ELSEVIER

International Journal of Pharmaceutics 175 (1998) 85–94

international journal of pharmaceutics

Influence of different penetration enhancers on in vitro skin permeation and in vivo photoprotective effect of flavonoids

Antonella Saija [a,*], Antonio Tomaino [a], Domenico Trombetta [a], Marcella Giacchi [b], Anna De Pasquale [a], Francesco Bonina [b]

[a] *Department Farmaco-Biologico, School of Pharmacy, University of Messina, Contrada Annunziata, Messina, 98168, Italy*
[b] *Department of Pharmaceutical Sciences, School of Pharmacy, University of Catania, Catania, Italy*

Received 24 April 1998; received in revised form 13 July 1998; accepted 30 July 1998

Abstract

In the present study the permeation of two flavonoids (naringenin and hesperetin) through excised human skin mounted in Franz diffusion cells and its possible optimization following skin pretreatment with two penetration enhancers (D-limonene and lecithin) were determined. Hesperetin and naringenin were able to permeate through excised human skin; moreover, skin pretreatment with D-limonene and lecithin increased, to different degrees, their cutaneous permeation. On the basis of findings obtained in these in vitro experiments, we designed a schedule for a series of in vivo experiments, in which the protective effect of topically applied naringenin and hesperetin against UV-B-induced skin damage was assessed monitoring the extent of erythema in human volunteers by means of reflectance spectrophotometry. Hesperetin and naringenin from formulations containing the flavonoid alone were completely ineffective in decreasing UV-B-induced erythema. Furthermore, both D-limonene and lecithin have enhanced, to a significant extent, the photoprotective activity of naringenin and hesperetin. Taken together, these data demonstrate that hesperetin and naringenin may be successfully employed as topical photoprotective agents. However their topical activity needs to be optimized by using suitable penetration enhancers.

Keywords: Flavonoids; Penetration enhancers; In vitro skin permeation; UV-B radiation-induced skin erythema; Human

1. Introduction

Skin is a biological interface with the environment and, due to its barrier function, is a potential target organ of oxidative stress from external

* Corresponding author. Tel.: + 39 90 6766530; fax: + 39 90 3533142; e-mail: saija@pharma.unime.it

0378-5173/98/$ - see front matter
PII S0378-5173(98)00259-2

insults, such as UV-irradiation, ozone, ionizing radiation, various toxic chemicals, etc. This oxidative stress, which could be an initiator in the pathogenesis of skin cancer and photoaging (Dalle Carbonare and Pathak, 1992; Darr and Fridovich, 1994), is referred to as a condition of imbalanced prooxidant/antioxidant equilibrium, in favor of the former. In fact prolonged skin exposure to UV light results in a severe decrease of its antioxidant content and in a striking formation of active oxygen intermediates (Fuchs and Packar, 1991; Shindo et al., 1994); moreover, in chronically sun-exposed human skin products of lipid peroxidation are elevated (Mefferth et al., 1976).

Topical administration of antioxidants provides an efficient way to enrich the endogenous cutaneous protection system and thus may be a successful strategy for diminishing ultraviolet radiation-mediated oxidative damage in the skin (Iurkiewicz et al., 1995; Montenegro et al., 1995, 1996; Bonina and Montenegro, 1996; Weber et al., 1997).

Flavonoids, a group of plant polyphenolic compounds possessing broad biological properties (Middleton and Kandswami, 1994), have been demonstrated to exert beneficial effects on some diseases involving uncontrolled lipid peroxidation (Middleton and Kandswami, 1994). The capability to interact with protein phosphorylation and the antioxidant, iron-chelating and free radical scavenging activity may account for the wide pharmacological profile of flavonoids (Saija et al., 1995a; van Acker et al., 1996).

Recently, much research has been focused on the potential use of these drugs as free radical scavengers to prevent oxidative skin damage (Schoemaker et al., 1995; Mortimer, 1997) and their topical application has met with considerable interest (Della Loggia et al., 1986; Casley-Smith et al., 1993; Li and Birt, 1996; Li et al., 1996).

However, to date little work has been done to assess the in vivo protective effect of topically applied flavonoids against photooxidative stress in the skin.

In the present paper we investigated the ability of topically applied flavonoids to reduce, in human volunteers, UV-B-induced skin erythema (one of the most suitable models for studying in vivo skin damage after acute UV exposure and a useful tool to assess radical scavenger activity of topically applied compounds). The flavonoids tested were chosen because they had been proven, in a previous in vitro study, to be good candidates for successful employment as protective agents against photooxidative damage (Bonina et al., 1996; Kootstra, 1994). Moreover, flavonoids show the additional advantages of possessing good anti-inflammatory activities (Middleton and Kandswami, 1994) and being free of side-effects and harmless to the skin (Jager et al., 1988).

Suitable percutaneous absorption is known to be an essential requirement for satisfactory topically applied photoprotective agents (Bonina et al., 1996) and may be improved by selecting the appropriate penetration enhancers (Barry, 1988, 1991). Thus in this study we evaluated the influence of some penetration enhancers on the photoprotective effect of the flavonoids tested.

