BENEFICIAL EFFECTS OF PRO-/ANTIOXIDANT-BASED NUTRACEUTICALS IN THE SKIN REJUVENATION TECHNIQUES

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Abstract - Modern technologies of skin rejuvenation include many physical and chemical intervention tools - laser irradiation, oxygen and ozone therapy, chemical peels, plastic surgery operations - affecting by different mechanisms the sensitive physiological free radical / antioxidant balance in the skin. All these interventions induce from mild to severe tissue damage, providing beneficial biochemical stimuli for skin re-epithelization and rejuvenation. Paradoxically, free radical production in the course of tissue inflammation helps to combat free radical damage consequent to the ageing process. We have studied two animal models (experimental burn and trichloracetic peeling), reproducing on the Wistar rat the effects generated by the commonly practiced aesthetic medicine procedures of laser resurfacing and chemical peels, demonstrating that the severe oxidative stress induced both systemically and on skin can be modulated by the oral pre- and post treatment administration of specific nutraceutical formulations. Potent antioxidants (RRR-α-tocopherol, coenzyme Q_{10}), enhancing antioxidant defences, coupled with mild pro-oxidants, enhancers of a specific immune defense (soy phospholipids, L-methionine), at the blood and the skin levels, proved in fact to be beneficial in vivo, on the rat, for skin healing, trophism and accelerated re-epithelization. Data obtained allow us to predict the possibility of innovative protocols for dermocosmetology, enabling successful lowering of the risk of permanent adverse effects, and prolonging the duration of the beneficial effects of dermocosmetologic procedures.

Keywords: aesthetic medicine, antioxidants, chemical peels, cosmetology, free radicals, laser resurfacing, nutraceuticals, skin

INTRODUCTION

The skin tissue damage induced by environmental free radical (FR) species of physical, chemical and microbial origin, as well as by endogenously formed reactive species, is strictly implicated in the skin aging process (8, 20). With age, increased formation of oxidation products, altered expression of antioxidant (AO) enzymes, decreased low-molecular weight AO levels, enhanced transcriptional factors and stress proteins are observed in the different organs and body fluids, as well as in the skin (21). On the other side, skin aging is also associated with a progressive decline of the immune function and of the capability to produce physiologically active FR by competent cells (9).

The major goal of aesthetic medicine is to prevent and/or attenuate the symptoms of skin photo- and chrono-ageing. Literature data allow to identify the modulation of the FR/AO balance, both at systemic and skin levels, as a key factor for the success of interventions (3, 5, 12, 16).

The aim of this work was to demonstrate FR unbalance after physical (skin resurfacing) and chemical (trichloracetic acid peeling) dermocosmetological procedures, and the consequent need for selected antioxidant-based pre- and post- treatments, to prevent excess tissue damage with the frequently occurring undesired side effects, such as alterations of pigmentation, scarring, microbial super-infections, etc. A specific formulation for oral administration was selected (17) following a thorough *in vitro* screening of its antioxidant properties, moderating tissue damage, and mild pro-oxidant activity, stimulating skin cell growth and immune response. The effects of the pre- and post- administration of this specific formulation, to control and modulate local and systemic inflammatory damage, was tested on two animal models (Wistar rat) of aesthetic medicine procedures, the skin burn and skin chemical peeling.

MATERIALS AND METHODS

In vitro experiments

Samples of whole blood (5 mL, in heparin) were drawn from 10 healthy adult volunteers. Granulocytes were isolated by differential centrifugation on a Histopaque gradient (density: 1.119). Washed cells were re-suspended in Hank's balanced salt solution (HBSS), 3:1 with heat-inactivated calf serum (5%), at a concentration of 10^7/ml, and analyzed immediately. Cells counts were performed by Coulter counter, and cell viability was determined by trypan blue exclusion test, the percentage of viable granulocytes being always $\geq$ 95%. All measurements were performed in the presence of single components and of the complete formulation (hereinafter named "AO formulation"). Ubiquinone - CoQ_{10}, RRR-α-tocopherol, L-methionine, soy phospholipids (lecithin) and the mixture of these compounds (granular form), in appropriate concentrations reproducing the recommended daily dose for human adults as nutraceutical (ubiquinone - 50 mg; RRR-α-tocopherol, 50 mg; L-methionine, 200 mg, soy phospholipids, 200 mg, daily), were tested.

