Chapter

The Effect of Antioxidants on Ischemia-Reperfusion Injury in Flap Surgery

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Abstract

Flap surgery has wide use in plastic surgery in the closure of tissue defects. In spite of the major advances in plastic surgery in the past years, flap surgery is still associated with significant mortality. Ischemia-reperfusion (I/R) injury, which is a complex injury associated with flap blood flow, is one of the most important causes of flap failure. The main pathophysiology underneath I/R injury is associated with reactive oxygen species, which can be prevented by certain antioxidant applications. Antioxidants have been widely used in flap surgery and I/R injury previously. There have been a lot of articles showing positive effects of antioxidants on I/R injury. In this chapter, we focus the mechanism of I/R injury and how antioxidants can able to diminish the damage, moreover demonstrating the effect of certain antioxidants on I/R injury that has been investigated previously.

Keywords: antioxidants, flap, flap failure, ischemia, reperfusion

1. Introduction

Flaps have been increasingly used in plastic and reconstructive surgery for tissue defects. Although the success rate of flap surgery reaches up to 99%, complications can occur even in the most experienced hands [1]. Vascular blood flow insufficiency is the leading cause of partial or total flap losses [2]. Ischemia-reperfusion (I/R) injury is the most important reason of vascular insufficiency to considerate. It is also the remarkable cause of flap compromise and organ dysfunction during organ transplantation and free flap surgery [3, 4].

The ischemic area of flaps is generally more distal to the region of vascular supply. Even the ischemia occurs for a short time period, it can generate reactive oxygen species (ROS). The reperfusion period, which occurs after ischemia, causes the restoration of blood flow and oxygen influx in the ischemic tissue and can finalize in cellular, inflammatory, and metabolic changes in the living cell. These changes caused by free radicals execute structural and functional alterations in the cell and may contribute to tissue necrosis [5]. This process can be prevented or decelerated with the use of antioxidant drugs that decrease the toxic metabolites responsible for tissue damage. Because postsurgical I/R injury is one of the most important causes of flap failure, in this review, we aim to show the pathophysiology of I/R injury and how the antioxidants show their beneficial effects on flap salvage.

2. Pathophysiology of I/R injury

Tissue perfusion is the most important parameter of flaps. When it is interrupted for a period of time and abruptly restored, I/R injury occurs. As long as there is timely reperfusion, ischemia results in reversible cellular damage. However, restoration of blood flow after a period of time results in an incident whereby reperfusion ends up with greater tissue injury than that which is produced by ischemia itself. I/R injury is a complex interplay between biochemical, cellular, and vascular endothelial factors. Although the clinical sequelae are organ specific, it may also involve systemic inflammatory responses [6].

The tissue damage in I/R injury is like a double-edged sword and is divided into two parts: ischemia injury and reperfusion injury. Ischemic injury may initially cause hypoxia and hyponutrition. After prolonged ischemia, the metabolic products from cells are retained and cause metabolic acidosis. When the blood supply is reestablished, local inflammation and reactive oxygen species production increase. Those lead to an activation of neutrophils and a consecutive adhesion between granulocytes and endothelial cells causing segmental vessel occlusion in postcapillary venules, transendothelial leukocyte migration, nitric oxide (NO) depletion, and the release of tissue-damaging enzymes leading to secondary injury [7] Reactive oxygen species are potent oxidizing and reducing agents that directly damage cellular membranes by lipid peroxidation [8]. The cell response is dependent on the severity of total tissue injury [9]. Cell damage induced by prolonged ischemia-reperfusion injury may lead to apoptosis, autophagy, and necrosis (**Figure 1**). Cell survival systems (control ROS generation and cell damage) are activated in short duration of I/R injury [10]. Moderate I/R injury may cause cell dysfunction by autophagy and also activate recovery systems for survival [11]. But if damage is severe, cell death may be induced via apoptotic or necrotic pathways [12].

I/R injury has effect on the microcirculation of the entire flap because of the inflammatory process and the rise in ROS in the early stages of reperfusion [13]. For this reason, insufficient microcirculation usually occurs mainly in the distal parts of the flap, which is a common cause of partial flap necrosis.

