PHOTOAGING AND FREE RADICALS: CORRELATIONS BETWEEN SKIN BIOMECHANICAL PROPERTIES AND PLASMATIC LEVELS OF SKIN OXIDATIVE SPECIES AND ANTIOXIDANT COMPOUNDS

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Summary

Literature data suggest that human skin elasticity decreases with age on both face and forearm. Because it is well known that free radicals are involved in cutaneous aging and skin cancers, we investigate: correlation between bio-mechanical properties of human skin and blood levels of an oxidant product malondialdehyde (MDA) and antioxidants: vitamin E, superoxide dismutase (SOD) and glutathione peroxidase (GPx-SE); blood levels of SOD, vitamin E, GPX-SE, and MDA during aging.

Sixty-seven healthy subjects (44 women aged from 21 to 86 years and 23 men from 21 to 74 years) entered the study after giving written, informed consent. All the volunteers had no vitamins or antioxidants supplementation in the diet before six months. The measurements of the bio-mechanical properties were performed with a Cutometer Sem 474 on three different anatomic locations: forehead, cheek and volar forearms, then 7cc of blood were drawed for High-Performance Liquid Cromatography measurement of vitamin E (mg/ml), Standard Spectrophotometric Methods evaluation of SOD (U/ml) and MDA (nmol/ml) and ELISA investigation of GPx-SE (mg/ml).

RESULTS. We found no correlation between bio-mechanical properties of the skin and aging, sex, skin photo-types and plasma levels of MDA and antioxidants. About biochemical data, we found that vitamin E and SOD levels increase progressively during aging in women population while in men population the increase is not statistically significant. GpxSE levels also increase during aging but only in men population. MDA levels showed no correlation with any of the previous parameters.

Riassunto

Secondo i dati della letteratura sembrerebbe che l'elasticità della cute si riduca con l'età sia sul viso che sulle braccia. Dato che, come è noto, esiste un coinvolgimento dei radicali liberi sia nei processi di invecchiamento che nella formazione dei tumori, sono state controllate le correlazioni esistenti tra

le proprietà meccaniche della cute umana ed il livello di ossidazione dei lipidi circolanti, legata alla minore o maggiore presenza di antiossidanti quali la vitamina E, la superossidismutasi (SOD) e la glutatione perossidasi (GPx-SE).
67 soggetti sani (44 donne di età compresa tra 21 e 86 anni, e 23 uomini tra 21 e 74 anni) sono stati inseriti nello studio dopo aver dato il loro consenso scritto. Tutti hanno interrotto l'eventuale uso di vitamine e/o antiossidanti sei mesi prima dell'inizio dello studio. Le proprietà bio-meccaniche della cute sono state controllate mediante l'uso del Cutometer Sem 474 su tre diverse aree cutanee: fronte, guance e avambraccio. La concentrazione di vitamina E, di SOD e di MDA è stata controllata sul siero di 7 cc di sangue mediante l'utilizzazione dell'HLPC.
Non è stata riscontrata alcuna correlazione tra le proprietà biomeccaniche della cute, l'invecchiamento, il sesso, il fototipo cutaneo ed i livelli di MDA e di antiossidanti nel sangue. La vitamina E ed i livelli di SOD aumentano progressivamente con l'età nella donna mentre nell'uomo tale incremento non è statisticamente significativo. Anche i livelli di GpxSE aumentano con l'aumentare dell'età non soltanto nell'uomo. I livelli di MDA non sembrano correlati a nessuno dei parametri controllati.

INTRODUCTION

Skin aging is represented by the amount of the progressive clinical, functional and structural modifications of the main cutaneous strata which depends on aging itself and on many environmental external factors (environmental pollution, ionizing radiations, cigarettes' smoke, carcinogens, drugs, sunlight) [PHOTOAGING] (1): in both cases is to be considered the alterations of two particular genes (c-fos proto-oncogene, SPR2, Il-1ra, c-myc, GADD, IL-1 beta) involved in the regulation of the cellular immuno-modulation, growing, differentiation and UV reaction (2).

From these short premises, that partly explain the predisposition to photo-induced carcinogenesis, especially for the aged skins, comes the growing interest for the molecular events aging responsible, for its prevention and for an appropriate photo-protection.

The theory of Harman (3), considering the modern knowledge and the most accurate methodologies in determining free radicals and the relevant antioxidant enzymes, nowadays assumes an ongoing interest and actuality: aging seems to be the result of the progressive accumulation of free radicals and/or cutaneous lesions they caused.

As it is known, free radical is any molecule or atom able to live by itself and containing one or more unpaired electrons in the outer orbital (anion superoxide O_2, peroxide of hydrogen H_2O_2, radical hydroxilic OH-, singlet oxygen O_2, nitric oxide NO). It should be remembered that they are produced separately by multiple exogen stimulation even in presence of: a) physiological reactions catalyzed by cytocrom P-450, cyclo-oxigenase, xantinaoxidase; b) oxidation of endogenous substances; c) mitochondrial chain of electron (mitochondrial leaking). Free radicals activity is characterized by: inactivation of enzymatic activities containing groups SH, interaction with DNA and consequent mutations and aberrations, destruction of cellular membranes by a direct damage and per-oxidation of unsaturated lipids. The organism defends itself against these attacks by efficacious mechanisms of protection both enzymatic and not enzymatic (free radicals scavengers): the first ones act at cytoplasmatic level (SOD, catalysis, glutatione peroxidase) the second ones act at cellular membranes level (tocopherol, carotenoids, ubichinon, ascorbic acid, pantotenate calcium, nicotinic acid, pyridoxine, biotin, glutatione).

