THE RADICAL PROTECTION FACTOR FOR INNOVATIVE NUTRICEUTICALS

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Summary

Oxidative stress is defined as a molecular alteration generated by oxidant molecules.

This oxidative damage can occur by direct chemical oxidation, or it can involve a more insidious chain reaction in molecules that are oxidatively reactive for the presence of a single unpaired electron: the free radical.

Since free radicals are essentially caused by UV and environmental oxidants, the free radical-induced oxidative damage is always present.

Moreover, the oxidative stress is characterized by both reversible and irreversible cell damage and can, with time and sufficient severity, lead to cell death.

Biological systems are protected from the oxidative assault by a diversity of mechanism designed to suppress pernicious oxidative pathways.

Among these mechanisms there are the antioxidant systems represented by carotenoids.

The object of the study was to control the efficacy against free radicals of a dietary supplement based on carotenoids and antioxidant vitamins, determining also its real activity "in vivo" together with its radical protection factor (RPF) according to Herrling et al.

The radical oxygen species (ROS) before, during, and after the diet supplementation was determined by the new Ros-meter-system (Dermotech - Italy) at 505 nm. on the blood serum of 36 smoker volunteers (women and men) aged between 30 and 45.

The obtained results proved that the carotenoids based dietary supplement is able to reduce the oxidative stress of about 30/40 % during the first week of treatment, and the methodology used is also useful to determine, in a quite fast way, the RPF of the diet supplement used.

This way it seems possible to label the diet by RPF values strictly depending on the different needs, as it already happens with the sun products. At the same time it is possible to classify diet supplements as "nutriceuticals" and to demonstrate their efficacy.

Riassunto

Lo stress ossidativo è dovuto ad un'alterazione molecolare provocata da molecole ad attività ossidante. Il danno ossidativo può verificarsi per un'ossidazione chimica diretta o può coinvolgere una serie di reazioni provocate da molecole particolarmente reattive per la presenza di un elettrone

spaiato: il radicale libero.
Siccome i radicali liberi sono provocati essenzialmente dai raggi UV, dalla luce e dagli ossidanti presenti nell'ambiente, il danno ossidativo indotto dal radicale libero è sempre presente. Inoltre lo stato di stress ossidativo è caratterizzato dal danno cellulare sia reversibile che irreversibile; danno che se molto grave, può portare alla morte della cellula.
I sistemi biologici sono protetti da diversi meccanismi necessari per eliminare tali danni. Tra questi meccanismi vi sono i sistemi antiossidanti rappresentati dai carotenoidi. Controllare l'efficacia contro i radicali liberi di un dietetico basato su carotenoidi e vitamine antiossidanti, determinando "in vivo" sia l' attività che il fattore di protezione "antiradicale libero" (RPF) secondo la metodica di Herrling et al.
La presenza di radicali liberi (ROS) prima, durante e dopo la dieta è stata determinata utilizzando il ROS-meter-system a 505 nm sul siero di sangue di 36 fumatori volontari (uomini e donne) di età compresa tra 30 e 45 anni. I risultati ottenuti hanno dimostrato che la dieta basata sui carotenoidi è in grado di ridurre lo stress ossidativo di circa il 30/40 % durante la prima settimana di trattamento.
In questo modo sembra possibile caratterizzare il prodotto dietetico con valori di RPF strettamente collegati con le diverse necessità, come già si verifica con i prodotti solari.
È così possibile classificarli, ad esempio, come veri e propri nutriceutici, dimostrandone anche una loro eventuale efficacia protettiva.

BACKGROUND

Radicals are among the most important intermediates in the mechanism of toxicity for a vast number of chemicals. Oxygen-derived radicals are obligatory intermediates in the mechanism of toxicity for a vast number of chemicals.
Biologically speaking, oxidative stress is defined as a molecular alteration generated by oxidant molecules.
This oxidative damage can occur by direct chemical oxidation, or it can involve a more insidious chain reaction in molecules that are oxidatively reactive for the presence of a single unpaired electron: the free radical.
Since free radicals are essentially caused by UV light and by environmental oxidants the radical-induced oxidative damage is always present (1-3).