As mentioned above, total flap necrosis is most often caused by thrombosis of the pedicle causing vascular insufficiency. Immediate revision of the anastomosis is crucial to reestablish blood flow. Nevertheless, such complications can increase

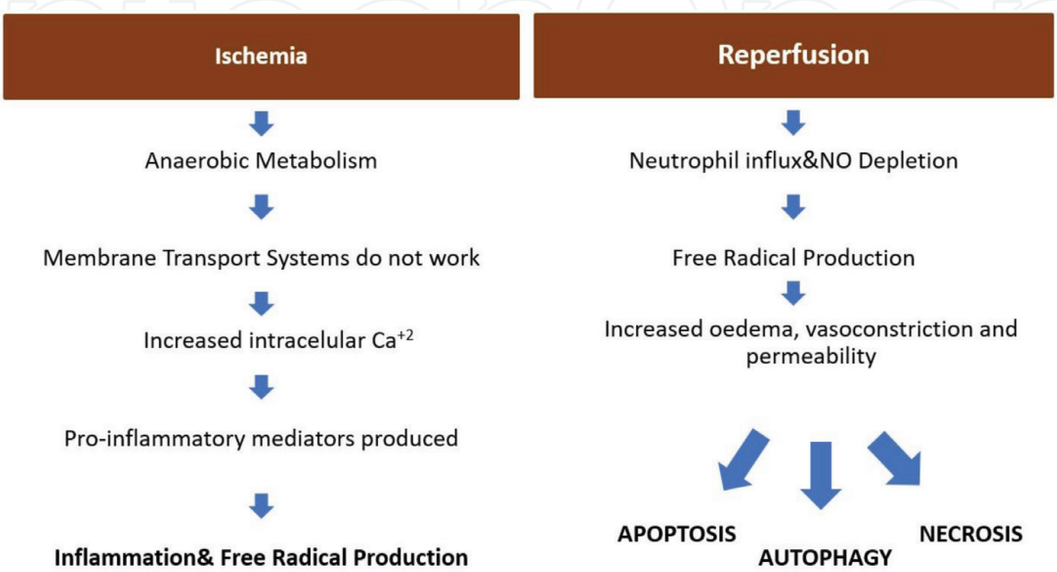

Figure 1.
Ischemia-reperfusion mechanism.

• Ischemic preconditioning
• Remote ischemic preconditioning
• Pharmaceutical preconditioning
• Thermic preconditioning
• Surgical delay
• Growth factors
• Extracorporeal shock waves
• Stem cells

Table 1.
Types of tissue conditioning.

I/R injury, which can lead to intravascular hemoconcentration, swelling of endothelium, increase in interstitial edema as well as inflammatory reactions because of the reperfusion injury. After a critical period, I/R injury can lead to a no-reflow phenomenon, which also leads to complete flap loss [14–16].

Several methods and techniques are described to protect flap from the dangerous effects of the I/R injury or minimize the stress during and after ischemia. Tissue conditioning, which is the most acceptable one, consists of preoperative, perioperative, and postoperative techniques to adapt the tissue to the ischemic stress. Multiple methods were described for tissue conditioning in **Table 1**.

Nitric oxide donation is another technique that NO administered through inhalation. NO plays a protective role via its antioxidative and anti-inflammatory functions [17], but it is not in common use in flap surgery and still remains experimental.

There are also studies aiming to prevent anti-inflammatory mediators released by leukocytes. Anti-leukocyte therapy limits leukocyte-mediated I/R injury and has focused on inhibition of inflammatory mediator release or receptor engagement, leukocyte adhesion molecule synthesis, or leukocyte-endothelial adhesion [18]. Many drugs act in this manner and have been shown to be very effective.

3. Free radical and antioxidant connection

Antioxidants that have been extensively studied in I/R injury are also found to be effective in various studies [19]. Because high amount of ROS is the primary supplement of the injury, it can neutralize the effect or prevent the mechanism from happening.

