BOTANICALS FOR INNOVATIVE COSME-NUTRICEUTICALS

Aldo Cristoni and Paolo Morazzoni
Scientific Dept., Indena SpA, Milan, Italy

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Summary

Cutaneous aging is a complex biological process affecting various layers of the skin, but the major changes are seen in the dermis. There are two biologically independent aging processes that occur simultaneously. The first is intrinsic aging and affects skin as it probably affects the internal organs. The second is extrinsic aging, the results of exposure to the elements, primarily ultraviolet irradiation.

There is the need to protect the skin from premature aging by tools able to counteract the action of the proteases on the dermis and the disappearance of the antioxidant defences from the skin.

On the basis of recent findings it is possible to suggest the use of some active ingredients of botanical origin which, besides to be strong and bioavailable antioxidants, are well known to be vascular protectors and anti-senescence as well.

LEUCOSELECT™ PHYTOSOME® is the linkage between the pharmaceutical usage of natural polyphenols in the field of vascular protection and the epidemiological evidences on their high dietary intake and low incidence of chronic vascular diseases.

Moreover, it is a potent quencher of reactive oxygen species, it preserves the integrity of PUFAs, and it prevents the erythema development.

LEUCOSELECT™ PHYTOSOME® is also a bioavailable form, both by oral and topical route of administration, which guarantees grape procyanidins the reaching of target tissues. There they can exert their antioxidant and antiproteasic properties, having a potential role in the prevention of photoaging and, probably, photocarcinogenesis.

Riassunto

L’invecchiamento cutaneo è un complesso processo biologico che influenza i vari strati della pelle; i maggiori cambiamenti avvengono però nel derma. Vi sono due processi di invecchiamento che avvengono simultaneamente. Il primo è l’invecchiamento intrinseco e influenza la pelle come pure gli organi interni. Il secondo è l’invecchiamento estrinseco, risultato dell’esposizione agli elementi, principalmente le radiazioni ultraviolette.

Vi è la necessità di proteggere la pelle dall’invecchiamento prematuro con strumenti capaci di contrastare l’azione delle proteasi nel derma e la scomparsa delle difese antiossidanti della pelle.

Sulla base di dati recenti è possibile suggerire l’uso di alcuni ingredienti attivi di origine vegetale che, oltre ad essere antiossidanti potenti e biodisponibili, sono ben noti come protettori vascolari e sostanze anti-senescenza.

Il LEUCOSELECT™ PHYTOSOME® è l’anello fra l’uso farmaceutico dei polifenoli naturali nel
Il campo della protezione vascolare e le prove epidemiologiche basate sulla loro elevata assunzione con la dieta, accompagnata da una bassa incidenza di malattie vascolari croniche.

Inoltre, è un potente "quencher" di specie reattive dell’ossigeno, protegge l’integrità dei PUFA, e previene lo sviluppo dell’eritema.

Il LEUCOSELECT™ PHYTOSOME® è anche una forma biodisponibile, sia per via di somministrazione orale che topica, garantendo alle procianidine il raggiungimento dei tessuti bersaglio. Esse possono così realizzare le loro proprietà antioxidanti e antiproteasiche, svolgendo un importante ruolo potenziale nella prevenzione del fotoinvecchiamento e, con tutta probabilità, della fotocarcinogenesi.
INTRODUCTION

A remarkable new phenomenon is occurring throughout the world: older ages are becoming far more common; and in several countries the fastest growing age group is the oldest. Many fears about aging are related to the concept that everything declines with aging. In fact, a number of important physiologic variables do decline with aging mainly affecting central nervous and cardiovascular systems, and also the skin. Cutaneous aging is a complex biological process affecting various layers of the skin, but the major changes are seen in the dermis. There are two biologically independent aging processes that occur simultaneously. The first is intrinsic aging and affect skin as it probably affects the internal organs. The second is extrinsic aging, the results of exposure to the elements, primarily ultraviolet irradiation (Figures 1, 2). In

![UV-DAMAGES](image)

**Fig. 1**

**HYPOTHETICAL MODEL OF THE PATHOPHYSIOLOGY OF DERMAL DAMAGE AND PHOTOAGING INDUCED BY UV IRRADIATION**

![Hypothetical Model](image)


**Fig. 2**
areas exposed to the sun, particularly the face and the backs of the hands, damage from photoaging is superimposed on tissue degeneration from innate aging. Thus, the most noticeable changes on facial and neck skin, the primary area that patients are concerned about, results from a combination of intrinsic and extrinsic aging processes.

From a biochemical standpoint, photoaging is thought to be provoked mainly by:

- the action of some proteases, mainly metalloproteases, overproduced by keratinocytes and fibroblasts because of the interaction with ultraviolet irradiation (these proteases are degradative enzymes able to irreversibly damage collagen, elastin and hyaluronic acid, bringing about dermis scars and visible wrinkles);
- the oxidative stress, able to eliminate the normal skin antioxidant defences in a very short time.

