypoxia is thought to be one cause of the early and obvious changes in appearance of the mitochondrial membrane during hypoxia.[22,28,37,38,40] Damage to these pumps and (more so) to the mitochondrial membrane may be the result of the hypoxia-induced activation of a number of proteases and phospholipases.[14] Progressive failure of the mitochondrial membrane hastens the progressing failure of the metabolic machinery within the mitochondrion,[14] leading to ever-increasing problems for the entire cell.

In addition to the metabolic failure of the mitochondrion during hypoxia, a number of proteins, including heat shock proteins, are expressed.[22,37,57] These stress proteins are used internally by cells (ie, not secreted)[22] and are expressed as a result of virtually any form of cellular stress.[13–16,22,37,57] Although these proteins, particularly those in the heat shock protein 70 family, have many roles in attempting to ''save'' malformed or damaged cellular molecules, others (eg, ubiquitin) play a key role in tandem with proteasomes in degrading intracellular molecules whose damage is beyond salvage.[22,45,46] Hypoxia-induced expression of ubiquitin and activation of proteasomes may play important roles in protein catabolism within the injured cell.

Oxidative or Reperfusive Injury. Although hypoxia is certainly capable of causing mitochondrial damage, most authors agree that more damage actually occurs after the return of perfusion and oxygen to the previously hypoxic area (ie, during reperfusion).[13–16,22,44,57,58] Reperfusive injury is caused by the action of free radicals and is, therefore, often referred to as oxidative injury. The biochemical pathway for reperfusive injury has been well described[13–16,22,37,50,57] and involves the production of oxygen-derived free radicals, unstable and extremely reactive molecules with an unpaired electron in their outermost orbit.[22] In biological systems, free radicals are produced in 2 different ways.[59] The first is related to the impact of radiation and has little importance in this review. The second is electron transfer involving transition metals (usually Fe[II] or Cu[II]) or enzymes.[13,22,44,59] In the cell, one of the principal locations for the enzymatic-transition metal pathway

for creation of oxygen-derived free radicals is the mitochondrion.[22,28,53,59] As potent oxidizers, free radicals irreparably react with lipids, proteins, carbohydrates, and even DNA molecules, causing a conformational change that alters the function of these molecules, in many cases completely disabling them.[13,22,50] Lipids are prime molecular targets of free radicals, making cell and organelle membranes particularly vulnerable to attack and lipid peroxidation.

During posthypoxia reperfusion, an unusually large quantity of oxygen-derived free radicals is produced.[22,28,44,50,53,59] Because a larger quantity of free radicals is produced than can be combated with our normal antioxidant defenses, tissue damage results.[22,28,44,59] One of the primary sites of reperfusive free-radical damage is the mitochondrial membrane,[53] damage to which leads to a loss of functionality of the mitochondrion.[22,28,50,53] Reperfusive injury may also be exacerbated by a nitric oxide–mediated postinjury period of vasodilation or hyperperfusion.[58]

Calcium Influx Injury. During the past decade, the role of calcium ions in both mitochondrial and cell injury has been examined in detail.[14,22,31,43,48,54,60–63] At one time, it was thought that an influx of calcium ions was primarily part of the oxidative injury pathway involving hydrogen peroxide, but calcium influx injury has subsequently been shown to also be a separate threat over and above its role in oxidative injury.[43,61] Also, calcium influx plays an important role in apoptosis-induced cell death.[62,64]

Under normal homeostatic conditions, the intracellular concentration of calcium is very low (less than 10^{-7} mol/L).[22,47] Under pathologic conditions, however, as cytosolic calcium levels increase as a result of various pathophysiologic and second-messenger mechanisms, the endoplasmic reticulum and mitochondria act as calcium sinks, whereby a complex array of calcium transporters allows them to absorb large quantities of calcium ions.[22,51,60,63] Because calcium is a key regulator of mitochondrial enzymes,[31,58,61] increased mitochondrial calcium poses a number of challenges to mitochondrial function, including activation of calcium-dependent proteases and phospholipases.[13–16,22,43] These disruptive enzymes may not be the greatest threat to mitochondrial function, however. Bernardi et al[31,60,63] have shown that an increase in mitochondrial calcium leads to opening of the permeability transition pore, an inner mitochondrial membrane channel, which is thought to lead to membrane depolarization, osmotic swelling, and outer membrane rupture,[31] which would amplify the apoptotic death cascade.[64] Clearly, calcium plays a major role in mitochondrial causes of cell death; however, the mechanics of calcium's role in traumatic injury are not well defined. It is possible that secondary injury after musculoskeletal trauma may be triggered through apoptosis, as appears to be the case with burn injuries.[35]

What About Exercise-Induced Muscle Damage?