3.1 Free radical formation

Free radicals were discovered less than 50 years ago [20]. At first, they were assumed as completely harmful. Later on, advantageous biological effects of free radicals were reported. In recent studies, the role of free radicals is being researched commonly both in physiological conditions and in diseases. In molecular biology, a molecule that has an unpaired electron in its outer valence orbital that needs an extra electron to restore stability is called a free radical. They are short lived and highly reactive. The situation of instability because of the unpaired electron creates energy that has to be released instantly. In cell physiology, normal low levels of free radicals are used in autophagy, cell signaling, and antimicrobial oxidative bursts [21]. But, at higher free radical levels, the interaction with neighbor molecules

such as lipids, proteins, and DNA for releasing the energy causes damage. So, free radicals are the products of normal cellular metabolism. In order to reach stability, an electron has to be stolen. The attacked molecule engages itself in a chain reaction to steal an electron from another molecule.

In aerobic organisms, oxygen free radicals launch autocatalytic reactions that finally damage the living cell. The unsaturated carbon-carbon double bonds in the exposed end groups are particularly sensitive to free radicals forming a covalent single bond at a carbon atom to form a free radical at the opposite carbon atom [22]. Free radicals interact with molecular cross-linking for increased structural organization by reducing the transport of oxygen. ROS can be produced from endogenous or exogenous sources. Endogenous ROS is produced in different cellular organs where oxygen consumption is high such as mitochondria, peroxisomes, and endoplasmic reticulum. Most of the intracellular ROS are derived from mitochondria. The amount of free radicals is determined by many factors. In periods of irregular hypoxia in mitochondrial energy synthesis, excess electron production can develop free radicals that can damage lipids, proteins, and greatly increase molecular size in increasing vicious cycles to further reduce oxygen availability for mitochondria during energy synthesis. Another major type of free radical in a living cell is reactive nitrogen species (RNS). Nitric oxide (NO) radical is formed by the enzyme nitric oxide synthase and involves in smooth muscle relaxation and various other cGMP-dependent functions [23].

Free radicals are prominent in many pathological conditions such as cancer, diabetes, cardiovascular diseases, neurodegenerative diseases, cataracts, asthma, rheumatoid arthritis, inflammation, burns, intestinal tract diseases, progerias, and ischemic and postischemic pathologies. In particular, ROS is substantial for the pathogenesis of atherosclerosis. Low density lipoprotein (LDL) accumulates within plaques and contributes to the inflammatory state when ROS concentration is high and ROS oxidizes neighbor LDLs [24]. Also, it is believed that aging is a process mediated by free radicals [25]. At the present time, each chemical step has been investigated meticulously in order to prevent cell damage and clarify free radicals.

Different reactive oxygen species are formed in biological tissue (**Table 2**). Superoxide radical (O_2^-) can be formed by adding an extra electron to the oxygen molecule. Hydroxyl radical (·OH) can be formed in two different ways. First, it can be formed from O_2^- and H_2O_2 with a reaction catalyzed by a metal such as iron (Fe). Second, it can be formed from singlet oxygen ($^1O_2$) reaction. Moreover, oxygen free radicals may also be formed by polymorphonuclear leukocytes in ischemic tissue.

3.2 Human body defense mechanisms

Although accumulation of this substances are harmful to cell viability, it is important to know that human body is equipped with a defense system consisting of several antioxidative enzymes, to fight with these ROS. Superoxide dismutase

Reactive oxygen species (ROS)	
Superoxide radical (O_2^-)	$O_2 + e^- \rightarrow O_2^-$
Hydroxyl radical (·OH) 1st	$2O_2^- + 2H \rightarrow O_2 + H_2O_2/Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + OH^- + \cdot OH$
2nd	$O_2^- + H_2O_2 \rightarrow OH^- + \cdot OH + {}^1O_2$

Table 2.
Reactive oxygen species.

(SOD), glutathione peroxidase, glutathione reductase and catalase are some of these enzymes. SOD catalyzes dismutation reaction where O_2^- transforms into O_2 + H_2O_2 molecules. Catalase (heme-containing enzyme) also catalyzes the H_2O_2 reaction. H_2O_2 can also be reduced by glutathione peroxidase (GSH-P) which is selenium dependent enzyme, transforms reduced glutathione to oxidized glutathione. After that reaction oxidized glutathione is transformed into reduced for with help of nicotinamide adenine dinucleotide phosphate (NADPH). Additionally, NADPH regenerated from glucose 6-phosphate catalyzed by the enzyme glucose 6-phosphate dehydrogenase (**Table 3**).