The spontaneous and PMA-activated formation of free radical products of hydrogen peroxide (H_2O_2) decomposition was measured by luminol-amplified chemiluminescence (CL) with a LKB/Wallac 1251 luminometer (Wallac Oy, Finland), according to Korkina et al. (13). Briefly, granulocytes (adjusted to $5x10^6$ cells/ml) were incubated in HBSS (pH 7.4) at 37°C in the presence of luminol (5×10^{-5} M), with continuous mixing for 5 min. to measure spontaneous CL intensity. Then, protein kinase C activator phorbol 12-myristate 13-acetate (PMA, 10 ng/ml), was added to stimulate NADPH-oxidase association and ROS formation. The results were expressed as the ratio between CL intensity (I CL) in the presence and in the absence of the test compounds.

Superoxide anion ($O_2^{\bullet-}$) production by granulocytes was evaluated by SOD-sensitive cytochrome c reduction (15). Fifty μM cyt C, 2 mM sodium azide, 100 μl WBC suspension, and HBSS (total volume 1 ml) were placed into plastic tubes and incubated at room temperature for 1 hour. Reaction was performed in the absence or presence of CuZnSOD (20 μg/ml), with or without PMA (0.1 μg/ml in DMSO). After 10 min. centrifugation at 600 x g, absorbance at 550 nm was read on the clear supernatants, against a water blank, with a LKB/Wallac Ultraspec 4050 spectrophotometer (Wallac Oy, Finland). The amount of $O_2^{\bullet-}$ produced by 10^6 granulocytes was calculated using ε_{550} (reduced cyt C) = 21.0 mM^{-1} cm^{-1}.

All chemicals were purchased from Sigma Chemical Co. (St. Louis, MO, USA). All reagents were of analytical grade.

Animal experiments

The skin burn model was performed on the Wistar rat. On the shaved abdomen skin, pressure (40 sec.) by a pre-heated (85°C) aluminum bar produced a burn of severity corresponding to a IIIB grade wound of 20% of the total body surface (17). Twenty-four male rats (300-320 g) were divided into 2 groups: Control (n = 12), receiving intra-esophageally a pure saline solution for 7 consecutive days before burning; Experimental (n = 12), treated with the AO formulation (granular form) described in the *in vitro* experiments, administered by esophageal injection for 4 days before burning, and continued till the last day of experiment, in the same daily dosage pro kg of body weight recommended for human adults as nutraceutical (ubiquinone - CoQ_{10} - 50 mg; RRR-α-tocopherol, 50 mg; L-methionine, 200 mg; selenium aspartate, 50 mg; soy phospholipids, 200 mg. daily). The rats of the control and experimental groups were sacrificed by over-dosage of barbiturates 24 hours (day 1) after trauma, or followed up for 12 days, with analyses on the 4th, 6th, 8th, 12th day.

The speed of wound healing was quantified by measuring the damaged surface by the planimetric method. The generalized inflammatory reaction was assessed by measuring the total FR production in the whole blood, following activation with PMA, by luminol-amplified CL (13). The Total Antioxidant Activity of plasma was measured by spectrophotometry (10). Egg yolk homogenised in 20 volumes of 0,04 M PBS (pH 7,4). 100 μl of the suspension were added to 700 μl of PBS, 100 μl of plasma, and 100 of 46 μM $FeSO_4$. (sample). Control contained 100 μl PBS instead of plasma, blank contained yolk in PBS. After 30 min incubation at RT, 500 μl 20% trichloracetic acid (TCA) were added, in the presence of BHT to block lipid oxidative reactions. After thorough mixing and centrifugation (15 min. at 900 x g), 700 μl of supernatant were mixed with 600 μl 0.5% thiobarbituric acid (TBA), boiled for 30 min and cooled. Absorbance was measured at 532 nm, with the following formula for

calculations: AOA,% = (Control absorbance-Experimental abs.) / (Control abs.-blank abs.) x 100%.