INTRODUCTION

The strong reactivity of free radicals makes their determination difficult.
The most popular methodology is spin trapping, whereas a diamagnetic organic molecule, called the spin trap, reacts with the radical to be identified to produce a secondary more stable radical called spin adduct, which is more readily detectable by Paramagnetic Resonance Spectroscopy (EPR) (4).
This method, though difficult to apply "in vivo", provides direct information regarding the identity of free radicals generated in a system (5, 6).
A big limit of this method is that free radicals, being too unstable, practically react topically where they are produced, that's why the "ubiquitary diffusability" of the "traps" should be assured, thing not at all demonstrated.
It has been tried to solve the problem differently, by using the hydroperoxides.
The fact that lipids in presence of free radicals, easily oxidize, has to be considered natural target; among these, the essential fatty acids (EFA), for their own characteristic, are gradually oxidized in the so-called reaction of propagation that leads to the formation of a hydroperoxide radical L00°, surely more stable than the OH° radical beginning the process of oxidation (7).
This hydroperoxide could be defined as "marker" of the oxidative stress in the biological systems, since its increase is proportional to the production of OH°.
Differently to other ROS (Reactive Oxygen Species), the hydroperoxide L00° is relatively stable and, in order to produce other radicals, it must react with an iron ion.

AIMS

The object of this study was to control the efficacy of a diet supplement based on carotenoids, antioxidant vitamins, and polyphenols against free radicals determining also its real activity "in vivo" together with its Radical Protection Factor (RPF) according to Herling et al. (8).

MATERIAL AND METHODS

Before, during ,and after, the dietary supplementation was detected by the new ROS-meter System (Dermotech-Italy) at 505 nm., on the blood serum of 60 smoker volunteers (women and men) aged between 30 and 45, according to Morganti P. and Fabrizi G. (9).

ROS MEASUREMENT

As it is known free radicals are extremely reactive and have a very short life. Because of its unopposed electron, a radical is slightly attracted to a magnetic field; it is paramagnetic. This unique physical property allows for its detection and analysis by electron paramagnetic resonance spectroscopy (EPR). But only a small num-

ber of radicals are stable enough to be detected by such spectroscopy in aqueous solution at room temperature. Fast flow techniques are used to detect short-lived radicals. The most popular methodology is spin trapping, in which a diamagnetic organ molecule, called the spin trap, reacts with the radical to be identified, to produce a secondary but more stable radical called a spin adduct which is more readily detectable by EPR.
One U. CARR is equal to a hydrogen peroxide concentration of 0,08% mg.
The methodology has been controlled and validated by the Electronic Spin Resonance (ESR) (10-12).

Table I

MEAN ASSESSMENT OF ROS IN STRONG SMOKERS

SMOKERS	N	Average ROS mg% ± SD*
20 Cigarettes/day	20	27.04 ± 0.81
30 Cigarettes/day	20	30.43 ± 0.74
40 Cigarettes/day	20	43.84 ± 0.66

Standard deviation on 3 different analysis for each of the 20 subjects controlled

SCREENING PROCEDURE

Before starting the study, the ROS present in the blood serum of different groups of volunteers (men and women) smoking from 20 up to 40 cigarettes a day were controlled.
A small quantity of blood was sampled from each volunteer, whose serum has been tested through three different determinations using the ROS-Meter System (Dermotech-Rome, Italy) (9).
The obtained results are reported on Table I.
The blood sampling was effected in a medical office always at 10.00 a.m. and on fasting.

TEST PROCEDURE

Sixty healthy smoker volunteers age range 30 and 45 (35 women and 25 men), were selected for the study. Each volunteer smoked at least 40 cigarettes a day. The subjects were randomly divided into 6 groups of 10 people and to each group were given sufficient capsules for 1

month of treatment. Neither the operator nor the subjects were able to identify the product. Eight weeks before starting the study, the subjects suspended all drugs or diet supplements taken by oral route.
The groups were subdivided in:

I	group	1 capsule/day	PRODUCT A[1]	(betacarotene 6 mg.)
II	group	2 capsule/day	PRODUCT A	(betacarotene 12 mg)
III	group	3 capsule/day	PRODUCT A	(betacarotene 18 mg)
IV	group	1 capsule/day	PRODUCT B	(carotenoids 10 mg.)
V	group	1 capsule/day	PRODUCT C	(carotenoids 15 mg.)
VI	group	1 capsule/day	PRODUCT D[2]	(carotenoids15mg + C and E vitamins and polyphenols)

Each subject took 1, 2 or 3 capsules per day for one month, according to the group of affiliation. The ROS were photometrically evaluated in the blood serum at week 0 (baseline value) and respectively at week 1, 2, 3 and 4 by ROS-meter System at 505 nm, according to Carratelli et al. methodology (10), formerly used by our group (9).

The RPF provided by the tested products, were determined by calculating the ROS reduction obtained with the diet supplement used, and interpolating those values in the standard curve determined at different concentrations of carotenoids taken by oral route (Fig. 1).

