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Oxidative Stress after Moderate to Extensive Burning in Humans

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Lipid peroxidation products, lipid antioxidants, and hematologic and blood chemistry changes were evaluated in plasma of patients after acute burning injury involving 10% ($n=8$), 20% ($n=8$), and 40% ($n=5$) of total body surface area (TBSA), 24 h after burning (baseline) up to 30 days after. Markedly increased plasma levels of malondialdehyde (MDA) were observed at baseline in all patients, according to the extent of the injury, then the values declined progressively. However, levels of MDA remained above normal up to 30 days even in less injured patients. On the other hand, the plasma level of conjugated diene lipid hydroperoxides was only slightly higher than control at the baseline, then dropped under the control value in all patients. Cholesterol showed a marked fall at baseline, followed by a rapidly progressive decrease, indicating a massive loss of circulating lipids by the acute thermal injury. Because of such an extensive and rapidly spreading oxidative degradation of lipids, decomposition of conjugated diene hydroperoxides, produced in early stages of the peroxidation process, occurs, so these compounds cannot be a suitable index to value lipid oxidation in burned patients.

Aldehydic products of lipid peroxidation act as endotoxins, causing damage to various tissues and organs. Damage to liver and decrease of erythrocyte survival were assessed by increased plasma levels of asparate and alanine transaminases, within 7–15 days after injury, and by a decreased number of red blood cells, which remained under the normal value at 30 days.

A marked decrease of lipid antioxidants, β-carotene, vitamin A and vitamin E was observed at baseline. The level of β-carotene remained low in all patients at the end of the 30-day observation. A complete recovery of vitamin A did not occur at 30 days post-burn, even in the patients with 10% of burned TBSA. Plasma levels of vitamin E decreased significantly in 1–7 days after burn in all patients, but these levels increased thereafter, with almost total recovery at 30 days.

These data show evidence of a marked, long-lasting oxidant/antioxidant imbalance in burned patients, in accordance with the severity of the injury, which is also reflected as systemic oxidant stress.

Keywords: Inflammation, burn injury, oxidative stress, lipid hydroperoxides, lipid antioxidants, vitamin A, vitamin E

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INTRODUCTION

Acute thermal injury of skin causes a number of pathophysiological events, leading to a local response, development of burn shock syndrome, and distant organ damage.[1] Experimental evidence has associated these events with the inflammatory reaction which is triggered by the acute injury.[2] Complement activation and the intravascular stimulation of neutrophils result in the production of reactive oxygen species (ROS), which in turn bring about oxidation and release of cell components. Plasma lipid hydroperoxides have been shown to increase after the thermal injury in many animal models,[3–5] as well as in patients.[6–10] On the other hand, damage to cell components and the imbalance between the oxidative injury and body defenses may rapidly lead to depletion of protective antioxidants. Enzymes such as superoxide dismutase (SOD) and catalase have been found markedly depressed respectively in plasma[3] and liver[11] of burned rats, whereas a decrease of antioxidant vitamins[7,12,13] and sulfhydryl groups[7] has been observed in plasma of burned patients. However, we have no evidence of a long-term evaluation of oxidative stress, as compared to hematologic and blood chemistry evidence, which investigates the time-course of restoration of normal conditions.

The aim of this study was to monitor the time-course of plasma levels of lipid peroxidation products in patients with acute thermal injury involving 10%, 20%, and 40% of total body surface area (TBSA), and to evaluate the level of β-carotene, vitamin A and vitamin E since the thermal injury up to 30 days after. Some hematologic and blood chemistry parameters were also evaluated within the same time period to compare the systemic response with the oxidative stress.

MATERIALS AND METHODS

Patients

Burned patients ($n = 21$) admitted to the Burns Centre participated in this study, with informed consent. Patients ranged in age from 8 to 78 years. An adequate therapeutical protocol was applied to maintain patients free of shock and infections. Patients were divided into three groups on the basis of the extent of burns ranging from 10% to 40% TBSA. Eight patients had minor burns (10% TBSA), eight patients had major burns (20% TBSA), and five patients had serious burns (40% TBSA). Degree of thermal injury was not less than second degree in 10% and 20% TBSA burned patients, and third degree in those whose burned TBSA was 40%. All patients were immediately treated with a standard protocol of fluid resuscitation (5% plasma protein solution), and received enteral nutrition without additional micronutrient supplementation, with the exception of vitamin C, 1 g *per os*, twice a day. Blood samples were obtained within 24 h from admission (baseline, day 1), and at least twice a week during the 30-day study period. Blood from 25 healthy subjects, aged 12–70, who did not take any medication, was used as control.