Skin biopsies were taken from undamaged skin surface, at a distance of 10 mm from lesion border, at days 0, 1, 4 and 12 after experimental burning. Separated epidermis was homogenized immediately in a glass homogenizer, and the supernatant obtained by centrifugation (10 min. at 400 x g) was analyzed by spectrophotometric methods for the following enzymatic activities, catalase (CAT) (1), superoxide dismutase (SOD) (6) and glutathione peroxidase (GPx) (7). The local level of inflammation was assessed by measuring the activity of myeloperoxidase (MPO) in the epidermis (22). The local nitric oxide (NO) formation in skin biopsies was determined by electron spin resonance (ESR) spectroscopy, employing the spin probe 2-phenyl-4,4,5,5-tetramethylimidazoline-1-oxyl 3-oxide (PTIO) (2). Protein content was measured by spectrophotometrical method according to Lowry (14).

The peeling model was performed on the Wistar rat. On the shaved abdomen skin, the chemical peeling preparation based on TCA at 18% was applied on an area corresponding to 20% of the total body skin surface. Thirthy-six 24 male rats (300-320 g) were divided into: Control (n = 12, no local application, no systemic treatment); TCA (n = 12, two applications of the 18% gel form, at a 20 min. interval, without neutralization or washing with water, till reaching "frosty" appearance of skin); TCA + AO formulation (n = 12, TCA treatment as described, pre-treatment for 4 days with the nutraceutical formulation in the granular form, as described for the burn experiments, continued till day 8 of observation. Total Antioxidant Activity of plasma (10) and MPO activity (22) on skin biopsies at 10 mm from the peeled area were measured as reported above.
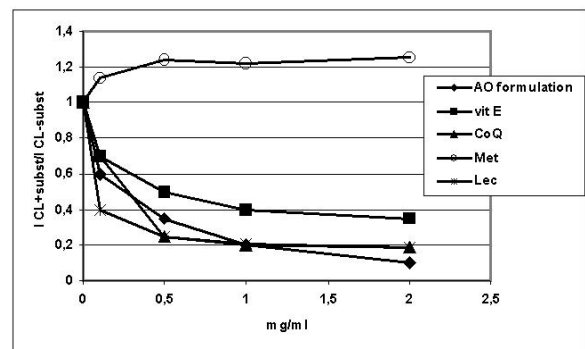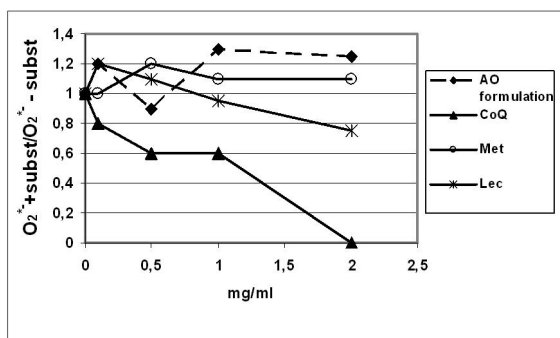
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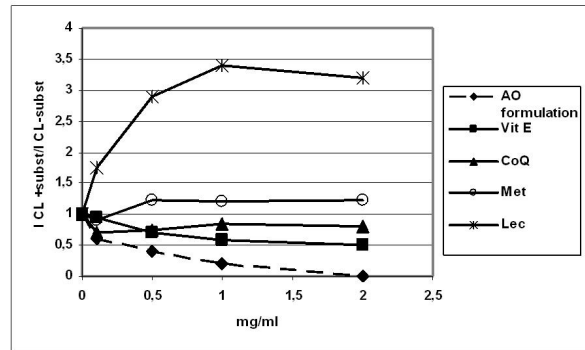
Figure 1A. Dose-dependent influence of the antioxidant (AO) formulation and its single components on the Intensity of luminol-amplified Chemiluminescence (I CL), spontaneously generated by isolated granulocytes of healthy adult subjects.

Results of 5 independent experiments in triplicate, SD was always < 5%.

Vit E = RRR-α-tocopherol; CoQ = Ubiquinone (CoQ10), Met = L-methionine; Lec = lecithin (soy phospholipids).

Figure 1B. Dose-dependent influence of the antioxidant (AO) formulation and its single components on the Intensity of luminol-amplified Chemiluminescence (I CL), generated after PMA activation, by isolated neutrophils of healthy subjects.

