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REVIEW ARTICLE

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The role of free radicals in cold injuries

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Abstract Cold injury is a tissue trauma produced by exposure to freezing temperatures and even brief exposure to a severely cold and windy environment. Rewarming of frozen tissue is associated with blood reperfusion and the simultaneous generation of free oxygen radicals. In this review is discussed the current understanding of the mechanism of action of free oxygen radicals as related to cold injury during rewarming. Decreased energy stores during ischaemia lead to the accumulation of adenine nucleotides and liberation of free fatty acids due to the breakdown of lipid membranes. On rewarming, free fatty acids are metabolized via cyclo-oxygenase and adenine nucleotides are metabolized via the xanthine oxidase pathway. These may be the source of free oxygen radicals. Leukocytes may also play a major role in the pathogenesis of cold injury. Oxygen radical scavengers, such as superoxide dismutase and catalase, may help to reduce the cold induced injury but their action is limited due to the inability readily to cross the plasma membrane. Lipid soluble antioxidants are likely to be more effective scavengers because of their presence in membranes where peroxidative reactions can be arrested.

Key words Cold injury and rewarming · Oxygen radicals · Lipid peroxidation

Introduction

Peripheral tissues cool more rapidly than the core tissues on exposure to cold. The limbs are generally more susceptible to the development of cold injuries. Both wet and dry cold conditions combined with a series of environmental factors produce local cold injuries. Environmental conditions such as temperature, precipitation and wind together with the activity of the individual, the duration of exposure, amount of protection and level of fitness, all contribute to overall susceptibility to cold injuries (Boswick et al. 1979). Hypoxia has been shown to play an important role in the reduction of blood flow to the extremities and therefore may modulate the cold injury (Durand and Martineua 1971). When the temperature of the extremities falls below approx. 10° C a transient vasodilatation may occur which allows a surge of blood flow to the area in the short-term. However, this phenomenon is followed by severe vasoconstriction leading to a reduction in blood flow to the extremities and tissue hypoxia. Tissue hypoxia may further render the limbs vulnerable to cold injuries. Structural and functional changes in the microvascular system may thus have an important bearing on recovery from cold injuries (Kulka 1965). Cold injury is often asssociated with irreversible cell damage. Cell death due to freezing and thawing of tissues at the time of cold exposure may be the primary cause of tissue death leading to cell necrosis (Weatherly – White et al. 1964).

On prolonged exposure to freezing temperatures, the cells become hyperosmolar and then freeze due to a shift of water from the intracellular to the extracellular space (Gadarowki and Esce 1973). If there is severe freezing, haemoconcentration, vascular stasis and microcirculatory insufficiency leading to tissue necrosis in the frozen part may follow (Bellman and Adams-Ray 1956). Furthermore, a shift of the haemoglobin dissociation curve to lower oxygen partial pressures will prevent the release of oxygen, leading to tissue hypoxia (Iyengar et al. 1990), producing an effect that is similar to ischaemia of the tissue vasculature. Rapid rewarming as a treatment measure for cold injuries and frozen tissues has been prescribed (Mills et al. 1960). Upon rapid rewarming, ischaemia is relieved and reperfusion takes place. Recent studies have indicated that the rewarming of frozen tissues is associated with the generation of oxygen-derived free radicals and may lead to further cellular injury (Iyengar et al.

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1990). The effect is similar to those described to occur during reperfusion of ischaemic tissues (McCord 1985). This review attempts to explain the mechanism of oxygen radical generation during rewarming of frozen and cold injured tissues and the role of various therapeutic measures against cold injury as related to oxygen-derived free radicals.