The schedule of in vivo experiments was designed on the basis of findings obtained in preliminary in vitro experiments, carried out to determine the flavonoid permeation profile through excised human skin and its possible optimization following skin pretreatment with some appropriate enhancers.

2. Materials and methods

2.1. In vitro skin permeation experiments

The experiments were carried out according to the previously described method (Bonina and Montenegro, 1994). Briefly, adult human skin samples (mean age 33 ± 6 years) were obtained from breast reduction operations. Subcutaneous fat was trimmed and the skin samples were immersed in distilled water at 60 ± 1°C for 2 min; then stratum corneum and epidermis (SCE) were peeled off, since the dermis in vitro can act as a significant additional barrier to the absorption of lipophilic drugs (Bronaugh and Stewart, 1984). The SCE samples were dried at room temperature in a desiccator maintained at approximately 25%

RH. The dried samples were wrapped in aluminum foil and stored at 4 ± 1°C until use. Samples of dried SCE were rehydrated by immersion in distilled water at room temperature for 1 h before being mounted in Franz diffusion cells (LGA, Berkeley, CA, USA). The exposed skin surface area was 0.75 cm^2 and the receptor volume was 4.7 ml. The receiving compartment contained ethanol/water solution (1:1, v/v) to ensure sink conditions (Touitou and Fabin, 1988). The receiving solution was stirred and thermostated at 35°C during the experiments. Skin barrier integrity of the SCE samples used in this study was assessed by determining their tritiated water permeability coefficient (K_p). K_p values were found to be $1.5 \pm 0.3 \times 10^{-3}$ cm/h and were consistent with those previously reported (Bronaugh et al., 1986; Bonina and Montenegro, 1994).

The enhancer effects on flavonoid (hesperetin, naringenin and quercetin) permeation was studied by the pretreatment technique (Bonina and Montenegro, 1994). Pretreatment was performed by applying on the skin surface 100 μl of the following enhancer solutions: D-limonene (1% w/v ethanol solution), lecithin (1% w/v aqueous solution). In order to determine if ethanol might have a significant enhancing effect, two controls were run; one consisted of pretreating the SCE sample with the same amount of water, the other with the same amount of ethanol. The enhancer solution was left on the SCE sample for 6 h and the excess enhancer wiped off with tissue paper. Then, 100 μl of a solution (4 mg/ml) of quercetin, naringenin or hesperetin in acetone were applied to the skin surface; after which the solvent was allowed to evaporate. Due to their poor solubility in aqueous medium, flavonoids were dissolved in acetone, which has been demonstrated not to affect human skin permeability (Bond and Barry, 1988). Each experiment was run in duplicate on three different skin donors. After 24 h all the receiving solution was withdrawn and analyzed for flavonoid content by means of an HPLC with UV/visible detection, to calculate drug cumulative amounts permeated through the skin during 24 h (Q_{24}). Statistical analysis was performed using Student's t-test. The effectiveness of penetration enhancers was determined by comparing, for each flavonoid, Q_{24} values in the presence and absence of enhancers; this was defined as the enhancement ratio (ER):

$$ER = \frac{\text{flavonoid } Q_{24} \text{ in the presence of enhancers}}{\text{flavonoid } Q_{24} \text{ in the absence of enhancers}} \quad (1)$$

2.2. HPLC analysis

The HPLC apparatus consisted of a Varian 5000 system (Varian, Walnut Creek, CA, USA) equipped with a 20-μl loop and a Polychrom 3060 UV/VIS detector (Varian). Integration of the chromatographic peaks was achieved with a 4290 integrator (Varian). Chromatography was performed on a Pecosphere HS-5 HC ODS column (particle size: 10 μm; 15 cm × 4.6 mm i.d.; Perkin-Elmer, Norwalk, CT, USA). The mobile phase was methanol-water (45:55) containing 1% acetic acid. The flow-rate was set at 1.0 ml/min. Each sample was filtered prior to injection using a Millex HV13 filter (Waters-Millipore Corporation, Milford, MA, USA) and an aliquot (20 μl) was injected into the HPLC apparatus. Detection was effected at 254 nm (quercetin), 282 nm (hesperetin), or 287 nm (naringenin).

2.3. In vivo photoprotective effect

2.3.1. Preparation of aqueous gel

Carbopol gels were prepared by dispersing Carbopol 934 (0.8% w/w as final concentration) and triethanolamine (0.9% w/w as final concentration) in the opportune amount of distilled water with constant stirring. Separately, flavonoids (1.0% w/w as final concentration) were solubilized in ethanol (50.0% w/w as final concentration). Both solutions were well mixed, and the resulting gels were stored at room temperature for 24 h under air-tight conditions prior to use. To prepare gels containing penetration enhancers, D-limonene (1.0% w/w as final concentration) or lecithin (1.0% w/w as final concentration) were dissolved in the ethanolic phase and in the aqueous phase respectively. The gel formulation used as control contained neither flavonoids nor penetration enhancers.