Sample Collection

Whole blood from each patient was withdrawn by venipuncture and collected in 5 ml tubes containing 0.5 mg heparin. The tubes were gently mixed and immediately centrifuged at 2000*g* for 10 min at 4°C. The analytical determinations were either performed immediately, or plasma was stored at −80°C and used within 72 h.

Biochemical Analysis

All-*trans* retinol and α-tocopherol were extracted from 200 µl of plasma samples diluted to 1.0 ml with 0.15 M NaCl, with two volumes of absolute ethanol and eight volumes of petroleum ether. The organic extracts were gathered, dried under nitrogen, resuspended in suitable solvent, and analyzed by a LC-18 HPLC column as above, with methanol at 1.0 ml min^{-1}. All-*trans* retinol and α-tocopherol were detected at a wavelength of 320 and 290 nm, respectively; all-*trans* retinol eluted at 5 min and α-tocopherol at 12 min. An automatic

wavelength change after 8 min allowed the detection of both compounds in the same sample. All procedures were performed under red dim light to preserve light-sensitive vitamins. β-Carotene was extracted from 500 µl serum samples, diluted with 0.15 M NaCl, with 1 vol of methanol and 3 vol of hexane : diethyl ether (1 : 1, vol : vol). The extracts were then dried under nitrogen, resuspended with a mixture of acetonitrile : methanol : tetrahydrofurane (58.5 : 35 : 6.5, vol : vol : vol), and analyzed with the same solvent by a HPLC Supelco Supelcosil LC-18 column (0.46 × 25 cm) (Bellefonte, PA), at a flow rate of 2.5 ml min^{-1}. Recording was at 450 nm.

Conjugated diene (CD) lipid hydroperoxides were extracted from 500 µl of plasma by $CHCl_3$: MeOH (2 : 1, vol : vol). The organic extract was dried under a nitrogen stream, resuspended in cyclohexane, and quantitated spectrophotometrically at 234 nm; $\varepsilon = 27{,}000\ M^{-1}\ cm^{-1}$. Malondialdehyde (MDA) was evaluated in 50 µl plasma samples by colorimetric reaction with thiobarbituric acid (TBA), followed by neutralization of samples by equivalent volumes of a mixture consisting of 4.5 ml 1.0 M NaOH and 45.5 ml methanol. Isocratic high performance liquid chromatography (HPLC) separation of the MDA adduct was performed by a Supelco Supelcosil LC-18 column (0.46 × 25 cm) (Bellefonte, PA), eluted with 40% methanol in 50 M potassium phosphate buffer, pH 6.8, at 1.5 ml min^{-1}. The MDA–TBA adduct was followed at 532 nm and quantified by reference to a calibration curve of 1,1,3,3-tetraethoxypropane (TEP).

Total plasma cholesterol, aspartate transaminase (AST) and alanine transaminase (ALT) were evaluated in serum from fasting individuals by using commercial analytical kits from Sigma (St. Louis, MO).

Statistical Analysis

Results are expressed as means ± standard deviation (SD). Comparison between controls and burned patients was performed by the unpaired Student's *t*-test.

RESULTS

The extent and time-course of lipid peroxidation in burned patients were monitored by plasma levels of MDA and of CD hydroperoxides, and by measuring the plasma concentration of cholesterol. MDA showed a sharp rise at the baseline (day 1) in all patients according to the extent of the burned TBSA (Figure 1). Levels of MDA, from a control value of 1.0 ± 0.11 µM to 2.27 ± 0.16, 3.07 ± 0.29, and 3.77 ± 0.35 µM were measured in patients with 10%, 20%, and 40% of burned body surface, respectively (Figure 1). The peak value was observed at day 1 after burn, then the level started decreasing. However, plasma MDA remained above normal at the end of the 30-day observation in all patients.