Results of 5 independent experiments in triplicate, SD was always < 5%.

Vit E = RRR-α-tocopherol; CoQ = Ubiquinone (CoQ$_{10}$), Met = L-methionine; Lec = lecithin (soy phospholipids).

Fig. 1 (A-B) shows the inhibitory effect of coenzyme Q_{10} and of the natural isomer of vitamin E on the reactive oxygen species (ROS) production by granulocytes isolated for the blood of healthy adult donors, both in spontaneous (A) and PMA-stimulated (B) systems. Lecithin (from soy phospholipids) exerted, unexpectedly, a marked stimulatory effect on the spontaneous luminol-amplified chemiluminescence (CL) experiments, whilst L-methionine induced a moderate increase in the PMA-stimulated experiments.

Figure 2A. Influence of the antioxidant (AO) formulation and the single components on the spontaneous superoxide anion (O_2^{*-}) release by isolated neutrophils of healthy subjects.

Results of 5 independent experiments in triplicate, SD was always < 5%.

CoQ = Ubiquinone (CoQ_{10}), Met = L-methionine; Lec = lecithin (soy phospholipids).
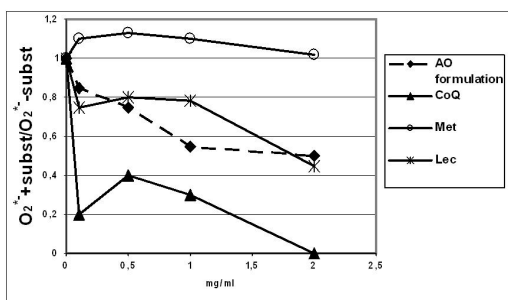
Figure 2B. Influence of the antioxidant formulation (AO) and the single components on the PMA-activated superoxide anion (O_2^{*-}) release by isolated neutrophils of healthy subjects.

Results of five independent experiments in triplicate, SD was always < 5%.

CoQ = Ubiquinone (CoQ_{10}), Met = L-methionine; Lec = lecithin (soy phospholipids).

Fig. 2 (A-B) shows the inhibitory effect of coenzyme Q_{10} on the superoxide anion release from granulocytes both in spontaneous (A) and PMA-stimulated (B) systems. L-methionine exerted, again, a moderate stimulatory effect respectively in the spontaneous and PMA-stimulated experiments. The complete formulation (ubiquinone - CoQ_{10}, RRR-α-tocopherol, L-methionine, soy phospholipids - lecithin), at the concentrations recommended for human use as a nutraceutical, resulted inhibitory in all experiments (Figs. 1-2), though to different extents, with the exception of a moderately stimulatory effect, at the higher concentrations tested, on the spontaneous release of superoxide by granulocytes without PMA-stimulation (Fig. 2 A).
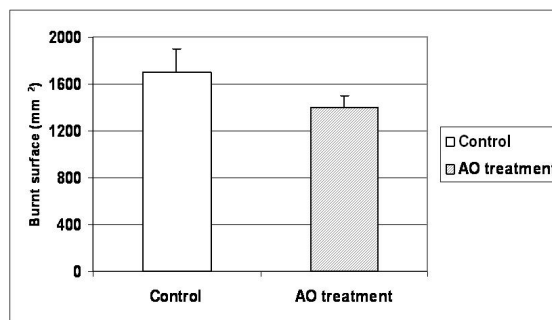
Figure 3. Planimetric measurements of the burnt area on the shaved abdomen of Wistar rats of the Control group (n= 12) and the Experimental group (n= 12, AO formulation treatment), at day 12 after experimental burn. p < 0.05 (AO treatment *vs.* Control). Results of three analyses for each animal.
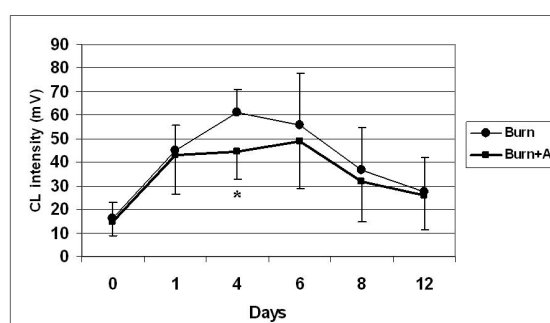
Figure 4. Time course of the PMA-activated free radical production (luminol-amplified Chemiluminescence – CL-Intensity) in the whole blood of Wistar rats of the Control group (n= 12) and the Experimental group (n= 12, AO formulation treatment). p < 0.05 at day 4 (AO treatment *vs.* Control). Results of three analyses for each animal
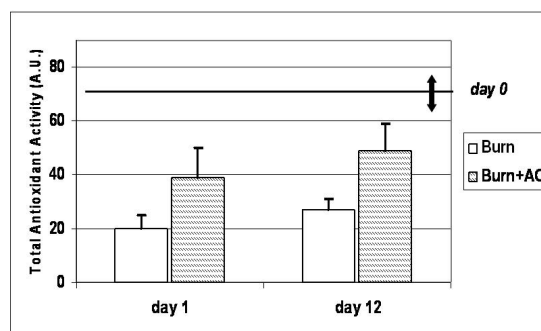
Figure 5. Plasma Total Antioxidant Activity of Wistar rats of the Control group (n = 12) and the Experimental group (n= 12, AO formulation treatment), before and at day 1 and 12 after experimental burn. Results of three analyses for each animal.