Classification of cold injuries

Cold injury may be differentiated into the following major categories: non-freezing (wet cold), freezing (dry cold), which are subsequently subdivided into localized and generalized injury (hypothermia). Non-freezing cold injuries include, in ascending order of severity, chillblains, pernio, and trenchfoot (immersion foot). Examples of freezing injury are frostnip, frostbite and high altitude frostbite. Hypothermia comprises whole body cooling, and may terminate in total body freezing. Non-freezing types of cold injury may be defined as tissue trauma produced by prolonged exposure to wet cold at temperatures above freezing (Francis 1984). Freezing cold injury is directly associated with very low temperatures, hypoxia and dehydration. This type of cold injury is very complex and occurs due to a direct freezing effect of cold on the exposed parts and due to anoxia resulting from circulatory insufficiency. Decreased circulation may lead to stasis in the tissue blood vessels and capillaries which may result in thrombosis. Thrombosis further aggravates the hypoxia, invariably leading to necrosis and gangrene demarcated by mummification or drying of the affected parts, all within a period of 2 to 3 weeks (Meryman 1957). If thrombus formation is prevented, the cold induced injuries, such as oedema and tissue loss, can be prevented to a significant extent (Mileski et al. 1993).

Both the severity of cold and duration of exposure are important for determining the magnitude of tissue damage (Manson et al. 1991). The rewarming of cold or frozen tissue exacerbates the cellular injury that has already occurred to some extent during cooling of the tissue due to volume expansion and ice formation. Such injury would also include microcirculation damage due to intravascular thrombosis. However, the significance of the rewarming injury may be obscured in the case of severe cold injuries e.g. frostbite. In such cases, revascularization can be largely inhibited due to vascular stasis and thrombus formation and rewarming injury thus becomes insignificant relative to severe cold injury. This is similar to the situation occurring when a tissue is subjected to severe ischaemic insult and reperfusion does not occur following ischaemia due to the 'non-reflow' phenomenon. Rewarming injury is of particular importance in the case of non-freezing cold injuries, because in such cases the tissues are subjected only to short-term ischaemia (Russell et al. 1993).

Mechanism for free radical formation during ischaemia and rewarming

Role of electron transport chain

In mitochondrial respiration, cytochrome oxidase is involved in reducing the oxygen to water without production of free oxygen radicals. The presence of oxygen at the terminus of the chain favours the maintenance of the carrier system in an oxidized state. During ischaemia, when oxygen supply is limited, the electron transport chain of the inner mitochondrial membrane becomes highly reduced and oxygen radicals may be formed. The ubiquinone-cytochrome *b* region of the electron transport chain is the major site of oxygen radical formation when mitochondria are in a maximally reduced state (Cino and Dalmaestro 1989).

Role of free fatty acid metabolites

Severe ischaemia is associated with failure of ATP-dependent ionic pumps, resulting in the influx of Na^+, Cl^-, Ca^{2+} and efflux of K^+ (Hochachka and Dunn 1983). One of the postulated consequences of calcium influx during ischaemia is the initiation of pathways involved in the breakdown of lipid membrane constituents and the accumulation of free fatty acids. With the onset of ischaemia, a decrease in phosphatidylethanolamine (PE), phosphatidylcholine (PC) and total phospholipids in muscle has been demonstrated to occur (Das et al. 1991a). The exact mechanism for the breakdown of lipid membranes is controversial. In an environment relatively poor in ATP, calcium influx may be responsible for the activation of phospholipase C with consequent breakdown of phospholipids in the cell membrane and the liberation of free fatty acids (Jennings and Reimer 1981). Activated phospholipase may further potentiate an ionic redistribution in the cell and mitochondrial membranes (Kettlecamp et al. 1971). Ischaemia may induce adenosine 3′5′-cyclic monophosphate (cAMP) dependent activation of phospholipase A_2. The cAMP-effect is increased by the release of K^+, adenosine and catecholamines, all of which increase with ischaemia (Traystman et al. 1991). Ischaemia has been shown to lead to a rapid increase in free fatty acids and an increase in arachidonic acid (Das et al. 1991a). Arachidonic acid readily intercalates into membranes and produces changes in the packing of lipid molecules (Klausner et al. 1980).

During rewarming, blood flow returns to the ischaemic tissues, and although necessary for tissue recovery, may also lead to additional tissue injuries. During the re-establishment of the circulation there is rapid utilization of free fatty acids, in particular, arachidonic acid. Arachidonic acid accumulated during ischaemia is metabolized upon rewarming via the lipoxygenase and cyclo-oxygenase pathways and has been shown to produce