BURNS, ENDOTHELIAL DYSFUNCTION, AND OXIDATIVE STRESS: THE ROLE OF ANTIOXIDANTS

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SUMMARY. Background. Burns cause a systemic inflammatory response, endothelial dysfunction, and increased microvascular permeability which results in oedema being formed; these effects are probably the result of a complex interplay between the direct effects of heat on the microcirculation and the action of chemical mediators, including reactive oxygen species. The use of antioxidants can reduce these changes, which are considered a promised step in burns management. Patients and methods. Forty-eight burn patients of either sex and with varying burns percentages were involved in the study. They were each allocated to one of four groups: A, B, C, and D, each group composed of 12 patients. Groups B, C, and D were treated with antioxidants: allopurinol, melatonin, and N-acetylcysteine respectively, while group A was treated according to normal hospital policy, without antioxidants; 12 healthy subjects (group E) served as a control group for comparison. In each group, serum malondialdehyde and serum glutathione levels were measured and liver and kidney function tests were performed, as well as microalbuminuria tests, using standard methods. Results. The administration of antioxidants to burns patients produced significant improvements in the parameters studied compared to group A parameters (no antioxidant given). Conclusion. This study clearly demonstrates the role of reactive oxygen species in endothelial dysfunction occurring in burn patients and the beneficial effect of antioxidants in reducing it, as shown by the reduced microalbuminuria and reduced resuscitation fluid in antioxidant-treated burn patients; the study also supports newly emerging evidence regarding the use of microalbuminuria as an indicator for endothelial dysfunction in burn patients.

Introduction

The pathophysiological mechanisms underlying the development of oedema in burn patients are mainly due to the inflammatory response which activates cytokines, with subsequent stimulation of phagocytic cells. This results in the formation of reactive oxygen species (ROS) leading to lipid peroxidation; another source of ROS in burns is the enzyme XO, produced from xanthine dehydrogenase under ischaemic conditions producing ROS, which causes lipid peroxidation. 1 A close relationship has been demonstrated between the intensity of lipid peroxidation and postburn complications, and it has been possible to document the role of ROS leading to lipid peroxidation as a causative agent in the mechanism of local and systemic complications in burns, including increased vascular permeability. 2 The increase in vascular permeability leads to immediate and continuous loss of substances ranging from water to macromolecules. 2 Generalized vascular permeability to macromolecules has been described during surgery in animal models, and microalbuminuria following acute inflammatory insults such as surgery has been demonstrated in human models, leading to the concept that microalbuminuria is a reflection of systemic vascular endothelial dysfunction. 3 It was recently reported that monitoring microalbuminuria in the very early post-burn period had potential as a sensitive and rapidly responding marker of systemic endothelial dysfunction. 7 It was also found that increased microalbuminuria matched the severity of the insult and correlated with the degree of organ failure - microalbuminuria reflects the evolution of systemic capillary leakage and could be a useful parameter for assessing the effectiveness of therapies for reducing endothelial damage. 7 Various antioxidants are used to antagonize ROS: allopurinol, a well-known XO inhibitor; melatonin, a product of the pineal gland that has powerful antioxidant action through its direct scavenging effect and its stimulation of antioxidant enzymes; 8 and N-acetylcysteine, used in the past as a mucolytic agent that rapidly metabolized to cysteine, a direct precursor in the synthesis of intracellular glutathione (GSH), the natural antioxidant. 9 The involvement of these antioxidants in burns treatment may have improving effects on endothelial dysfunction and burn outcome in general.

Patients and methods

The study was carried out on 48 burn patients of either sex; the age range was 20-45 yr, and the burn per-
percentage varied from 15 to 40% total body surface area (TBSA), estimated according to the rule of nine.