Table 1. Myeoloperoxidase (MPO) activity, NO production (PTI/PTIO), catalase (CAT), superoxide dismutase (SOD) amd glutathione peroxidae (GPx) activities in skin biopsy epidermis (10 mm from the peeled area) of Wistar rats of the Control group (n = 12) and the Experimental group (n= 12, AO formulation treatment), before and 4, 12 days after experimental burn

MPO, µmol/g prot.

Intact (before burn)	117,6±46,7	
days post burn	Control	AO
4	247,5±80,9*	272,2±75,1*
12	109,4±55,1**	123,4±35,7**

*-p<0,05 *v.s.* Intact
**-p<0,05 *v.s.* 4 days post burn

PTI/PTIO (NO), relative units/90 µg prot.

Intact (before burn)	0,37±0,1	
days post burn	Control	AO
4	0,19±0,05*	0,34±0,13
12	0,24±0,03*	0,30±0,02**

*-p<0,05 *v.s.* Intact
**-p<0,05 *v.s.* Control group

|	CAT U/mg prot.		SOD U/mg prot.		GPx U/mg prot.	
Intact (before burn)	57,5±25,7		4,9±3,4		0,45±0,12	
days post burn	Control	AO	Control	AO	Control	AO
4	83,0±39,8	65,2±11,7	43,9±19,6	28,8±10,7*	0,68±0,14	0,40±0,01
12	85,3±18,9	80,7±38,7	32,3±15,1	22,9±6,6	1,04±0,18*	0,63±0,25**

*-p<0,05 *v.s.* Intact
**-p<0,05 *v.s.* Control group

Figs. 3-5 and Table 1 illustrate the results obtained on the model of experimental burning. In fig. 3, planimetric measurements show the marked acceleration of wound healing and re-epithelization in the group of rats treated with intra-aesophageal adminstration of the AO formulation (p<0.05 vs. control group), at day 12 after burn. Data obtained from the evaluation of the systemic (blood) parameters demonstrate, over the 12 days of observation, a significant increase of whole blood ROS production in the rats subjected to experimental burn, as compared to baseline values before burn, and a significant decrease of ROS production in the AO formulation treated group, as compared to control group (p<0.05 vs. control, at day 4 after burn) (Fig. 4). Fig. 5 shows a significant (p<0.05) increase of the Total Antioxidant Activity in the plasma of the AO treated rats, as compared to the untreated control group, both at day 1 and at day 12 after burning. In the skin biopsies obtained from the skin area surrounding the lesions (Table 1), the inflammation marker myeloperoxidase (MPO) activity, peaking at day 4 after burning, was not significantly affected by AO treatment, whereas the labile free radical NO production was significantly increased (p<0.05 *vs.* controls at day 12), and antioxidant enzyme defenses, in