On the basis of these general considerations, it looks clear the need to protect the skin from premature aging by tools able to counteract the action of the proteases on the dermis (increasing at the same time the capability of fibroblasts to produce and release new collagen fibers) and the disappearance of the antioxidant defences from the skin.

Even though it is maybe impossible to totally abolish skin damage due to age, on the basis of recent findings it is possible to suggest the use of some active ingredients of botanical origin which, besides to be strong and bioavailable antioxidants, are well known to be vascular protectors and anti-senescence as well.

In formulating cosmetic products containing these herbal derivatives, the main problem is to guarantee the better possible bioavailability, in order to be sure that the active principles effectively can reach their site of action: in this case, the dermis. Quite surprisingly, the experience demonstrated that both by oral and topical route the vehiculation with the phospholipids can improve absorption of several classes of substran-

ces. In the first case, because gastrointestinal absorption is favored when the substance is in a lipophilic form, in the second, because this form is able to enter the skin barrier. So, conceptually there are no significant differences between treating the dermis with a topical or an oral formulation, provided that the actives arrive in a concentration able to give the desired effects.

**FROM VITAMIN P TO THE "FRENCH PARADOX"**

Some decades ago Hungarian investigators discovered that flavonoids, commonly present in the human diet, were provided with relevant properties in respect of the cardiovascular system. Flavonoids and other polyphenols were than described to exert a sort of vitaminic action, which the investigators called vitamin P activity, mainly connected with the capacity to improve capillary resistance and to normalize an altered capillary permeability. In the following years, a controversial debate took place in the scientific community about the validity of the term vitamin P, and finally this denomination was rejected since the role of polyphenols were recognized to be not comparable to that of vitamins. Nevertheless what remained well demonstrated by a number of pharmacological tests, pharmacokinetics evidences and clinical trials was the tropism of some polyphenols for the cardiovascular system. Within this huge family of natural products, some specific compounds such as the case of grape procyanidins, demonstrated a particularly elevated specificity in the targeting to the cardiovascular system. This constituted the basis for the development of prescription drugs containing standardized extracts from grape seeds (the part of the plant particularly rich in procyanidins) and devoted to the relief of disturbances of the microcirculation. These pharmaceuticals are widely used in European countries and particularly in France they are still within the top reimbursed products.
Recently, another interesting finding coming in this case from epidemiology suggested that grape polyphenols, present also in red wine, could act as preventive agents in respect of the development of chronic diseases of the cardiovascular system, such as atherosclerosis (2).

This evidence came out mostly from the evaluation that the low incidence of cardiovascular diseases in France, is paralleled by a very high dietary intake of lipids, but concomitantly also by an elevated consumption of red wine (Figure 3). Scientists agree that this last event well explains the apparent “French Paradox” and they suggest that the “antioxidant properties” of red wine polyphenols could be good candidates as responsible for the preventive effect (3,4).

Nowadays, hundreds of papers are available dealing with the antioxidant properties of natural polyphenols, most of the studies being conducted in vitro and concentrated on monomeric products. In this respect studies on grape procyanidins constitute a unique among natural antioxidants since they deal with oligomeric polyphenols and include both in vitro and in vivo investigations.

At the light of these considerations it is not an overestimation to indicate grape procyanidins as a really unique example of pharmacologically and clinically proved biologically-active antioxidant, provided with a specific indication in the prevention and therapy of cardiovascular diseases.

**GRAPE SEEDS EXTRACT AS A STANDARDIZED SOURCE OF GRAPE PROCYANIDINS**

Most of the studies performed on grape procyanidins were made possible by the availability of standardized extracts which could guarantee the constancy of composition of a so complex chemical mixture. Recently, the definition of the industrially produced grape seeds extract LEUCOSELECT™ has been completely elucidated by using fractionation with Sephadex® and by analyzing the isolated fractions with HPLC-UV, GPC, HPLC-TSP MS and ES-MS (5).

The composition of LEUCOSELECT™ (Figures 4, 5) has the following pattern:
- (+)-catechin, (-)-epicatechin (15%)
- (-)-epicatechin gallate, dimers, trimers, tetramers and their gallates (80%)
- pentamers, hexamers, heptamers and their gallates (5%)
Botanicals for innovative cosmenutriceuticals

**HPLC-UV PROFILE OF LEUCOSELECT™**

![HPLC-UV Profile](image)


**Fig. 4**

**GPC PROFILE OF LEUCOSELECT™**

1. Monomers
2. Monomers, dimers and their gallates
3. Trimers, tetramers... heptamers and their gallates

![GPC Profile](image)