Levels of CD appeared slightly higher than the control value at the baseline, in all patients (Figure 2(a)). Then a rapid decrease was observed. The incremental decrease was higher in the most injured patients, and plasma CD levels markedly lower than control were observed at day 15 in 20% and 40% TBSA burned patients (Figure 2(a)). This may indicate a rapid cleavage of oxidatively degenerated lipids. Information on the lipid status of the patients were obtained by measuring cholesterol, as an index of circulating lipids, during the post-burn period. A massive loss was evident

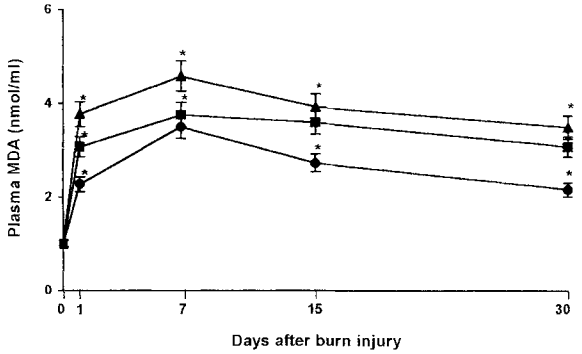

FIGURE 1 Plasma levels of MDA in burned patients. Each point is the mean ± SD of the values obtained from *n* patients; 10% TBSA burned patients (-●-, $n = 8$); 20% TBSA burned patients (-■-, $n = 8$); 40% TBSA burned patients (-▲-, $n = 5$). With respect to control, values are: *, significant with $p < 0.001$.

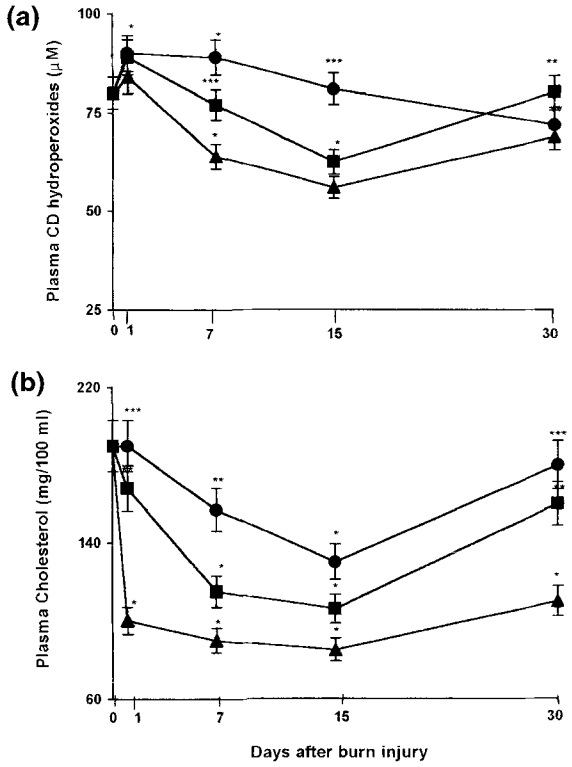

FIGURE 2 Plasma levels of CD hydroperoxides (a) and cholesterol (b) in burned patients. Each point is the mean ± SD of the values obtained from n patients; 10% TBSA burned patients (-●-, $n=8$); 20% TBSA burned patients (-■-, $n=8$); 40% TBSA burned patients (-▲-, $n=5$). With respect to control, values are: *, significant with $p<0.001$; **, significant with $p<0.05$; ***, non-significant.