The biochemical reaction of glutathione (GSH) is crucial. An intermolecular disulfide non-radical end product, glutathione disulfide (GSSG), is formed, which can either be exported from the cells or transformed back to glutathione by the combined action of glutathione reductase and the NADPH cofactor. Glutathione can react directly with ROS and RNS by its thiol group; also, it can aim the disulfide bridges formed inside and between proteins by the action of free radicals [26].

3.3 Antioxidants

Antioxidants are molecules against free radicals and are capable of securing or deactivating free radicals before damaging the cells. There are many antioxidant systems that work synergistically with each other to protect the body's organs and organ systems against free radical damage. There are highly complex enzymatic and non-enzymatic antioxidants: the enzymes such as SOD, glutathione peroxidase, and catalase, as well as non-enzymatic compounds such as α-tocopherol (vitamin E), β-carotene, ascorbic acid (vitamin C), and glutathione. Referred enzymes aim free radicals to delocalize their proteins into side chains and peptide bonds. Also, antioxidants may be endogenous or exogenous, such as part of a diet or dietary supplement. As we know aging is related to free radicals, nutrients rich with antioxidants contend with aging. Under oxidative stress, endogenous antioxidants may not be sufficient and dietary antioxidants may be required to maintain optimal cellular functions. According to literature, exogenous antioxidants comprise the secondary defense system against oxygen free radicals. Moreover, it is believed that ischemia-reperfusion is associated with generation of excess amounts of reactive oxygen species, the removal of which is beyond the capacity of the existing antioxidant defense system [19]. So, contribution of secondary defense system is crucial for the injury associated with ischemia-reperfusion.

Some dietary compounds that do not neutralize free radicals but increase endogenous activity can also be classified as antioxidants. An antioxidant should eliminate free radicals and be absorbed easily, and chelate redox metals at physiologically

Human body antioxidative enzymes	
Superoxide dismutase (SOD)	$2O_2^- + 2H \rightarrow O_2 + H_2O_2$
Catalase (heme-dependent enzyme)	$2H_2O_2 \rightarrow 2H_2O + O_2$
GSH-P (selenium-dependent enzyme)	$2GSH + H_2O_2 \rightarrow GSSG + H_2O$
Glutathione reductase	$GSSH + NADPH + H^+ \rightarrow 2GSH + NADP^+$
Glucose 6-phosphate dehydrogenase (G6PD)	Glucose 6-P + $NADP^+ \rightarrow$ gluconate 6-P + NADPH + H^+

Table 3.
Antioxidative enzymes and catalyzed reactions in human body.

relevant levels. Redox-active metals are involved in the generation of free radicals by binding strongly. It should also work in both aqueous and membrane domains and affects gene expression in a positive way.

It is a fact that all of the reactive oxygen species are formed in human body constantly but destroyed by these endogenous antioxidative mechanisms with the help of these enzymes. Endogenous antioxidants are products of the human metabolism. Except the ones that we mentioned before, human body have numerous different antioxidants. Alpha-lipoic acid (ALA), coenzyme Q, and melatonin are some of them. Alpha-lipoic acid is a disulfide derivative of octanoic acid and cysteine and a type of thiol antioxidant. ALA has significant functions such as scavenging free radicals, metal ion chelation, and antioxidant recycling. Coenzyme Q is the only lipid soluble endogenous antioxidant. It transfers electrons from complexes I and II to complex III within the mitochondria. Melatonin is produced in the pineal gland that is an indoleamine neurohormone. It has many physiopathological functions. One major function of melatonin is about oxygen metabolism to scavenge free radicals.