In addition to these lines of research regarding cellular injury, there is also a body of literature[54,65–67] describing exercise-induced muscle damage (referred to as *delayed-onset muscle soreness* in older literature). Exercise-induced muscle damage, typically caused by excessive eccentric muscular exercise, leads to significant ultrastructural changes within skeletal muscle, particularly to the cytoskeletal structural proteins.[65–67] Much of this damage is thought to be directly related to mechanical stress during the exercise.[65,66] Additionally, some evidence suggests that mitochondrial calcium may play a role in the muscle damage.[54] Although this research is progressing at a great rate, exercise-induced muscle damage theory may not be terribly applicable to other forms of injury in muscle tissue. The inflammation that accompanies exercise-induced muscle injury appears to be somewhat different than that observed with crush injury and other direct trauma.[68,69] Similarly, the progression of injury and the subsequent repair appear to be different from that seen with other types of injury.[40,43,66–69]

Examining Injury Sequelae

If cryotherapy and other short-term interventions are indeed effective at reducing metabolic demand and, therefore, altering the sequelae of injury, then we should be able to quantify this phenomenon experimentally. Researchers concerned with limb replantation have made a related attempt.[38] Using amputated cat hind limbs stored at temperatures of 22°C, 15°C, 10°C, 5°C, and 1°C, Sapega et al[38] quantified ATP and phosphocreatine levels using phosphorus 31 nuclear magnetic resonance. They reported that, with the exception of 1°C, lower temperatures resulted in better ATP sparing and there was no difference between storage at 5°C and 10°C. With storage at 1°C, tissues used more ATP than limbs stored at higher temperatures. Therefore, it would appear that when tissues are cooled to 1°C, stimulation of some ATP-degrading process may occur and there is a limit to desirable tissue storage temperatures.[38] Unfortunately, because Sapega et al only studied amputated, nonperfused limbs and only at temperatures of 22°C and below, it is difficult to generalize the findings of this study to other cryotherapy treatments. This is particularly true of treatments to thick muscular areas, in which in vivo temperatures in humans during cryotherapy rarely fall below 20°C.[1,6] There may be slightly more applicability to superficial ligamentous tissues, in which temperatures during cryotherapy do reach the range studied by Sapega et al.[6,70]

A second avenue into examining mitochondrial injury after trauma has involved examining the flux through the oxidative phosphorylation pathway.[5,28] When the mitochondria are damaged, the activity of mitochondrial enzymes is diminished.[5,21,28] Altered oxidative phosphorylation suggests an inability to produce adequate ATP by aerobic means.[5,28,51] Because so many of the pathophysiologic mechanisms that lead to cell death depend on mitochondrial function and ATP supply, this appears to be a promising technique for studying injury intervention and secondary injury. Although this approach holds some promise, in most of the literature on this technique,[28,40,54] researchers have used an ischemia model, which may not relate well to most musculoskeletal trauma. To date, only one group has applied these techniques to acute musculoskeletal trauma.[5] Merrick et al[5] demonstrated that 5 hours of continuous cryotherapy inhibited the loss of mitochondrial oxidative function that follows crush injury (Figure 2). It should be noted that these findings were limited to continuous cryotherapy for 5 hours. The effect of intermittent cryotherapy for other durations has not been examined.