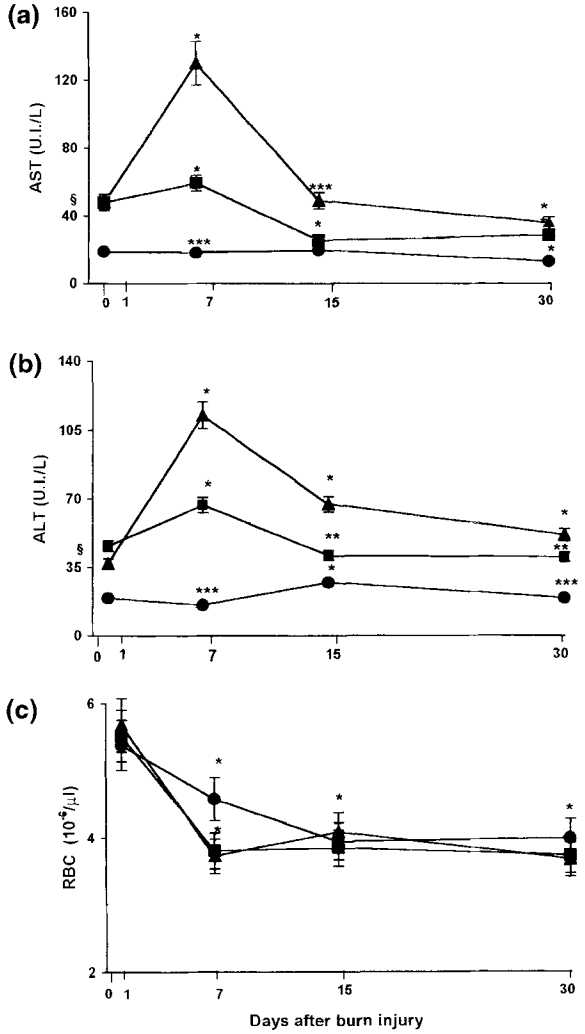

FIGURE 3 Plasma levels of AST (a) ALT (b) and red blood cells count (c) in burned patients. Each point is the mean ± SD of the values obtained from n patients; 10% TBSA burned patients (-●-, $n=8$); 20% TBSA burned patients (-■-, $n=8$); 40% TBSA burned patients (-▲-, $n=5$). With respect to the baseline, values are: *, significant with $p<0.001$; **, significant with $p<0.05$; ***, non-significant. § indicates the normal upper level.

at the baseline in 20% and 40% TBSA burned patients (Figure 2(b)). A progressive decrease was observed in all patients after one day from burn, and levels which were 68%, 55%, and 45% of the control value were measured at day 15 after injury, in patients bearing 10%, 20%, and 40% of burned TBSA, respectively. An increase of plasma cholesterol was monitored in 15–30 days, but a resumption of control levels was observed only in the less injured patients (10% TBSA, Figure 2(b)). The marked and rapid depletion of plasma lipids may account for the apparently small portion of circulating CD lipid hydroperoxides observed in burned patients, since these compounds, produced only in early stages during the lipid peroxidation process, decompose to give other products so that increased steady-state concentrations cannot be found.

Reactive aldehydes as MDA are agents of cytotoxicity.[14] Increased amounts of such compounds may cause damage to various tissues and organs, permitting leakage of enzymes into the bloodstream. To monitor liver damage, serum levels of ALT and AST were measured (Figure 3(a) and (b)).

The plasma levels of both enzyme activities did not appear significantly varied with respect to the normal values in the 10% TBSA burned patients, whereas increased levels were observed at 7 and 15 days in patients with 20% and 40% burned TBSA, the more the injury, the more the increase (Figure 3(a) and (b)). Oxidative alteration to erythrocytes cause premature destruction. Red cell count provided evidence that the erythrocyte number decreased markedly in all patients within 1–15 days, then the number of red blood cells remained significantly lower than the initial value at the end of the 30-days observation (Figure 3(c)).

The extent of oxidative stress may be estimated by measuring the total antioxidant potential of plasma.[15] However, such a global index was not considered by taking into account that the patients received plasma protein solution, and vitamin C. The time-course of changes in the level of the lipid-soluble antioxidants β-carotene, vitamin A, and vitamin E were monitored. The absolute plasma concentration was considered, because the extent of the fall of plasma lipids makes meaningless and misleading the variation of the levels in the groups of patients. All the compounds showed a marked fall, with respect to control, which was related to the severity of injury, within 1–7 days after burning (Figure 4). The levels of β-carotene remained low in all patients at the end of the 30-day observation (Figure 4(a)). Some resumption of vitamin A was observed, but a complete recovery did not occur at 30-days post-burn, even in the 10% TBSA burned patients (Figure 4(b)). On the other hand, plasma levels of vitamin E appeared less altered than those of vitamin A. Recovery of vitamin E occurred within 30 days in 10% and 20% TBSA burned patients, and a 75% resumption was observed in those with a 40% burned TBSA (Figure 4(c)).