The patients were allocated to four groups and treated as follows:

- Group A: 12 patients - this group was already present in the burns unit and treated according to standard hospital policy, by which antioxidants are not given
- Group B: 12 patients, treated with 100 mg/day allopurinol tablet
- Group C: 12 patients, treated with 3 mg/day melatonin capsule, taken at night
- Group D: 12 patients, treated with 500 mg/day N-acetylcysteine capsule

A fifth group, Group E, consisting of 12 healthy subjects in the same age range as the patients, served for control purposes.

All the treated groups received antioxidants in addition to other drugs prescribed according to the hospital drug policy.

Blood samples were collected from all subjects by venipuncture: 10 ml were taken on admission to the burns unit within the first 24 h post-burn, before starting drug treatment (zero time), and then at days 3, 7, and discharge day in order to check any parameter changes. Urine samples were collected from all subjects at the same time periods, as also blood samples for microalbuminuria tests.

Serum malondialdehyde (MDA) levels were measured according to the standard method of Stocks and Dormandy, as modified by Gilbert et al. Serum GSH levels were measured using the method of Godin et al.; liver enzyme activities (glutamate-pyruvate transaminase (SGPT) and glutamate-oxaloacetate transaminase (SGOT) were calculated calorimetrically according to the method of Reitman and Frankel; serum creatinine was determined by the method of Henry; blood urea by that of Fawcett and Scott; and microalbuminuria by that of McElderry et al., using for the purpose a ready-made kit. Statistical analyses were performed using Student’s t-test and the ANOVA test was used to examine the degree of significance (p value less than 0.05 was considered significant).

Results

Results showed that serum MDA levels were significantly increased (p < 0.05) at zero time in the burn patients compared to control; in group A (no antioxidant used), serum MDA levels decreased nonsignificantly at day 3 and day 7 compared to zero time, but at discharge day serum MDA levels decreased significantly by 62.2% compared to zero time; this result was still significantly higher than in control group E (Fig. 1). Results in groups B, C, and D showed a significant reduction in serum MDA levels at day 7 and discharge day compared to zero time and group A (no antioxidant).

Fig. 1 - Effects of antioxidants on serum malondialdehyde levels in burn patients.
Discharge day in group A: after more than 3 weeks; other groups: less than 2 weeks.

With regard to serum GSH, Fig. 2 shows that serum GSH levels were significantly reduced in burn patients at zero time compared to healthy subjects; in group A, serum GSH levels remained significantly lower than control until discharge day, while in antioxidant-treated groups serum GSH levels were significantly increased at discharge day compared to zero time.

Fig. 2 - Effects of antioxidants on serum glutathione level in burn patients.
Discharge day in group A: after more than 3 weeks; other groups, less than 2 weeks.
Figs. 3 and 4 show the changes in liver enzyme activity, with significant increases in all the burn patients at zero time; enzyme activity gradually returned to normal levels at discharge day in group A; in the antioxidant-treated group, liver enzyme activity returned to normal levels at day 3 post-burn - the same results profile as that obtained for serum creatinine and blood urea (Fig. 5, 6).

Fig. 7 shows a significant reduction ($p < 0.05$) in microalbuminuria levels in the antioxidant-treated groups (B, C, and D) at day 3, compared to zero time - this reduction continued gradually down to the level of healthy control levels at discharge day, while in group A (no antioxidant), the microalbuminuria level was still significantly higher than control at zero time, day 3, and day 7 but only significantly reduced at discharge day, compared to zero time.

**Discussion**

Thermal injury of the skin is an oxidation process, associated with biological and metabolic alterations; it gen-
erates free radicals from various cellular populations by a number of pathways; and the modulation of generated free radical activity with antioxidants may improve outcome.\textsuperscript{17}

In burn patients, alterations in antioxidant micronutrient status and in endogenous antioxidant defences against the deleterious effects of free radicals are crucial, as recent studies have shown.\textsuperscript{18}