**Fig. 5**
LEUCOSELECT™: ONE OF THE
MOST EFFECTIVE NATURAL
ANTIOXIDANTS

The antioxidant effect seems to be responsible for most of the biological properties reported for grape procyanidins. In this light the availability of a standardized extract such as LEUCOSELECT™ allowed the detailed in vitro definition of the antioxidant profile along with other biochemical properties such as the inhibitory effect on several proteases (including collagenase and jaluRonidase) which has been proved (Figure 6) in several experimental models (6,8):

- DPPH scavenging activity (Figure 7)

\[
\begin{array}{|c|c|c|}
\hline
\text{Protease} & \text{LEUCOSELECT™} & \text{Catechin} \\
\hline
\text{Xanthine oxidase} & 2.40 & 300 \\
\text{Elastase} & 4.24 & \text{uneffective} \\
\text{Collagenase} & 36.00 & 1800 \\
\text{Hyaluronidase} & 60.00 & \text{uneffective} \\
\text{β-Glucuronidase} & 1.10 & 75 \\
\hline
\end{array}
\]

Fig. 6

- hydroxyl radical entrapping capacity after water sonolysis (ESR study)
- superoxide anion quenching action in a non-enzymatic generating system
- peroxyl radical scavenging activity in PCL (phosphatidylcholine liposomes) and MLM (methyl-linoleate micelles) stimulated by ultrasounds and UV
- iron (II)/copper (II)-chelating properties
- sparing effect on α-tocopherol
- regenerating effect on α-tocopherol
- protection of rabbit heart from ischemia/reperfusion injury
- stimulation of prostacyclin release from isolated rabbit hearts

This panel of properties makes LEUCOSELECT™, at least in vitro, a complete natural antioxidant and from a biochemical standpoint one of the most efficient among botanical derivatives known until now. This was a strong basis to investigate the activity in vivo.

For this purpose we relied on our previous experience in the field of polyphenols which, once isolated, are very well known to be generally provided with a very low and erratic bioavailability (Table 1). In order to overcome this gap, we investigated several formulations and among these, one in particular (resembling the natural affinity of polyphenols for phospholipidic substrates), succeeded in improving the absorption at gastrointestinal level. This new formulation, PHYTOSOME®, is based on the complexation between the botanical derivative and the polar heads of the soy phospholipidic mixture which has used as lipidic vehicle (Figure 8).

By using this process, we obtained LEUCOSELECT™ PHYTOSOME® (EP 0 275 224; US Patent 4, 963, 527; WO 99/29331) which has been tested in vivo for its antioxidant properties both in experimental animals and in human volunteers.
MAJOR GAPS FOR THE USAGE OF NATURAL POLYPHENOLS IN VIVO

- Extensive intraluminal degradation
- Poor absorbability due to chemical-physical characteristics
- Intensive presystemic metabolism

Tab. 1

EFFECT OF COMPLEXATION WITH PHOSPHATIDYLCHOLINE ON THE BIOAVAILABILITY OF NATURAL POLYPHENOLS

Mean plasma levels of total silybin after oral administration of 200 mg/kg of silybin and silybin-phosphatidylcholine complex in rats (n=6)


Fig. 8
LEUCOSELECT™ PHYTOSOME®:
THE BIOAVAILABLE ANTI-OXIDANT FOR THE PROTECTION OF THE CARDIOVASCULAR SYSTEM

Several investigations have been performed in order to establish the capacity of the product to exert its properties after oral administration to both experimental animals and human volunteers:

• effect on total antioxidant capacity and physiological antioxidant defences of plasma in young and old rats (Figures 9, 10) fed on diet containing LEUCOSELECT™ PHYTOSOME® at the concentration of 2.4% for 30 days (10)
• effect on ischemia-reperfusion induced damages (Figures 11, 12) in the heart of rats fed on diet containing LEUCOSELECT™ PHYTOSOME® at the concentration of 2.4% for 30 days (10)
• effect on experimental atherosclerosis induced in rabbits by using balanced cholesterol-rich diets with controlled intermediate conditions of hypercholesterolemia (11)
• effect on experimental atherosclerosis induced in rabbits by using a cholesterol-rich diet with severe condition of hypercholesterolemia (12)
• effect on total antioxidant capacity and physiological antioxidant defences of plasma in healthy volunteers (Figure 13) with controlled dietary supply of polyphenols (13)
• effect on plasma oxidative status in healthy volunteers after a fat-rich meal (14)
• effect on LDL-susceptibility to oxidation (Tables 2, 3) in aged smoking men (15)
• effect on oxidative stress in diabetic (NIDDM) patients (16)
• preventive effect on the UV erythema (Figure 14) in healthy volunteers (17)

CONCLUSION

LEUCOSELECT™ PHYTOSOME® is the linkage between the pharmaceutical usage of natural polyphenols in the field of vascular protection and the epidemiological evidences on their high dietary intake and low incidence of chronic vascular diseases.