A third avenue into examining secondary injury has been adopted by Fisher et al.[24] They examined postinjury protein catabolism, frequently reported as posttraumatic atrophy. By using novel inhibitors of catabolic pathways, they attempted to identify the specific causes of the postinjury protein breakdown. They reported that immune cells, through digestion by lysosomic enzymes (cathepsin B), play a significant role in the break-

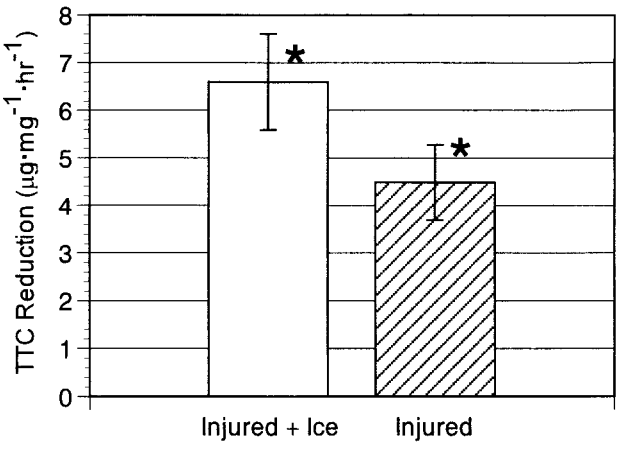

Figure 2. Cryotherapy inhibits the loss of mitochondrial oxidative function following crush injury. Reprinted with permission from Merrick et al.[5] TTC indicates triphenyltetrazolium chloride.

down. Inhibiting these enzymes resulted in a 44% decrease in protein catabolism. Similarly, they reported that selectively inhibiting calcium-activated proteases reduced protein catabolism by 18%, suggesting that calcium also plays a significant role.

CONCLUSIONS

The secondary injury model, dominant since its introduction more than 25 years ago,[11] is based on the modulation of the sequelae of musculoskeletal injury that is observed with cryotherapy. Knight[6,11] described these sequelae in terms of primary and secondary injury and framed these concepts around the most common pathologic models at the time. Although this theory is still strong and has proven to be largely correct, some tenets of the theory require reconciling with current literature.[22,39,40,43] The original theory meshed well with lysosomal and membrane permeability explanations of cell death. However, there are several limitations to these explanations, and several more recently described mechanisms need to be integrated into this injury model. When we examine the literature concerning the events leading to the death of the cell, it is clear that the mitochondria can play a major and early role. Mitochondrial function can be impaired by a number of mechanisms, although the role of these mechanisms in musculoskeletal trauma has yet to be examined.

Clinical and Research Implications

This review has primarily examined acute injury pathophysiologic theory rather than clinical practice. The omission of an examination of clinical techniques was not an oversight. The truth is that there is a conspicuous absence of research directly examining the efficacy of our clinical treatments on the cellular pathologic mechanisms associated with injury. Similarly, most of the pathophysiologic mechanisms identified in this review have yet to be examined in a musculoskeletal injury model at all. If we directly examine secondary mechanisms for cell death in future research, we may be able to specifically determine the efficacy of treatments, such as cryotherapy, on these secondary mechanisms. Moreover, we may be able to determine the specific treatments to produce the best suppression of secondary injury. For example, we do not currently have direct evidence of the best tissue temperature, treatment duration, or application pressure to suppress secondary injury after musculoskeletal trauma.

Knight's secondary injury theory[6,11] has been the cornerstone for cryotherapy research and for acute management of musculoskeletal injury for some time. As solid as this cornerstone has proven to be throughout the years, a cornerstone is not enough. We need to build up the rest of the structure around the cornerstone in the hope that better understanding of the pathophysiologic mechanisms may allow us to make meaningful improvements in the way we manage acute injuries. One area that may prove to be a valuable building block is examination of mitochondrial and metabolic function after trauma.[5] A second is the postinjury atrophy in muscular tissues.[24] A third is the role of the immune system in posttraumatic atrophy.[24] This area is particularly interesting because we may well learn that immune cells and other immune processes are a significant cause of secondary injury through direct means such as lysosomal digestion[24] or perhaps indirect means by inducing apoptosis, as is the case with burn injuries.[35] However, until we begin to examine these mechanisms in our research and teach them to our students, we will not know whether we can improve on the way that we treat acute injuries.

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