3.3.1 Redox homeostasis

ROS production and antioxidant capacity are in balance during the stable state of a cell and it is called "redox homeostasis" [27]. This stable state has to be reestablished in temporary derangement (**Figure 2**). When ROS concentration is detected to be high, gene expression is engaged for antioxidant activity. Signal cascades increase the amount of intracellular glutathione and other potent ROS scavengers. Thus, redox homeostasis is sustained. Another regulatory mechanism is feedback inhibition. Production of NO inactivates NO-producing enzyme, NOS. There are many physiological redox-responsive signaling pathways regulated by NO or ROS. The concentration level is modified by the balance between antioxidants and free radicals. If free radical production becomes uncontrolled, aging and diseases occur eventually.

Some major exogenous antioxidants are vitamin C, vitamin E, carotenoids, and polyphenols. Diet is the main source for exogenous antioxidants, especially fruits, vegetables, and grains [28]. Endogenous and exogenous antioxidants act in coordination in order to reach homeostasis. Vitamin C (ascorbic acid) involves in the reaction where intracellular glutathione levels raise thus protects protein thiol group against oxidation. Also, it works in cooperation with vitamin E and the carotenoids. α-Tocopherol is the most active form of vitamin E and is a membrane-bound antioxidant. The main function of it is to protect cell membrane against lipid peroxidation. Carotenoids are pigments known as the protector of plants against photooxidative processes. They contain conjugated double bonds, and in the human organism, their antioxidant activity arises due to scavenging singlet molecular oxygen and peroxyl radicals. There are many studies exhibiting that carotenoids protect the skin against photooxidative damage. Polyphenols are found in blueberry, and they facilitate increased neuronal signal transduction [29].

As previously described, the formation of high amount of ROS is the primary supplement of ischemia-reperfusion injury. Antioxidants can neutralize the effect or prevent the mechanism to develop. Considerable clinical and experimental data support the role of oxidative stress in I/R injury and emphasize the importance of antioxidant defense mechanisms in tissue protection [30]. In previous studies, it is shown that concentration of some antioxidants such as glutathione and uric acid decrease in response to ROS burst during skin flap ischemia-reperfusion [31]. So, supplementation of the antioxidants at I/R injury may help to neutralize the oxidative stress and reinforce tissue tolerance to reactive oxygen species [32].

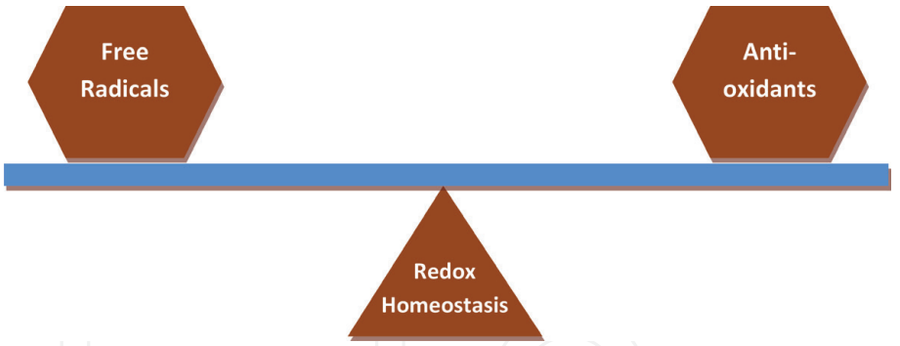

Figure 2.
Redox homeostasis.

4. Main antioxidants and effects on flap surgery

Most of the antioxidants have been proved to be beneficial versus I/R injury, with the exception of only few [19]. There are several important antioxidants that are investigated in I/R injury in previous studies as mentioned below.

5. α-Tocopherol

α-Tocopherol is the most popular one among the antioxidants; so, numerous studies were written about the positive effects of α-tocopherol in I/R injury. It can be found on foods, and also used in cosmetic and pharmaceutical industries. It is the bioavailable component of vitamin E, and appropriate consumption is considered to help to diminish risk of many chronic diseases associated to oxidative stress [33].

It is a lipid soluble antioxidant and stabilizer of membranes, has been found to decrease myocardial I/R injury and reverse contractile dysfunction by inhibition on cellular Ca^{2+} accumulation and reduce lactate dehydrogenase (LDH) release [34, 35]. Hydrophilic analog of α-tocopherol called Trolox has been studied before, and beneficial effects were shown on liver I/R injury [36].