Moreover, it is a potent quencher of reactive oxygen species, it preserves the integrity of PUFA's, and it prevents the erythema development. LEUCOSELECT™ PHYTOSOME® is also a bioavailable form, both by oral and topical route of administration, which guarantees grape procyanidins the reaching of target tissues. There they can exert their antioxidant and antiprotease properties, having a potential role in the prevention of photoaging and, probably, photo-carcinogenesis.
Young rats: total plasma antioxidant capacity (TRAP)

Fig. 9

Aged rats: total plasma antioxidant capacity (TRAP)

Fig. 10
Young rats: creatine kinase release in the perfusate

R. Maffei Facino et al.: Life Sciences 64, 627 (1999)

Aged rats: creatine kinase release in the perfusate

R. Maffei Facino et al.: Life Sciences 64, 627 (1999)
**EFFECT OF LEUCOSELECT™ PHYTOSOME® (300 mg/daily as LEUCOSELECT™) ON TOTAL ANTIOXIDANT PLASMA CAPACITY (TRAP) IN HEALTHY VOLUNTEERS**

![Graph showing the effect of Leucoselect™ Phytosome on total antioxidant plasma capacity (TRAP).](image-url)

*Fig. 13*

**EFFECT OF LEUCOSELECT™ PHYTOSOME® ON LDL-SUSCEPTIBILITY TO OXIDATION IN AGED SMOKING MEN**

**RANDOMIZED DOUBLE-BLIND CROSS-OVER STUDY**

**SERUM TOTAL CHOLESTEROL, LDL-CHOLESTEROL, HDL-CHOLESTEROL, TRIGLYCERIDES**

*(baseline and after 4 weeks of treatment)*

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Placebo baseline</th>
<th>Placebo week 4</th>
<th>LEUCOSELECT™ PHYTOSOME® baseline</th>
<th>LEUCOSELECT™ PHYTOSOME® week 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total cholesterol (mg/100 mL)</td>
<td>235 ± 35</td>
<td>234 ± 34</td>
<td>228 ± 39</td>
<td>235 ± 37</td>
</tr>
<tr>
<td>LDL-cholesterol (mg/100 mL)</td>
<td>160 ± 34</td>
<td>160 ± 33</td>
<td>156 ± 33</td>
<td>160 ± 36</td>
</tr>
<tr>
<td>HDL-cholesterol (mg/100 mL)</td>
<td>42 ± 11</td>
<td>42 ± 10</td>
<td>43 ± 11</td>
<td>43 ± 11</td>
</tr>
<tr>
<td>Triglycerides (mg/100 mL)</td>
<td>169 ± 66</td>
<td>160 ± 56</td>
<td>147 ± 55</td>
<td>163 ± 78</td>
</tr>
</tbody>
</table>


**TAB. II**
### EFFECT OF LEUCOSELECT™ PHYTOSOME® ON LDL-SUSCEPTIBILITY TO OXIDATION IN AGED SMOKING MEN

**RANDOMIZED DOUBLE-BLIND CROSS-OVER STUDY**

**FLUORESCENT LIPIDIC PRODUCTS (FLP), LDL-OXIDATIVE PARAMETERS AND MALONDIALDEHYDE**

(baseline and after 4 weeks of treatment)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Placebo</th>
<th>LEUCOSELECT™ PHYTOSOME®</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>baseline</td>
<td>week 4</td>
</tr>
<tr>
<td><strong>FLP (RFU/mL plasma)</strong></td>
<td>20.2± 6.0</td>
<td>20.3± 3.9</td>
</tr>
<tr>
<td><strong>Lag phase (min)</strong></td>
<td>59.9±13.0</td>
<td>57.7± 9.8</td>
</tr>
<tr>
<td><strong>Propagation Rate (nmol/min/mg LDL-C)</strong></td>
<td>7.2± 1.3</td>
<td>7.5± 1.3</td>
</tr>
<tr>
<td><strong>Malondialdehyde (nmol/mg proteins)</strong></td>
<td>0.56±0.10</td>
<td>0.57±0.08</td>
</tr>
</tbody>
</table>

RFU: Relative Fluorescence Unit. *p < 0.005 vs baseline. **p < 0.05 vs Placebo. ***p < 0.005 vs Placebo.


### MED VALUES ON LEUCOSELECT™ PHYTOSOME® PRETREATED HUMAN SKIN

- **Placebo**: 1.44 ± 0.09 *p < 0.01*
- **5% LEUCOSELECT™ PHYTOSOME®**: 1.84 ± 0.13

Fig. 14
References

9) EP 0 275 224; US Patent 4, 963, 527.

Author Address:
Dr. Aldo Cristoni
Viale Ortles, 12 - 20139 Milano - Italy
E-mail:aldo.cristoni@indena.com
Fax. +39.02.57496290

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