particular SOD and GPx activities, were significantly enhanced, *vs.* controls (p<0.05), till the last day of observation.
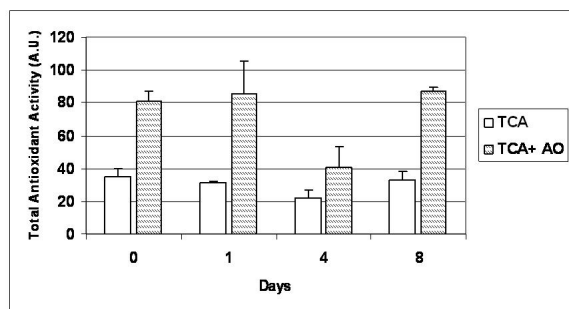
Figure 6. Plasma Total Antioxidant Activity of Wistar rats of the Control group (n = 12) and the Experimental group (n= 12, AO formulation treatment), before and at days 1, 4 and 8 after experimental chemical peeling of 20 % body skin surface with 18% trichloracetic acid (TCA). Results of three analyses for each animal.
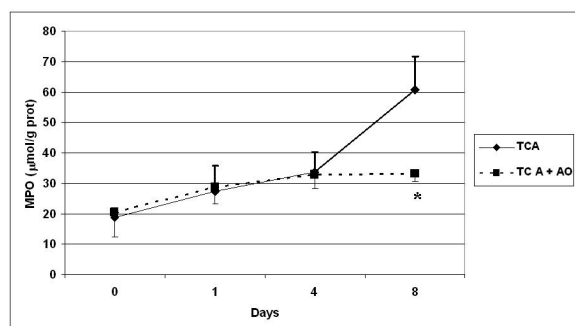
Figure 7. Myeoloperoxidase (MPO) activity in skin biopsies (10 mm from the peeled area) of Wistar rats of the Control group (n = 12) and the Experimental group (n= 12, AO formulation treatment), before and at days 1, 4 and 8 after experimental chemical peeling of 20 % body skin surface with 18 % trichloracetic acid (TCA). Results of three analyses for each animal.

Fig. 6 and 7 show data obtained for the TCA peeling experiments. Luminol-amplified CL intensity in the whole blood is decreased, though not significantly, in the AO treated group as compared to controls (28,9±6,0 *vs.* 16,2±1,1). Plasma Total Antioxidant Activity in the rats of the experimental group, receiving the AO formulation pre- and post- treatment, is significantly higher that the control group values (Fig. 6), throughout the whole 8 days of observation. In the biopsies from the skin at 10 mm from the peeled area, MPO activity values are significantly lower in the AO treated group as compared to controls, at day 8 after experimental peeling (p<0.05).

DISCUSSION

Results of the *in vitro* experiments predict that the formulation based on coenzyme Q_{10}, the

natural isomer of vitamin E, L-methionine and lecithin from soy fospholipids is able to exert a strong FR scavenging activity *in vivo*, due to the well-known potent antioxidants ubiquinone and vitamin E (9, 21), along with a mild pro-oxidant activity, to be ascribed to L-methionine, and lecithin, the latter possibly due to the presence of lipoperoxides in traces amounts, produced by the oxidation of the polyunsaturated phospholipid moiety (5).

As a whole, data from the skin burn experiments demonstrate that FR production and FR/AO homeostasis both in the blood and at the skin level were beneficially affected by the pre- and post-treatment with the pro-/antioxidant formulation studied. MPO activity in the skin was unaffected, reflecting that AO supplementation does not influence the granulocyte recruitment to the injured skin. The normalization of NO production in the skin affects positively the healing process. The AO administration improved the dynamics of all AO enzymes activities in the close vicinity of the burnt skin area, these data representing a positive sign for facilitating wound healing and decreasing the massive oxidative stress induced by burning. As a result, the treatment accelerated significantly the expected proper skin re-epithelization process, as compared to the untreated control.

In the TCA peeling experiments, results confirmed the efficacy of the AO formulation, in enhancing the systemic antioxidant defences, thus exerting a protective effect at the systemic level, after a procedure that stimulated FR level increase in the blood, and in moderating inflammation at the epidermal level, as demonstrated by the significant decrease of MPO activity at the border of the peeled area.