According to Franch et al. [37], the α-tocopherol was compared with control group on rat hepatic I/R injury model. The SOD after I/R period catalases after reperfusion period, and glutathione peroxidase in all periods showed lower activities than those of control group. Erkut et al. [38] came across similar results on their study too. In rabbit skeletal muscle I/R injury model, they found out superoxide dismutase, catalase, and glutathione peroxidase levels that show the cellular injury were lower in α-tocopherol group compared with control group.

5.1 Ascorbic acid (vitamin C)

Vitamin C, one of the most popular vitamins we have heard in everyday life, has powerful antioxidant effects too. Because of the potential benefits, people pay attention to consume certain amount of fruits and vegetables these days. Ascorbic acid can protect the endothelium from direct injury by oxidants (such as H_2O_2) and prevent microvascular dysfunction. Moreover, it is proven that administration of ascorbic acid helps to decrease I/R injury [39, 40].

Because of its beneficial effects, ascorbic acid has been used in hepatic, cerebral, and renal I/R injury models in the literature [41–43] before and demonstrated positive results for end-organ protection.

There are several studies related to the effect of ascorbic acid I/R injury skin flap model. Zaccaria et al. [44] demonstrated positive effects of ascorbic acid compared with control group in rat epigastric island skin flap model. They determined higher percentage of flap survival in ascorbic acid group. On the other hand, according to Yoshida and Campos [45], vitamin c and mannitol (antioxidant group) group did not prevent or reduce the necrosis area compared with control group in rat groin flap I/R injury model.

6. β-Carotene

Carotene is called a provitamin because it can be stored in the liver and converted into vitamin A when necessary. There are two main types of carotene: alpha-carotene (α-carotene) and beta-carotene (β-carotene). β-Carotene consists of two retinyl groups and is destroyed by beta-carotene dioxygenase in the small intestinal mucosa and transformed into retinol that is a type of vitamin A.

β-Carotene is also a lipid-soluble antioxidant as α-tocopherol, interferes with lipid peroxidation by clearing away singlet oxygen, and reacts with peroxy radicals. According to Kikugawa et al. [46], β-carotene plays a preventative role in the oxidative damage process. In the literature, beneficial effects of β-carotene were shown in I/R injuries in the liver, myocardium, kidneys, and ovaries.

Karabulut et al. [47] compared vitamin A with control group in rat epigastric island skin flap venous I/R injury model in rat. By the fact in their study, after reperfusion of flaps, surviving flap area was 16% in the control group and 90% in the vitamin A group, respectively. They also combined vitamin A and vitamin E, where it was 92%.

6.1 Glutathione

Glutathione is present in all mammalian cells and has a variety of cellular functions, including amino acid transport, the maintenance of sulfhydryl groups of proteins, and the protection against oxidizing molecules and electrophilic xenobiotics. It is a tripeptide composed of glutamic acid, cysteine, and glycine. Because of strong antioxidant features, it is very popular in media in terms of healthy nutrition.

Glutathione shows its functions by clearing away O_2^- and protecting thiol groups against oxidation so that it supplies cellular integrity. In order to protect the thiol groups of proteins, a relatively high concentration of GSH is necessary. Moreover, other free radical scavengers and antioxidants (such as α-tocopherol and ascorbic acid) converted to their reduced state by GSH. Pretreatment with exogenous GSH can provide protection against gross mucosal ischemia-reperfusion injury [48]. Also, it is shown that application of intravenous GSH reduces the myocardial infarct size and decreases postischemic left ventricular dysfunction [32].

On the other hand, according to Van den Heuvel et al. [31], I/R did not significantly alter GSH concentrations in their study. They had taken biopsies from 17 DIEP flaps at the different time of surgeries but there was no immediate change in GSH concentrations compared to the concentrations at the start of surgery. They explained this finding that because the skin is less sensitive to I/R than other tissues (such as muscle, liver, and fat tissue), it may prevent antioxidant defense reactions from occurring. That could be the reason for normal levels of GSH.

