Risk Factors Influencing the Oxidative Stress in Surgical Therapy of Skin Grafts

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ABSTRACT Recent experimental data sustain that twenty-four hours postburn, mitochondrial outer membrane damage was progressively increased and cytosolic cytochrome-c gradually accumulated to approximately three times more, indicating impaired mitochondrial integrity. Maximal decrease of mitochondrial SOD activity occurred 8 hours postburn, maximal decrease in glutathione peroxidase activity persisted 2–24 h postburn. Lipid peroxidation increased, suggesting burn-induced oxidative stress. Administration of antioxidant vitamin therapy prevents burn-related loss of membrane integrity and antioxidant defense in mitochondria and prevents organic dysfunction. Lipid peroxidation could be measured in mitochondria, by means of malondialdehyde level, which assess oxidative stress. The mitochondrial outer membrane damage and cytochrome-c translocation estimate mitochondrial integrity, and activities of SOD and glutathione peroxidase evaluate mitochondrial antioxidant defense. We used this data for observing the burn wound healing correlated with oxidative stress and antioxidant therapy.

KEY WORDS burn trauma, oxidative stress, malondialdehyde, antioxidants, skin grafts

Introduction

Abnormalities in mitochondrial structure and function have been found to occur in tissular injury produced after thermal burns (1). Actual reports demonstrated that burn injury induce dysfunction in several animal models and in humans (2, 3). Recently, it was identified apoptosis and increased mitochondrial calcium levels in cell plasma and in burn serum (4, 5). Apoptosis has been associated with mitochondrial injury, such as opening of mitochondrial permeability transition pore and release of apoptotic factor cytochrome-c (6), since intramitochondrial Ca\(^{2+}\) homeostasis plays a significant role in the regulation of mitochondrial permeability transition pore (7). Oxidative stress could appear, firstly in mitochondria as a result of increased production of reactive oxygen species (ROS). The defense mechanisms, against ROS consist in releasing of intracellular antioxidant enzymes, such as: glutathione peroxidase (GPx), catalase (CAT), and superoxide dismutase (SOD) (8, 9). These enzymes maintain mitochondrial integrity, and protect cells from apoptosis. In burn patients, oxidative stress contributes to the development of distant organ injury or failure (10). In experimental and clinical studies, burn-associated oxidative stress was observed downregulation of antioxidant activities and the appearance of oxidative modifications of proteins and lipids in multiple organs (11). Administration of antioxidant vitamin therapy assured a postburn recovery of organ function and a good burn-related imunosuppression (12).

Pre- and postburn administration of an antioxidant regimen provided significant organ protection by attenuating inflammation (13). In the present study, we determined whether a cutaneous burn injury, produced oxidative stress, and impaired mitochondrial defense against ROS. To further investigate whether these factors contributed to postburn cellular defects, we next applied antioxidant vitamin therapy to burn-and examined status organic functions at special time points. We hypothesized that burn injury promoted mitochondrial damage, in different organs, subsequently contributing to the impaired of their function. This study was also directed to identify potential targets of therapeutic interventions that may improve cellular function in burn injuries.

Material and Methods

We realized our study upon: healthy subjects, and surgical patients with the similar burned body surface, from Plastic and Reconstructive Surgery Department, from the Emergency County Hospital of Craiova. The study was approved by Ethics Committee of UMF Craiova.

We selected 100 patients from Plastic and Reconstructive Department, Emergency County Hospital of Craiova (having their accept and the agreement of Ethic Committee of UMF. Craiova). We divided them into three groups, depending on
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body surfaces of burns (BSB) and degree of burns, as follows:

Subgroup A (I): 5% BSB degree 1-2 Control.
Subgroup B( II): 27% BSB degree 3.
Subgroup C (III) 45% BSB degree 3.

For each subgroup there was a different surgical technique applied such as:
- Subgroup A: toilette (cleaning and elimination of necrotic tissues) of wound, application of a sterile and moist dressing and outcome after three days.
- Subgroup B: excision of altered zones and application of skin grafts. These patients have need of blood administration (2 units: BU), during surgical intervention.
- Subgroup C: cleaning of wounds, debridation and expectative for tissues granulation.

For transfusions we used stored blood deplaketated and leukoreduced. We used for analgesedation: opioids, anti-inflammatory and hypnotics, the doses administrated, beeing adequate to the degree of pain, expressed by each patient.

For patients from group B, who needed blood transfusion, we associated nutritional support with arginine: 10-22 g/day, zinc 90mg and Vitamin C 500 mg. x 3/day after 48 hours after skin grafting. We added antioxidants necessary to block the oxidants elements brought by blood transfusion. (exogenous sources).

Criteria of evaluation of skin grafts healing:
- efficient healing: 90% after 7 days and 100% after 14 days, from the skin grafts application.
- inefficient healing after 6 weeks associated with reintervention for restauration of skin grafting.
- no healing and bad evolution after 2 months.