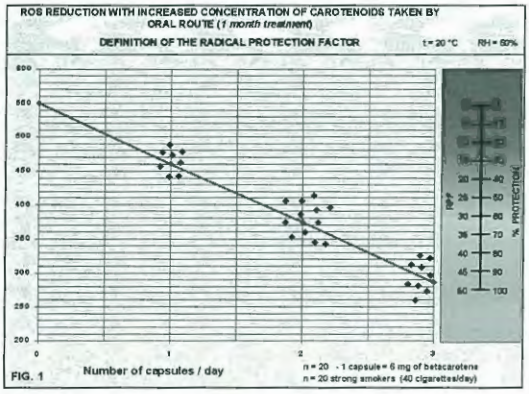

Fig. 1

RESULTS AND COMMENTS

As it is possible to see from Tab. I, the values of ROS, found in the smokers, seems to change from mg 27.04 ± 0.81 to 30.43 ± 0.74 up to mg 43.84 ± 0.66 ($p<0.05$) in depending of the number of cigarettes smoked during the day: 20, 30 or 40.

In fact, as it is known, the presence of carotenoids, found in the smokers' blood, decreases over 50% for the ROS provoked by the combustion of the tobacco substances.

Moreover, it can be observed an increasing of free radicals that seems to be directly proportional to the cigarettes smoked (9).

These data perfectly agree with what Carratelli (10) set in evidence, underlining how the smoke and the stress, or the beginning of different pathologies, can influence, in a remarkable way, the presence of free radicals in the blood serum. From that, the possibility to classify the different pathologic conditions, or the simple stress condition, in relation to the ROS checked (Tab. II) in the serum (10, 11).

What is interesting to observe is the positive influence, towards the ROS, not only of carotenoids but also of polyphenols, and vitamin C and E.

Table II

BASELINE VALUES: U.CARR	
300 to 320 CARR U.	Borderline value
320 to 340 CARR U.	Slight oxidative stress
340 to 400 CARR U.	Oxidative stress
400 to 520 CARR U.	Heavy oxidative stress
Above 500 CARR U.	Very heavy oxidative stress

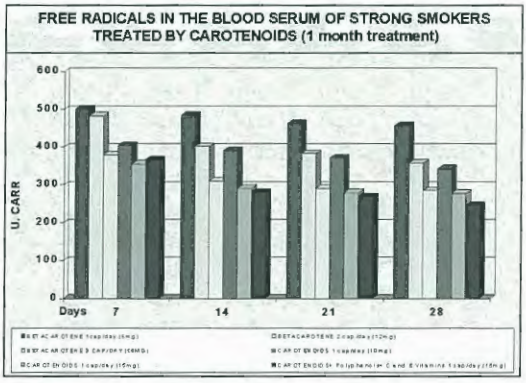

Fig. 2

1 Trade name Betaeffe Plus
2 Trade name Betaeffe Complex

In fact, from the Tab. III and Fig. 2, it can be easily observed how the assumption by oral route of an antioxidant, such as Betacarotene, at the dosage of 6 mg/day for a month, can reduce of about 16% ($p < 0.05$) the ROS content in the serum blood of strong smokers (RPF 15).
The above reduction is even higher in a daily intake of 12 mg (RPF 30) and reaches values of 50%, if increasing the dosage at 18 mg per day (RPF 50).
What is interesting to underline is that, even with just 15 mg/ day of carotenoids is possible to obtain a reduction of the ROS of about 50 % ($p< 0.05$), reduction increasing in a remarkable way ($p < 0.05$), when the carotenoids are added with polyphenols, vitamin C and E (RPF 60) (Tab. III).
In conclusion by means of this study, following the suggestion of Herling et al. (8), it has been tried to determine the RPF of the dietary supplements in order to classify their activity towards the ROS in the serum.
It was defined a conversion table through which it seems possible to pre-determine the RPF in depending of the more or less activity they solve towards free radicals present in the blood serum (Fig. 1).
This way it seems possible to label the diet by RPF values strictly depending on the different needs, as it already happens with the sun products. At the same time it is possible to classify diet supplements as "nutriceuticals" and to demonstrate their efficacy.

Table III

EFFECT OF 1 MONTH TREATMENT REGIMEN ON RPF VALUE IN STRONG SMOKERS (40 cigarettes/day)

TREATMENT (1)	REGIMEN	GROUP	ROS	RPF
6 mg Betacarotene	1 cap/day for a month	I	36.56 ± 0.072	15
12 mg Betacarotene	2 cap/day for 1 month	II	26.84 ± 0.081	30
18 mg Betacarotene	3 cap/day for 1 month	III	22.30 ± 0.059	50
10 mg Carotenoids	1 cap/day for 1 month	IV	27.30 ± 0.034	35
15 mg Carotenoids	1 cap/day for 1 month	V	21.80 ± 0.046	55
15 mg Carotenoids + C and E Vitamins + Polyphenols	1 cap/day for a month	VI	16.30 ± 0.018	60

(1) All treatments consisted of a diet supplement containing BETACAROTENE or various concentrations of CAROTENOIDS or/and vitamins and polyphenols

Tab. II

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