Furthermore, from the literature results examined, during skin aging free radicals not always show a real and constant increase (5-6), even if augmented in many physio-pathological conditions (inflammation, cancers, autoimmune diseases, photosensitivity and photo-toxicity, immunoallergical reaction) (4). By the way these data do not exclude their importance since a reduction with age of the antioxidative capabilities together with a contemporaneous increase of phenomena of lipidic peroxidation, could make pathological the physiologic concentration of free radicals produced (7).

Moreover, we have poor news about the correlation existing between cutaneous damage induced by free radicals, istopathological alterations and consequent modifications of the skin biomechanical properties.

At this regard, we carried out a study aimed to correlate the skin biomechanical properties to age, sex and plasmatic levels of SOD, vit. E, Se-GPx and products of lipoperoxidation, and to verify eventual tissue variations of oxidant and antioxidant substances in the global idea of aging.

MATERIALS AND METHODS

We enrolled 67 subjects, 44 men (aged between 21 and 86) and 23 women (aged between 21 and 74), healthy, with negative anamnesis for systemic and metabolic diseases and assumption of vitamins supplementation and/or antioxidants for at least six months.

Working anamnesis, food habits, life style, and photo-type were evaluated.

Skin biomechanical properties were evaluated

by CUTOMETER SEM 474 on three different areas: forehead, cheek and volar armpit area. After measuring in an environment at temperature and humidity controlled, were drawn 7 cc of blood in order to evaluate vit. E in HPLC, SOD and MDA in spectro-photometry and Se-GPx in ELISA*.

Skin Mechanical Properties

Were evaluated by Cutometer Sem 474 equipped with a 2mm-diameter probe. The measurements consisted in three cycles of 5 second's suction each at negative pressure of 400 mbar every 5 seconds pause. The values reported represent the average of the three measurements.

*Evaluation of the vitamin E ematic levels

The analytic determination was done in HPLC. 0,2 ml of plasma treated with 200 micro-liters of tocopherol avetate (20 microg/ml in ethanol) [inner standard] and with 400 micro-liters of butanol-etil-acetate (1:1; V:V). After shaking for 20 seconds were added 20 mg of sodium solfate followed by a 60 seconds shaking.
Further to a centrifugation at 15.000 xg for 5 minutes at 4°C was recuperated the organic stratum and 50 micro-liters of the same were injected in the HPLC column (ultratecfere C18, 250x4,6mm, 5 micron). The mobile phase was represented by a mixture of methanol-water (97:5; V:V) with a flux of 1ml/min at environment temperature. The detector was at wavelength of 280 nano-meters. The plasmatic concentrations of vitamin E are expressed as µ/ml

*Measurement of the MDA ematic levels

The plasmatic levels of malondialdehyde were evaluated by spectrophotometry using a kit colorimetric Lipid peroxidation assay (n° 437634 - Calbiochem-Novabiochem Corporation, San Diego, and USA). The plasmatic concentrations of MDA are in n/ml.

*Determination of the SOD ematic levels

The activity of super-oxide dismutase was evaluated in spectro-photometry at 525 nm using a kit colorimetric Superoxide Dismutase Assay (n° 574600 - Calbiochem-Novabiochem Corporation, San Diego, USA). The plasmatic activity of SOD is in ml.

*Analysis of the SeGPx ematic levels

Plasmatic levels of glutatione peroxidase selenium were evaluated by ELISA using a kit SP1-GPX EIA (Calbiochem-Novabiochem Corporation, San Diego, USA). The plasmatic concentrations of Se GPx are in µg/ml.

RESULTS AND CONCLUSION

The results obtained can be summarized as follows:

- Decrease of the elasticity (tab.I) and increased of the extensibility (tab. II) with aging;
- vit. E (tab. III) and SOD (tab. IV) showed a progressive increase and statistically significant in the female subjects only;
- Se-GPx (tab. V) increases in men only;
- MDA (tab. VI), constantly increasing in strong smokers, it did not showed significant correlation with the former parameters;
- Tissue levels of vit. E , in all the samples tested, were always less of one mcg/g (weight not sufficient of the material?); data concerning SOD and MDA presented wide variability even if at high levels of SOD corresponded high levels of MDA.