6.2 Coenzyme Q_{10} (CQ10)

Coenzyme Q_{10} is an organic, natural, fat-soluble, antioxidant, endogenous vitamin-like substance (similar structure to vitamins K and E). Also called ubiquinone,

it is an auxiliary factor in the intercellular electron transport chain. It has become one of the most popular nutritional supplements in recent years. It was shown in the literature as an effective antioxidant for the prevention of oxidative damage. What is more, CQ10 breaks down macromolecules to prohibit inflammatory responses [49]. Also, CQ10 is able to balance mitochondrial Ca^{2+}-dependent ion channels and prevents energy depletion in the cell [50]. In cardiovascular diseases, exogenous CQ10 has been widely applied as a dietary supplement, and it may be suggested as a therapeutic agent [51].

CQ10 has inhibitory effects for tumor necrosis factor-α (TNF-α), which may be responsible for muscle damage in I/R injury as well [52]. Moreover, it prevents the peroxidation of the cell membrane and subcellular lipids, which happens during I/R injury [53]. Hwang et al. [54] were proved that pretreatment with CQ10 had positive effects on spinal cord I/R injury and improved neurological function.

According to Ozalp et al. [55], who compared CQ10 with control group in rat inferior epigastric island flap I/R injury model, mean flap survival ratios were 88% in CQ10 group, markedly higher than the control group (51%). They also emphasized that CQ10 group had high levels of SOD and GSH compared with control group because of CQ10's antioxidant effect, which prevented lipid peroxidation during the initial phase (reduce GSH and SOD destruction).

6.3 Alpha-lipoic acid (ALA)

ALA is an antioxidant that is found in various foods and also can be synthesized in the human cells. It is also an endogenous short-chain fatty acid and a cofactor for multiple mitochondrial dehydrogenase enzymes [56]. ALA shows its antioxidant properties by the use of free radical scavenging, chelating with metals, increasing the reusability of other antioxidants, and repairing oxidative damage.

There have been a lot of articles written about ALA because of its wide range of antioxidant capabilities. The efficiency of ALA has been shown in atherosclerosis, diabetes mellitus, I/R injury, multiple sclerosis, and senile dementia [57].

In severe oxidative damage caused by I/R, levels of malondialdehyde (MDA) and nitric oxide (NO) increases [58]. ALA is capable of cleaning up MDA and NO within brain tissue [59]. Deng et al. [60] found out diminished MDA and NO levels in ALA group compared with control group in rat brain I/R injury model. They also discovered that ALA has enhanced the activities of total antioxidant capacity and SOD in rat brains. According to these findings, administration of ALA before skin flap surgery may have benefits to improve I/R ınjury.

6.4 Melatonin

Melatonin is a type of ethionamide that is secreted from the pineal gland, which determines the biorhythm. Moreover, it is an effective free radical scavenger and received significant attention because of its antioxidative feature. In spite of its ability to neutralize free radicals directly, also it has indirect effects, such as stimulating the activity of antioxidative enzymes (such as glutathione peroxidase and SOD) [61, 62].

Mitochondrion, which is an organelle for ATP production in the cell, has been proved to play critical roles in I/R injury. Therefore, the protection of mitochondrion can decrease I/R injury in vital organs [63]. Melatonin has been shown to restore the disturbance caused by I/R injury in mitochondria and has become a remarkable therapeutic strategy [64].

The effect of melatonin on I/R injury has been investigated in the literature for a long time. It has been shown that melatonin could be an effective neuroprotective agent for treatment of ischemic spinal cord injury [65]. Singhanat et al. [66]

explained that melatonin has cardioprotective effects against cardiac I/R injury. However, they also mentioned that the mechanism of the cardioprotective effects of melatonin were still unclear. Gurlek et al. [67] showed beneficial effects of melatonin in rat inferior epigastric flap I/R ınjury model. The determined melatonin replacement therapy causes reduction in I/R-induced flap injury.