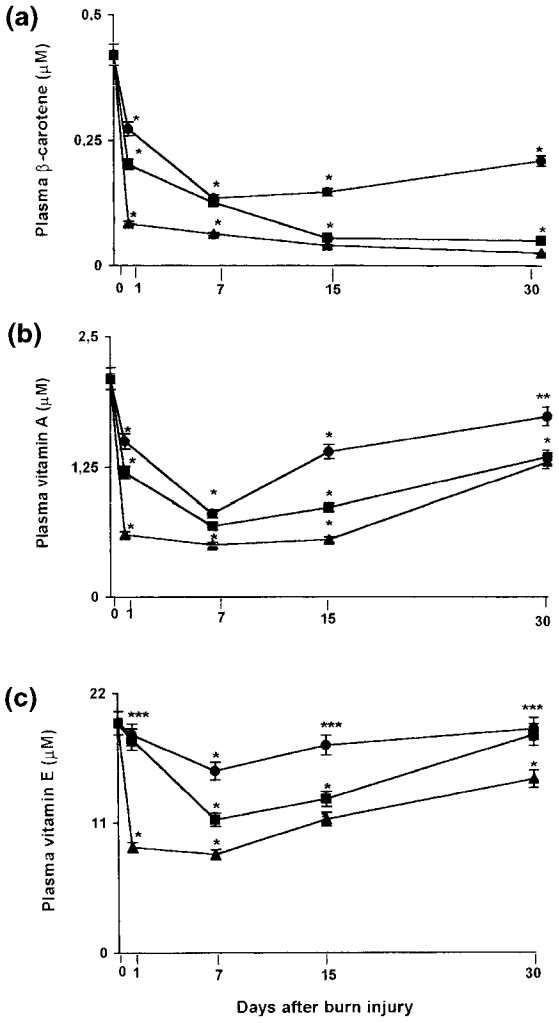

FIGURE 4 Plasma levels of β-carotene (a), vitamins A (b) and vitamin E (c) in burned patients. Each point is the mean $\pm$ SD of the values obtained from n patients; 10% TBSA burned patients (-●-, $n=8$); 20% TBSA burned patients (-■-, $n=8$); 40% TBSA burned patients (-▲-, $n=5$). With respect to control values are: *, significant with $p<0.001$; **, significant with $p<0.05$; ***, non-significant.

DISCUSSION

There is evidence that immunological and biochemical changes are associated with the thermal injury. Formation of xanthine oxidase-derived oxidant and release of burn toxin from the site of injury are considered the main responsible of local burn edema, and of distant inflammatory damage with further oxidant release, leading to chronic inflammation and systemic organ dysfunction.[16–18] This work provided information on the time-course of various biochemical

parameters of oxidative damage, and compared the systemic response with the oxidative stress, in burned patients for a 30 days long period.

Oxidant-induced lipid peroxidation occurs immediately after burn injury,[3–5,7–9,18–24] however the initial increase of lipid peroxides last only a few hours, and again increase with the onset of burn tissue inflammation.[5] Systemic lipid peroxidation has been reported in burned patients at 5 and 10 days after burn.[6] In accordance, plasma levels of MDA were very high in all our patients at day 1 from injury, and showed a peak 7 days post-burn. More importantly, our study reports that, although plasma levels of MDA declined within 15–30 days, they remained over normal even in burned patients who exhibited only a 10% burned TBSA. This may be indicative that the systemic inflammation is not completely done even in the less injured patients. The decreased number of erythrocytes, which is still observed in the late post-burn period, may be accounted for by the long-lasting increased level of toxic MDA.

Extracts from burn plasma at $\frac{1}{2}$ and 3 h yield CDs which, although non-specific and transient, have been considered as an indication of the magnitude of initial trauma or of the ensuing leucocyte-mediated inflammatory response.[25] We have evidence that levels of CDs that appeared slightly higher than control at the baseline (day 1), underwent a paradoxical sharp decrease during the post-burn period. The parallel fall of circulating lipids, as expressed by the cholesterol levels, during the entire period of observation, provides evidence that lipid peroxidation is so extensive and rapid that the steady-state level of CD hydroperoxides, produced in early stages of the oxidation process, is very low. Therefore, while MDA may show progress of oxidative systemic dysfunction, CD cannot be a long-term index for assessing the oxidative status of burned patients.