We evaluate the evolution of skin grafts, not only the local aspects of wounds, but verifying clinical status, presence of organic dysfunctions (calculating SOFA scores), level of hematocrit, and hemoglobin.

We noted clinical parameters, calculated SOFA scores, and correlated them with the values of hematological and biochemical parameters: hematocrit, leucocytes count, INR (obtained from Emergency County Hospital Craiova Laboratory ) and Superoxide dismutase, Malondialdehyde (from the Research Laboratories for oxidative stress). We selected Oczan Erel (14) technique for oxidative stress analysis.

Results

Clinical parameters:
Local macroscopic aspects of wounds:
Subgroup A: outcome after 3 days.

Subgroup B: After grafting, the ends of wound bring together: 80% cases developed an efficient healing; for 20% patients the duration of healing was longer, because of organic dysfunctions (13% cases) and infectious complications (7% cases).

Subgroup C: The ends of wounds were situated at a distance, and separated by large zones, with absence of substance. These kind of wounds had need of a long period of evolution to permit to the tissue to granulate for applying the skin grafts.

Clinical general manifestations:
Fever: 40 cases
Organic dysfunctions:
Pulmonary: tachypnea, cough, bronchitis: 24 cases
Renal: oligoanuria, 10 cases
Miocard: tachycardia, dyspneea, 6 cases.

Biochemical parameters:
Superoxide dismutase values

Malondialdehyde values

Figure No. 1 SOD values (U/ml)

Figure No. 2 MDA values (Micromol/l)
Discussions

Burn injury caused significant impairment of antioxidant defense in the site of burn trauma. Imbalance between ROS production and scavenging leads to oxidative stress, ROS mediated injury. Previous NMR spectroscopy findings the injury does not alter mitochondrial respiratory function (15). Recent results sustain that there are no changes in the activity and expression of cytochrome-c oxidase, belonging to the mitochondrial respiratory complex that means there is a little effect on ROS production in mitochondria. Rapid downregulation of mitochondrial SOD activity, and significant rise in lipid peroxidation, sustain the hypothesis that burn injury increases mitochondrial oxidative damage by decreasing ROS scavenging.

Antioxidant vitamin therapy preserved postburn SOD, suggesting that mitochondrial SOD activity is regulated by factors other than enzyme expression. This hypothesis is supported by several studies indicating posttranslational nitration of MnSOD that could lead to its inactivation (16).

Burn injury promotes lipid oxidation, not as a consequence of overproduction of hydroxyl radicals, but as a defect in antioxidant activities (9).

Antioxidant vitamin therapy in burns suppressed increased lipid peroxidation 8 and 24 h, but not 2 and 4 h, after burn injury. These data indicate that other enzymes may also contribute to the increased mitochondrial MDA after injury.

Recently, peroxiredoxin family proteins (Prxs) have been identified as antioxidant enzymes, which protect against hydrogen peroxide, peroxynitrite, and a wide range of organic hydroperoxides (17,18). These Prxs could be antioxidant enzymes involved in the regulation of lipid peroxidation in mitochondria during burn trauma. This hypothesis sustains the necessity of the administration of antioxidant vitamin therapy, to maintain mitochondrial integrity, after burn injury.

Conclusions

1. The most important pathological mechanism in burn patients is integral disturbance of O2 metabolism, in all its parts: bringing , transport, intake and utilization by cells, because of decreasing of erythrocytes number, of hemoglobin level and hipercoagulability in microcirculation.

2. Good effects of blood transfusions consist in O2 extraction by tissues and utilized in mitochondria.

3. Transfusions with blood stored about maximum 48-72 h. open the ways for an efficient wound healing, without complications.

4. Longer period of waiting for the applications of skin grafts more the leukocytes accumulation and increasing of their degranulation in regions of grafts. Intensity of oxidative stress is amplified by blood transfusions which determine oxidative lesions and death of healthy cells.

5. ROS overproduction increases the degree of oxidative stress and decrease of CAO, situation which could be considered as an indicator prognostic for wound nonhealing.

6. MDA concentrations, marker for lipid peroxidation, significantly elevated in plasma, and persist in postburn period (72 h), suggesting a prolonged oxidative damage, in group.

7. Antioxidant vitamin therapy prevented fall of ROS defense and oxidative damage in the region of skin graft. burn injury severely impaired SOD activity.

8. There is a relationship between skin grafts, cutaneous dysfunction and beneficial effects of antioxidant vitamins in maintaining cellular and tissular integrity and function.

9. It is necessary to find efficient and more sensitive therapeutic strategies to improve cutaneous function in grafts skin care.

10. Antioxidant vitamin therapy associated with zinc, promoted SOD activity 4, 8, and 24 h postburn. Antioxidant vitamin therapy prevents the installation of tissular dysfunction.

References


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