7. Discussion

Vitamins with antioxidant properties such as vitamins A, C, and E have positive effects on I/R injury but they are not adequately effective when given alone. If they are used in combination with other vitamins and drugs with antioxidant properties, they work synergistically to decrease I/R injury. For instance, combined use of vitamin A and E in an animal model showed increase in flap survival with the help of their synergistic effect on reducing lipid peroxidation [47]. Also, vitamin E and iloprost (synthetic analog of prostacyclin PGI_2) combination demonstrated that they attenuate reperfusion injury more efficiently than their separate use, in skeletal muscle I/R injury model [68]. In addition to these, Kayiran et al. [69] showed that combined usage of vitamins C, E, acetylcysteine, and prednisolone alleviated the results of ischemia and enhanced the flap survival on free radial forearm flap. There is a wide range use of substances in the literature to prevent I/R injury, but there is no single ideal drug that can overcome this damage; so, we think that the use of combined therapy is more successful in achieving impressive results.

Because high amounts of ROS are produced in I/R injury, the amount of certain antioxidants decreases. Depletion of antioxidants does not always mean oxidative tissue damage has happened: it might simply indicate that the antioxidant system has removed the ROS and thereby consumed the present antioxidants to protect the tissues [70]. Tissue damage occurs if the ROS amount outruns the capacity of the body defense system. In other words, administration of antioxidants prior to flap surgery may help to avoid unfavorable effects of I/R injury.

Trolox equivalent antioxidant capacity (TEAC) is a measure that shows the capacity of the present hydrophilic antioxidants. In other words, it demonstrates the antioxidant level on a certain amount of tissue. According to Van den Heuvel et al. [31], TEAC concentrations was not differ 30 min later after reperfusion but there was an important decrease after 1 h of reperfusion. The TEAC concentrations were recovered in the next 1 h, showing us the body was replacing the antioxidants. These findings suggest that first hour after reperfusion is the most vulnerable period of flap to I/R injury and timing of antioxidant replacement may have an important role in preventing this injury too.

Lipids are usually primary target of free oxygen radicals in oxidative damage and lipid oxidation occurs when they exposed free radicals. Lipid oxidation is a process that generates many end products. Between these products, MDA, which is an aldehyde, is the most studied one among them [71]. Because it is the end product of the lipid oxidation pathway, measurement of MDA levels is a commonly used indicator of oxidative damage [72]. Also, MDA might help us to monitor the reaction of the body against the antioxidant support. In other words, if MDA levels are high, we can increase the antioxidant support to body in I/R injury.

Measuring MDA levels helps us to determine antioxidant therapy protocol. However, we cannot increase the dosage of antioxidants easily because they are not only antioxidant but also have prooxidant properties. Helpful effects of antioxidants are usually higher in the studies that were done in vitro comparing with the studies that were done in vivo. This fact is called “The antioxidant paradox” [73]. Many antioxidants can produce ROS at higher doses. But if they are administered at

even higher doses, they can generate massive amounts of ROS that the body cannot handle. So, they can worsen oxidative stress and cause necrosis. For instance, ascorbic acid, which is widely known as a powerful antioxidant vitamin in human body, can reduce most of the ROS and other radicals. On the other hand, it has been shown that it has a prooxidant act in humans even at a dose of 500 mg/day [74]. Moreover, according to Mendes-da-Silva et al. [75], ascorbic acid was served as an antioxidant on rats at 30/mg/kg/day but they were discovered opposite effects at 60/mg/kg/day. In light of these findings, since the use of antioxidants is like a double-edged sword, careful dose adjustment is required for use in I/R injury. Further studies are necessary in order to determine completely how they act.

The effect of antioxidants on I/R injury is still under investigation. There are also substances with antioxidant properties such as flavonoids [76], thioredoxin [77], propofol [78], ebselen [79], edaravone [80], etc. which also have positive effects on I/R injury in addition to the antioxidants described previously.

8. Conclusion

I/R injury is still the most challenging problem in flap surgery. Prolonged ischemia of the flap causes irreversible infarct and flap loss, and this increases the patient morbidity. Because free radical formation is a significant step in pathophysiology of I/R injury, the help of antioxidants on I/R injury is cannot be disregarded. There have been a lot of studies proving this fact so there is a need for further studies for a better understanding of their certain effects. In this chapter, we tried to show how the antioxidants affect the I/R injury and how effective their use is. In conclusion, although the efficacy of antioxidants is not yet fully understood, we think that they will increase their medical use in the future.

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