Various early and delayed factors may account for the observed decrease of plasma cholesterol, including a rapid oxidation of low density lipoprotein, and demand by burn tissues to support new membrane synthesis. A decreased sterol synthesis because of liver damage, is also to be considered, at least in the most injured patients. The leakage into the bloodstream of enzymes such as ALT and AST, which has been observed in our burned patients, as well as in previous studies,[6,10] within 7–15 days from injury, is in accordance with this issue.

Bioactive molecules such as ROS are necessary to the cell defense,[26] and to cell growth and proliferation as well,[27] but a proper balance must be secured. Burned patients should face two major problems, i.e. the endotoxic shock and formation of hypertrophic dermal scarring. Excess of ROS and free radicals are involved in both processes.[28–31] Therefore, the marked decrease of plasma antioxidants, especially in the most seriously burned patients, which is the consequence of the oxidative injury, is also to be considered as a condition contributing to both a delayed healing and to the risk of hypertrophic scars. A dramatic fall of the plasma level of vitamin A is known after burn injury.[12] We observed that β-carotene, vitamin A and vitamin E are rapidly depleted after burning, and that the level of β-carotene and vitamin A remained lower than control at 30 days, although patients received a complete enteral nutrition. Liver is responsible for the release into the bloodstream of vitamin A, as a RBP complex, and of vitamin E into lipoproteins. Impairment of the liver function may concur to the decrease of blood levels of both vitamins. Since patients receive vitamin C as a supplement, some recycling of the oxidized vitamin E may occur,[29] which may explain why the level of vitamin E is more easily recovered, whereas that of vitamin A is not. On the other hand, the plasma levels of carotenoids reflect dietary intake and absorption,[32] and consumption by oxidative stress as well.

The importance of antioxidants under situation of burn injury may be appreciated in consideration that non-survivors (seven out of thirteen burned patients) demonstrated increased consumption of antioxidants compared with

survivors.[7] This may indicate inadequate antioxidant protection in non-survivors, and can also suggest antioxidant supplementation to promote maintenance of defense mechanisms. Antioxidants may be of help at various levels after thermal injury. Deficit of cell ATP and total adenine nucleotides in burn-induced systemic organ dysfunction in rats may be prevented by administration of water-soluble antioxidants,[33] suggesting a cause and effect relationship between increased oxidant release with inflammation, decreased antioxidant activity and altered cell energetics. Moreover, other reports showed that, with respect to untreated patients, the administration of SOD to burned patients prevented raise of plasma CDs formed as the result of oxygen radical production.[34] Administering β-carotene enterally has appeared useful to a rapid recovery of plasma level of β-carotene and lycopene.[13] Vitamin A and vitamin E have roles in processes involved in immune and inflammatory responses.[35,36] This appears of interest in that post-burn immunosuppression occurs after a major burn injury.[37] Therefore, besides their activity against oxidation of plasma and LDL lipids,[38,39] both vitamins are of help in the post-burn care. Plasma levels of α-tocopherol were inversely correlated with the level of the pro-inflammatory interleukin-8 in burned patients.[13] In addition, vitamin A and vitamin E may affect production of collagen fibers,[40,41] which could control formation of hypertrophic scars. Maintenance of adequate levels of both vitamins may be advised.

References

[1] M. Allgower, G.A. Schoenenberger and B.G. Sparkes (1995) Burning the largest immune organ. *Burns*, **21**, S7–S47.

[2] P.A. Ward and G.O. Till (1990) Pathophysiologic events related to thermal injury of skin. *Journal of Trauma*, **30**, S75–S79.

[3] O. Cetinkale, A. Belce, D. Konukoglu, C. Senyuva, M.K. Gumustas and T. Tas (1997) Evaluation of lipid peroxidation and total antioxidant status in plasma of rats following thermal injury. *Burns*, **23**, 114–116.

[4] K. Yagi, J.-Y. Guan, Q. Sun, S. Komura, N. Ohishi and I. Nishigaki (1997) Change in serum lipid hydroperoxides in thermal injury. *Journal of Clinical Biochemistry and Nutrition*, **23**, 139–143.