Biochemical pathways and molecular machinery induced by widely used chemical and physical methods of skin rejuvenation are still poorly understood. Thorough evaluation and better understanding of basic mechanisms underlying the routine procedures of esthetic medicine is bound to ameliorate the efficacy, and lower the incidence of undesired adverse effects. Depending on dosage and duration of the application, physical – chemical treatments can either induce or inhibit cutaneous free radical processes. Laser irradiation may either suppress or induce NO-synthase or a number of AO enzymes, depending on energy and spectral characteristics (3, 16, 18). Oxygen and ozone therapy exert either strong FR-activating or

suppressing effects (4). Chemical peeling cocktails include strong FR-generating molecules (11, 19). Paradoxically, FR produced upon the aesthetic medicine procedures may help to combat the free radical-mediated ageing process, but this process must be carefully modulated.

Our data provide evidences that the aesthetic procedures, such as chemical peeling/laser resurfacing, alter the systemic and cutaneous FR status and the AO defense system of the patient to different degrees, inducing a state of acute oxidative stress both locally and systemically. The inflammatory cells, mainly "primed" granulocytes, are recruited to the damaged skin from the circulating blood and bone marrow. They release extremely reactive oxygen and nitrogen species, which aggravate the oxidative damage to cellular and non-cellular material of epidermis and derma. Then, the oxidized residues of cellular membranes, DNA, and extracellular polysaccharides stimulate consequently gene expression, protein synthesis, cell proliferation, and function that results in the skin regeneration. Adverse effects, such as activation of "dormant" viral and bacterial infections, persistent erythema, hyper/hypopigmentation, and scarring are presently the main concern of the dermatologist performing aesthetic procedures for skin rejuvenation. Nevertheless, the risk of remote undesirable effects for example, the reduced skin capacity to regeneration, immunodeficiency, and tumor formation should also be taken into consideration.

In conclusion, the oral administration, pre- and post-treatment, with selected antioxidants (RRR-α-tocopherol, coenzyme Q_{10}) potentiating antioxidant defence system, coupled with enhancers of aspecific immune defense (soy phospholipids, L-methionine), is beneficial to skin healing and trophism. These combined treatments may well represent an efficient tool to mitigate both FR-mediated skin ageing and adverse effects of aesthetic procedures, increasing the beneficial results, prolonging their duration, lowering the incidence of undesired side effects. It must also be considered that treatments must be tailored for the different aesthetical procedures, and possibly adjusted for the specific individual cutaneous and systemic FR/AO status.

REFERENCES

1. Aebi, H., Catalase, In: Methods of Enzymatic Analysis, Bergmeyer P. (ed.), Academic Press, N.Y., London, 1984. Vol.2, pp. 673-684.

2. Akaike, T., Yoshida, M., Miyamoto, Y., Kohno, M., Sasamoyo, K., Miyazaki, K., Ueda, S. and Maeda, H. Antagonistic action of imidazolineoxyl N-oxides against endothelium-derived relaxing factor/NO through a radical reaction. Biochem., 1993, 32: 827-832.

3. Altavilla, D., Galeano, M., Bitto, A., Minutoli, L., Squadrito, G., Seminara, P., Venuti, F.S., Torre, V., Calo, M., Colonna, M., Lo Cascio, P., Giugliano, G., Scuderi, N., Mioni, C., Leone, S. and Squadrito, F., Lipid peroxidation inhibition by raxofelast improves angiogenesis and wound healing in experimental burn wounds. Shock 2005, 24(1): 85-91.

4. Cotovio, J., Onno, L., Justine, P., Lamure, S. and Catroux, P., Generation of oxidative stress in human cutaneous models following in vitro ozone exposure. Toxicol. In Vitro 2001, 15(4-5): 357-362.

5. De Luca, C., Deev, A. and Korkina, L., Squalene II. Squalene as an acceptor of pro-oxidant effects towards human skin. Cosmetics & Medicine 2002, 6: 17-26 (in Russian).

6. Greenwald, R.A. (ed.), Handbook of Methods for Oxygen Radical Research , CRC Press, Florida, 1986, pp-291-302.

7. Gunzler, W.A. and Flohé, L., Glutathione peroxidase, in: Handbook of Methods for Oxygen Radical Research, Greenwald, R.A. (ed.), CRC Press, 1987, pp. 285-290.