It is known that circulating XO activity has been detected during skin burns, and this is the greatest source of free radicals in the serum of burn victims.\textsuperscript{19} Allopurinol, and its principal metabolite, oxypurinol, inhibit the enzyme XO, which catalyses the sequential oxidation of hypoxanthine to xanthine and of xanthine to uric acid,\textsuperscript{20} suggesting that allopurinol may exert a beneficial action in ischaemia reperfusion injury, as is the case in burns.\textsuperscript{21}

It has been reported that the pineal hormone melatonin may function as a powerful antioxidant: it functions as a scavenger of hydroxyl, peroxy, and superoxide radicals,\textsuperscript{22} as also of hydrogen peroxide\textsuperscript{23} and peroxynitrite - the ugly free radical.\textsuperscript{3}

Melatonin is also able to stimulate the activity of the antioxidant enzyme-like superoxide dismutase, catalase, GSH peroxidase, and GSH reductase;\textsuperscript{24} this explains the strong indication of melatonin as a rational antioxidant therapy in the treatment of burns - the rich free radical environment.

With regard to N-acetylcysteine’s antioxidant, it has been shown that N-acetylcysteine acts as a source of sulphydryl groups and facilitates GSH synthesis, in addition to being a direct scavenger for ROS.\textsuperscript{25}

The data obtained in the present study regarding reduction of the MDA serum level and elevation of the serum GSH level - the natural antioxidant - in burn patients are clearly compatible with the results obtained by other researchers.

Increased liver enzyme activity reflects cellular damage due to a burn - it is widely believed that enzyme activity normalizes before patients are discharged.\textsuperscript{26} The same observation was made in investigations of blood urea and serum creatinine in burn patients.\textsuperscript{27} It has also been shown that an increased concentration of the lipid peroxidation product MDA in the early post-burn period can affect the liver and kidney, resulting in the release of enzymes into the blood stream, and that this damage can be remedied with antioxidant, resulting in a return of enzymes to normal levels.\textsuperscript{28} This is exactly what happened in this study - precisely the same thing occurred as regards blood urea and serum creatinine.

The vascular endothelium, which regulates the passage of macromolecules and circulating cells from blood to tissues, is a major target of oxidant stress, playing a critical role in the pathophysiology of several vascular diseases. Oxidative stress increases vascular endothelial permeability and promotes leukocyte adhesion - these are coupled with alterations in endothelial signal transduction and the redox-regulated transcription factor such as NF-κB.\textsuperscript{29} Results obtained by Giner et al.\textsuperscript{30} in hypertensive patients showed that in microalbuminuric subjects the amount of reduced GSH was significantly lower than in normoalbuminuric subjects, which is compatible with the results of this study, as shown in Figs. 2 and 7. It has been suggested that reactive oxygen intermediate and reactive nitrogen intermediate are the final common pathway leading to endothelial cell dysfunction, premature endothelial cell senescence, and apoptosis, and that antioxidants represent an important therapeutic strategy for the prevention and correction of endothelial dysfunction.\textsuperscript{31}

It has been reported that oxidants produced endothelial redox imbalance and loss of vascular integrity by disorganizing several junctional proteins that contribute to the maintenance and regulation of the endothelial barrier; endothelial cell permeability significantly increased as a response to chemical redox imbalance; thiol depletion also resulted in disruption of the endothelial barrier; and the deleterious effects of intracellular redox imbalance were blocked by treatment with exogenous GSH.\textsuperscript{32}

In vascular endothelial cells, the cross-bridge movement between actin and myosin occurs via myosin light chain kinase (MLCK)-catalysed phosphorylation of the regulatory myosin light chain. This reaction results in cell contraction and opening of the intercellular junctions, facilitating the paracellular flux of fluid and macromolecules across the endothelium. Inflammatory agonists, including histamine, cytokines, ROS, and neutrophils, are capable of inducing MLCK activation concomitantly with endothelial hyperpermeability. Typical hyperpermeability factors like
ROS are potent activators of MLCK, and antioxidants may act to block myosin light chain phosphorylation, which may provide therapeutic intervention in burn patients with a goal of alleviating systemic inflammation-induced endothelial dysfunction. ²⁴