[5] R.H. Demling and C. LaLonde (1990) Systemic lipid peroxidation and inflammation induced by thermal injury persists into post-resuscitation period. *Journal of Trauma*, **30**, 69–74.

[6] R. Kumar, R.K. Seth, M.S. Sekhon and J.S. Bhargava (1995) Serum lipid peroxide and other enzyme levels of patients suffering from thermal injury. *Burns*, **21**, 96–97.

[7] T.T. Nguyen, C.S. Cox, D.L. Traber, H. Gasser, H. Redl, G. Schlag and D.N. Herndon (1993) Free radical activity and loss of plasma antioxidants, vitamin E, and sulfhydryl groups in patients with burns: the 1993 Moyer Award. *Journal of Burn Care and Rehabilitation*, **14**, 602–609.

[8] J.O. Woolliskroft, J.K. Prasad, P. Thompson, G.O. Till and I.H. Fox (1990) Metabolic alterations in burn patients: detection of adenosine triphosphate degradation products and lipid peroxides. *Burns*, **16**, 92–96.

[9] J. Sasaki, G.L. Cottam and C.R. Baxter (1983) Lipid peroxidation following thermal injury. *Journal of Burn Care and Rehabilitation*, **4**, 251–254.

[10] M. Hiramatsu, Y. Izawa, M. Hagihara, I. Nishigaki and K. Yagi (1984) Serum lipid peroxide levels of patients suffering from thermal injury. *Burns*, **11**, 111–116.

[11] R. Daryani, C. LaLonde, D. Zhu and R.H. Demling (1990) Changes in catalase activity in lung and liver after endotoxemia in sheep. *Circulatory Shock*, **32**, 273–280.

[12] K. Rai and A.D. Courtemanche (1975) Vitamin A assay in burned patients. *Journal of Trauma*, **15**, 419–424.

[13] C.L. Rock, J.L. Rodriguez, R. Khilnani, D.A. Lown and R.S. Parker (1993) Carotenoids and antioxidant nutrients following burning injury. *Annals of the New York Academy of Sciences*, **691**, 274–276.

[14] H. Esterbauer, R.J. Schaur and H. Zollner (1991) Chemistry and biochemistry of 4-hydroxynonenal, malondialdehyde and related aldehydes. *Free Radical Biology and Medicine*, **11**, 81–128.

[15] B. Halliwell (1996) Oxidative stress, nutrition and health. Experimental strategies for optimization of nutritional antioxidant intake in humans. *Free Radical Research*, **25**, 57–74.

[16] H.S. Jacob, P.R. Craddock, D.E. Hammerschmidt and C.F. Moldow (1980) Complement induced granulocyte aggregation. *New England Journal of Medicine*, **302**, 789–794.

[17] J.M. Klausner, L. Kobsik, C.R. Valeri, D. Shepro and H.B. Hechtman (1988) Selective lung leukosequestration after complement activation. *Journal of Applied Physiology*, **65**, 80–88.

[18] R.H. Demling and C. LaLonde (1990) Early postburn lipid peroxidation: effect of ibuprofen and allopurinol. *Surgery*, **107**, 85–93.

[19] R.H. Demling, C. LaLonde, Y. Liu and D. Zhu (1989) The lung inflammatory response from thermal injury (relationship between physiological and histological changes). *Surgery*, **106**, 52–59.

[20] I. Nishigaki, M. Hagihara and M. Hiramatsu (1980) Effect of thermal injury on lipid peroxide levels of rat. *Biochemical Research*, **24**, 185–189.

[21] L.-J. Jin, C. LaLonde and R.H. Demling (1986) Lung dysfunction after thermal injury in relation to prostanoid and oxygen radical release. *Journal of Applied Physiology*, **61**, 103–112.