8. Jenkins, G., Molecular mechanisms of skin ageing. Mech. Ageing Development 2002, 123: 801-810.

9. Kagan, V.E., Kisin, E.R., Kawai, K., Serinkan, B.F., Osipov, A.N., Serbinova, E.A., Wolinsky, I. and Shvedova, A.A., Toward mechanism-based antioxidant interventions: lessons from natural antioxidants. Ann. N.Y. Acad. Sci. 2002, 959: 188-98.

10. Klebanov G.I., Babenkova I.V. and Teselkin, Yu.O., Measurement of plasma antioxidant properties in the egg yolk lipoprotein test. Lab. Delo 1988, 5(C):59-62 (in Russian).

11. Kligman, L.H., Sapadin, A.N., Schwartz, E., Peeling agents and irritants, unlike tretinoin, do not stimulate collagen synthesis in the photoaged hairless mouse. Arch. Dermatol. Res. 1996, 288(10): 615-620.

12. Kockaert, M. and Neumann, M., Systemic and topical drugs for aging skin. J. Drugs Dermatol. 2003, 2(4): 435-441.

13. Korkina, L.G., Samochatova, E.V., Maschan, A.A., Suslova, T.B., Cheremisina, Z.P. and Afanas'ev, I.B., Release of active oxygen radicals by leukocytes of Fanconi anemia patients. J. Leukoc. Biol. 1992, 52(3): 357-362.

14. Lowry, O.H., Rosenbrough, N.J., Farr, A.L., Randall, R., J., Protein measurement with the Folin phenol reagent. J. Biol. Chem. 1951, 193(1): 265-275.

15. Malorni, W., Straface, E., Pagano, G., Monti, D., Zatterale, A., Del Principe, D., Deeva, I.B., Franceschi, C., Masella R. and Korkina, L.G., Cytoskeleton alterations of erythrocytes from patients with Fanconi's Anemia. FEBS Letters 2000, 468: 125-128.

16. Mc.Daniel, D.H., Ash, K., Lord, J., Newman, J. and Zukowski, M., Accelerated laser resurfacing wound healing

using a triad of topical antioxidants. Dermatol. Surg. 1998, 24(6): 661-664.

17. Mikhal'chik, E.V., Ivanova, A.V., Anurov, M.V., Titkova, S.M., Pen'kov, L.Y. and Korkina, L.G., Preventive and therapeutic effect of complex antioxidant preparation in rats with burn trauma. Bull. Exp. Biol. Med. 2004, 138(3): 264-266.

18. Naziroglu, M., Kokcam, I.and Yilmaz, S., Beneficial effects of intraperitoneally administered alpha-tocopheryl acetate on the levels of lipid peroxide and activity of glutathione peroxidase and superoxide dismutase in skin, blood and liver of thermally injured guinea pigs. Skin Pharmacol. Appl. Skin Physiol. 2003, 16(1): 36-45.

19. Park, K.S., Kim, H.J., Kim, E.J., Nam, K.T., Oh, J.H., Song, C.W., Jung, H.K., Kim, D.J., Yun, Y.W., Kim, H.S., Chung, S.Y., Cho, D.H., Kim, B.Y. and Hong, J.T., Effect of glycolic acid on UVB-induced skin damage and inflammation in guinea pigs. Skin Pharmacol. Appl. Skin Physiol. 2002, 15(4): 236-245.

20. Pugliese, P.T. (ed.), Physiology of the skin II. Revised Edition. Allured Publishing Corporation, Illinois (USA), 2001.

21. Thiele, J.J., Schroeter, C., Hsieh, S.N., Podda, M. and Packer L., The antioxidant network of the stratum corneum. In: Oxidants and antioxidants in cutaneous biology. Curr Probl Dermatol., Thiele, J. and Elsner, P. (eds), Karger, Basel, 2001, pp. 26-42.

22. Xia, Y. and Zweier, J.L., Measurement of myeloperoxidase in leucocyte-containing tissues. Anal. Biochem. 1997, 245: 93-96.