Data obtained in the present study show that treatment with antioxidants (groups B, C, and D) results in a reduction of resuscitation fluid from 4 ml/kg/% burn to 1 ml/kg/% burn, compared to the non-antioxidant-treated patients (group A), who received 4 ml/kg/% burn; this result may be supported by the data shown in Fig. 7 that indicate that microalbuminuria decreased significantly at day 3 post-burn in all antioxidant-treated groups, but not in group A where no antioxidant was used - this may explain the role of antioxidants in preventing endothelial dysfunction, increased microvascular permeability, and fluid loss.

On the other hand, results obtained by Till et al. ³³ demonstrated that, after thermal injury, permeability changes and oedema formation progressed over time, with peak changes occurring 60 min after thermal trauma; the plasma of thermally injured rats showed dramatic increases in the levels of XO activity, with peak values appearing as early as 15 min after thermal trauma - it has been suggested that vascular injury defined by increased vascular permeability is related to the activation of XO and to the generation of toxic metabolites that damage microvascular endothelial cells. Also, Berry and Hare ³⁴ showed that in ischaemia-reperfusion injury XO participated in endothelial dysfunction and that, in these conditions, XO inhibitors like allopurinol and oxypurinol attenuated the dysfunction.

Conclusion

In conclusion, the results of this study strongly suggest the role of reactive oxygen species in the endothelial dysfunction that occurs in burn patients and the beneficial role of antioxidants in the reduction of this endothelial dysfunction, as represented by a reduction in microalbuminuria and a reduction of resuscitation fluid in antioxidant-treated burn patients. The results of the study also support the newly emerging evidence regarding the use of microalbuminuria as an indicator for endothelial dysfunction occurring in burn patients.

RÉSUMÉ. Eléments de base. Les brûlures provoquent une réaction inflammatoire systémique, un dysfonctionnement endothéial et un incrément de la perméabilité microvasculaire qui porte à la formation d’œdèmes; ces effets sont probablement le résultat d’une interaction complexe entre les effets directs de la chaleur sur la microcirculation et l’action de médiateurs chimiques, y compris les espèces réactives oxygénées. L’utilisation des antioxydants peut réduire ces modifications, qui sont considérées le développement futur de la gestion des brûlures. Patients et méthodes. Quarante-huit patients brûlés de l’un et l’autre sexe atteints de brûlure d’extension variable ont été inclus dans l’étude. Les patients ont été assignés à un de quatre groupes: A, B, C et D (chaque groupe était composé de 12 malades). Les patients des groupes B, C et D ont été traités avec des antioxydants: allopurinol, mélatonine et N-acétylcystéine respectivement, tandis que le groupe A a été traité selon la normale politique de l’hôpital, c’est-à-dire sans antioxydant; en outre, 12 sujets sains (groupe E) ont été utilisés comme groupe témoin pour comparaison. Dans chaque groupe, les niveaux de malondialdéhyde du sérum et de glutathione du sérum ont été évalués et la fonction hépatique et rénale a été évaluée moyennant les méthodes standard; aussi la microalbuminurie a été évaluée. Résultats. L’administration d’antioxydants aux patients brûlés a produit une amélioration considérable dans les paramètres étudiés par rapport aux paramètres du groupe A (aucun antioxydant administré). Conclusion. Cette étude démontre clairement le rôle des espèces réactives oxygénées dans le dysfonctionnement endothéial qui se produit dans les patients brûlés et l’effet salutaire des antioxydants dans la réduction de ce type de dysfonctionnement endothéial, un résultat démontré par la réduction de la microalbuminurie et de la fluidothérapie pour les patients brûlés traités avec les antioxydants; cette étude en outre confirme des résultats récents qui concernent l’emploi de la microalbuminurie comme indicateur du dysfonctionnement endothéial des patients brûlés.

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