[22] G. Till, D. Beauchamp, W. Menapace, W. Tourtellotte, B.S. Robin Kunkel, K.J. Johnson and P.A. Ward (1983)

Oxygen radical dependent lung damage following thermal injury of rat skin. *Journal of Trauma*, **23**, 269–277.
[23] R.H. Demling, K. Ikegami and C. LaLonde (1995) Increased lipid peroxidation and decreased antioxidant activity correspond with death after smoke exposure in the rat. *Journal of Burn Care and Rehabilitation*, **16**, 104–110.
[24] H.P. Friedl, G.O. Till, O. Trentz and P.A. Ward (1989) Roles of histamine, complement and xanthine oxidase in thermal injury of skin. *American Journal of Pathology*, **135**, 203–217.
[25] G.O. Till, J.R. Hatherill, W.W. Tourtellotte, M.J. Lutz and P.A. Ward (1985) Lipid peroxidation and acute lung injury after thermal trauma to skin. Evidence of a role for hydroxyl radical. *American Journal of Pathology*, **119**, 376–384.
[26] B.M. Babior (1997) Superoxide: a two-edged sword. *Brazilian Journal of Medical and Biological Research*, **30**, 141–148.
[27] R.H. Burdon (1995) Superoxide and hydrogen peroxide in relation to mammalian cell proliferation. *Free Radical Biology and Medicine*, **18**, 775–794.
[28] D.R. Blake, R.E. Allen and J. Lunec (1987) Free radicals in biological systems. A review orientated to inflammatory processes. *British Medical Bulletin*, **43**, 371–381.
[29] B. Halliwell and J.M.C. Gutteridge (1999) *Free Radicals in Biology and Medicine*, 3rd edn. Oxford University Press.
[30] W.F. Petrone, D.K. English, K. Wong and J.M. McCord (1980) Free radicals and inflammation: superoxide-dependent activation of a neutrophil chemotactic factor in plasma. *Proceedings of the National Academy of Sciences USA*, **77**, 1159.
[31] K.C. Wan and J.H. Evans (1999) Free radical involvement in hypertrophic scar formation. *Free Radical Biology and Medicine*, **26**, 603–608.
[32] R.S. Parker (1996) Absorption, metabolism and transport of carotenoids. *FASEB Journal*, **10**, 542–551.
[33] C. LaLonde, U. Nayak, J. Hennigan and R.H. Demling (1996) Antioxidants prevent the cellular deficit produced in response to burn injury. *Journal of Burn Care and Rehabilitation*, **17**, 379–383.
[34] P.D. Thomson, G.O. Till, J.O. Woolliscroft, D.J. Smith and J.K. Prasad (1990) Superoxide dismutase prevents lipid peroxidation in burned patients. *Burns*, **16**, 406–408.
[35] A.C. Ross and U.G. Hammerling (1994) Retinoids and the immune system. In *The Retinoids*, 2nd edn. (Eds. M.B. Sporn, A.B. Roberts and D.S. Goodman), Raven Press Ltd., New York, pp. 549–561.
[36] S.N. Medyani and R.P. Tengerdy (1993) Vitamin E and immune response. In *Vitamin E in Health and Disease* (Eds. L. Packer and J. Fuchs), Marcel Dekker Inc., New York, pp. 549–561.
[37] O. Cetinkale, O. Senel and R. Bulan (1999) The effect of antioxidant therapy on cell-mediated immunity following burn injury in an animal model. *Burns*, **25**, 113–118.
[38] M.A. Livrea and L. Tesoriere (1998) Antioxidant activity of vitamin A within lipid environments. In *Subcellular Biochemistry*, Vol. 30 (Eds. P. Quinn and V. Kagan), Plenum Press, New York, pp. 113–143.
[39] G.W. Burton and K.U. Ingold (1981) Autoxidation of biological molecules. 1. The antioxidant activity of vitamin E and related chain-breaking phenolic antioxidants *in vitro*. *Journal of the American Society*, **103**, 6472–6477.
[40] H. Oikarinen, A.I. Oikarinen, E.M.L. Tan, R.P. Abergel, C.A. Meeker, M.L. Chu, D.J. Prockop and J. Uitto (1985) Modulation of procollagen gene expression by retinoids. *Journal of Clinical Investigation*, **75**, 1545–1553.
[41] K. Houglum, D.A. Brenner and M. Choikier (1991) d-Alpha tocopherol inhibits collagen alpha 1 (I) gene expression in cultured human fibroblasts. Modulation of constitutive collagen gene expression by lipid peroxidation. *Journal of Clinical Investigation*, **87**, 2230–2235.