To conclude, on a general level at least, abstracting from the preliminary data in our experience, which did not allowed to highlight any

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correlation between the mechanical skin bioproperties, age, sex and plasmatic levels of oxidants and antioxidants, we would like to underline the following aspects:

- Free radicals play an important role in skin aging particularly in photo-aging, through mechanisms not yet clearly known;
- other systems, at least at cutaneous level, beside SOD, contribute in an efficacious way to defend the skin against free radicals (tioredoxine-reductase -TR-, system Glutatione reductase- Glutatione peroxidase -GR-GP) (8);
- the protective action of the diet supplementation, even if encouraging, still represents an open and controversial problem: the studies done did not demonstrated in uncontestable and definitive way a clear correlation between the assumption of antioxidant substances, aging and cancers growing:
- the great antioxidant capability of the skin, relative to a major alpha-tocopherol concentration in the skin (90%) compared to derma (9), together with the recent demonstration that keratinocytes of the more differentiate strata have antioxidant properties higher than germinal ones (10), underlining the importance of the topical application of antioxidants able to reduce in a significant way the production of free radicals induced by UV (11).

If the topical application of these substances gives certainly a further real protection against photo-damaging, we hope that, together with a possible dietary supplementation, the future studies will be devoted to this research exploiting at the best the peculiar and natural characteristics anatomic-functional of the skin.

Tab. 1

WOMEN

ELASTICITY

Age	N.	Cheek	Forehead	Forearm
20-39	20	0.52 ± 0.25	0.49 ± 0.08	0.82 ± 0.18
40-59	12	0.55 ± 0.23	0.46 ± 0.14	0.70 ± 0.17
> 60	12	0.67 ± 0.42	0.41 ± 0.14	0.59 ± 0.14

1a

MEN

ELASTICITY

Age	N.	Cheek	Forehead	Forearm
20-39	7	0.55 ± 0.28	0.66 ± 0.16	0.73 ± 0.12
40-59	10	0.50 ± 0.11	0.55 ± 0.19	0.70 ± 0.14
> 60	7	0.61 ± 0.32	0.64 ± 0.20	0.62 ± 0.18

1b

Tab. 2

WOMEN

ESTENSIBILITY

Age	N.	Cheek	Forehead	Forearm
20-39	20	0.17 ± 0.06	0.29 ± 0.06	0.21 ± 0.07
40-59	12	0.18 ± 0.09	0.26 ± 0.09	0.21 ± 0.07
> 60	12	0.19 ± 0.07	0.24 ± 0.08	0.27 ± 0.08

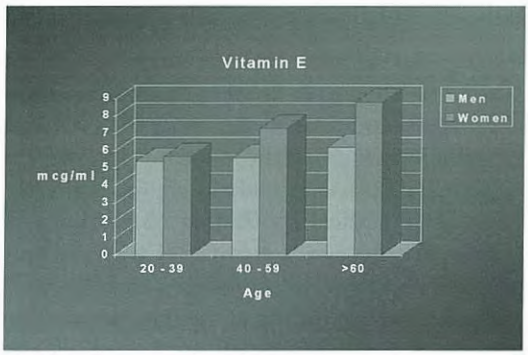
2a

MEN

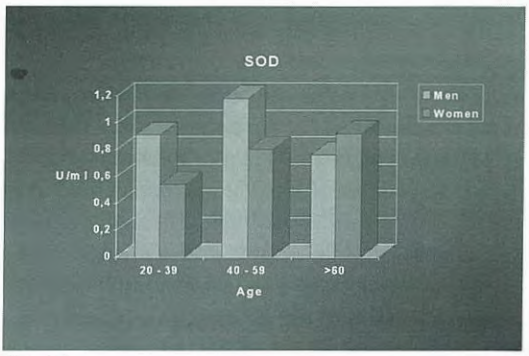
ESTENSIBILITY

Age	N.	Cheek	Forehead	Forearm
20-39	7	0.21 ± 0.08	0.28 ± 0.05	0.23 ± 0.05
40-59	10	0.23 ± 0.08	0.26 ± 0.08	0.22 ± 0.09
> 60	7	0.25 ± 0.12	0.27 ± 0.13	0.20 ± 0.08

2b



Tab. III



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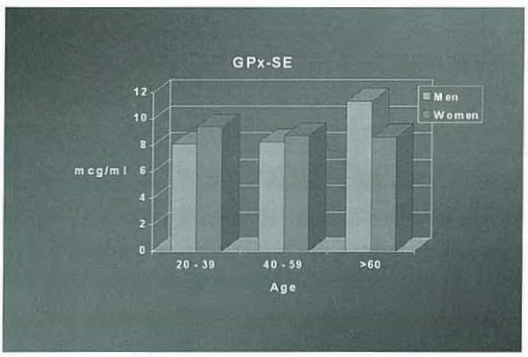

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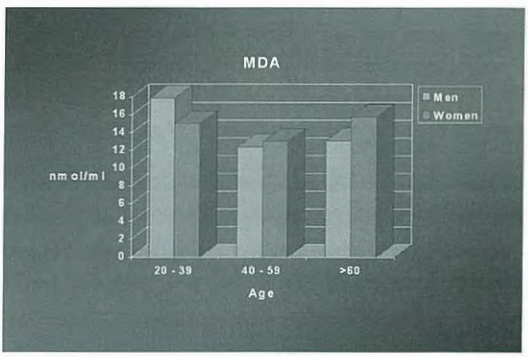

Tab. VI

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