2.3.2. Instrument

UV-B-induced skin erythema was monitored by using a reflectance visible spectrophotometer X-Rite model 968, having 0° illumination and 45° viewing angle as previously reported (Montenegro et al., 1995). The instrument was calibrated with a supplied white standard traceable to the National Bureau of Standard's perfect white diffuser. The spectrophotometer was controlled by a computer, which performed all color calculations from the spectral data by means of a menu-driven suite of programs (Spectrostart) supplied with the instrument. Reflectance spectra were obtained over the wavelength range of 400–700 nm using illuminant C and 2° standard observer.

2.3.3. Protocol

In vivo experiments were performed on six volunteers of both sexes in the age range of 25–35 years. The volunteer subjects were fully informed about the nature of the study and the procedures involved. No subject was known to exhibit abnormal sensitivity to sunlight, or was taking any medication at the time of the study. The experiments were performed under standardized room conditions (22 ± 2°C and 40–50% relative humidity) after a resting time of 15 min. Skin erythema was induced by UV-B irradiation using an ultraviolet lamp model UVM-57 (UVP, San Gabriel, CA). This source emits in the range of 290–320 nm with an output peak at 302 nm. The fluence rate measured at the skin surface was 0.80 mW/cm^2. For each subject, the minimal erythemal dose (MED) was preliminarily determined and an irradiation dose corresponding to the double of the MED was used throughout the study.

For each subject, eight sites on the ventral surface of one forearm and six sites on the other were defined using a circular template (1 cm^2) and demarcated with permanent ink. Skin sites were exposed to UV-B irradiation and the preparations to be tested (50 mg) were spread uniformly on the sites by means of a solid glass rod. For each volunteer, two of the eight sites of each forearm were used as controls (applying the gel without active compounds) and the other six sites were treated with the flavonoid-containing formulations. The sites were then occluded for 6 h, using Hill Top chambers (Hill Top Research, Cincinnati, OH), to prevent any loss of material from the skin surface. After the occlusion period, the chambers were removed and the skin surfaces were washed to remove the gel and allowed to dry for 15 min; after which the induced erythema was monitored for 72 h.

From the spectral data obtained, the erythema index (*EI*) was calculated using an equation similar to that reported by Dawson et al. (1980):

$$EI = 100\left[\log\frac{1}{R_{560}} + 1.5\left(\log\frac{1}{R_{540}} + \log\frac{1}{R_{580}}\right) - 2\left(\log\frac{1}{R_{510}} + \log\frac{1}{R_{610}}\right)\right] \quad (2)$$

where $1/R$ is the inverse reflectance at a specific wavelength (560, 540, 580, 510 and 610 nm).

EI baseline values were taken at each designated site before UV-B irradiation of gel formulations and they were subtracted from the *EI* values obtained after UV-B exposure at each time point, to obtain ΔEI values. For each site, plotting ΔEI versus time the area under the curve (AUC_{0-72}) was computed using the trapezoidal rule.

To better compare the efficacy of the different formulations tested, the percentage inhibition of UV-B skin erythema (PIE) was calculated from AUC_{0-72} values using the following equations:

$$\text{Inhibition\%(PIE)} = \frac{AUC_{(C)} - AUC_{(T)}}{AUC_{(C)}} \times 100$$

where $AUC_{(C)}$ is the area under the response-time curve on the vehicle treated site (control) and $AUC_{(T)}$ is the area under the response-time curve on the drug-treated site. Statistical analysis was performed by using Student's *t*-test.

2.4. Drugs

Quercetin dihydrate, hesperetin and naringenin (analytical grade) were purchased from Extrasynthèse (Genay, France), D-limonene, soybean lecithin, perchloric acid, Tris–HCl, ethanol, methanol, chloroform, dimethylsulphoxide, acetone and acetic acid from Sigma-Aldrich (Milan, Italy), and carbopol 934 from Biochim (Milan, Italy).

Table 1
Effect of different penetration enhancers on cumulative amount permeated after 24 h through excised human skin (Q_{24}, $\mu g/cm^2$) and percentage of the dose absorbed (%D) of quercetin, hesperetin and naringenin

Pretreatment	Parameters	Flavonoids		
		Hesperetin	Naringenin	Quercetin
Water	Q_{24}	18.32 ± 3.75	14.75 ± 3.89	2.21 ± 0.28
	%D	4.63 ± 0.95	3.75 ± 0.99	0.59 ± 0.07
	ER	–	–	–
Ethanol	Q_{24}	18.45 ± 3.35	14.92 ± 3.81	2.15 ± 0.36
	%D	4.55 ± 0.84	3.67 ± 0.89	0.55 ± 0.09
	ER	–	–	–
D-Limonene	Q_{24}	29.31 ± 3.84**	42.83 ± 6.18**	2.54 ± 0.26
	%D	7.31 ± 0.98**	10.72 ± 1.57**	0.85 ± 0.08
	ER	1.60	2.90	–
Lecithin	Q_{24}	52.38 ± 7.19*	22.83 ± 2.47*	2.66 ± 0.29
	%D	13.10 ± 1.77*	5.74 ± 0.69*	0.70 ± 0.07
	ER	3.21	1.58	–

Enhancement ratio values (ER) represent the effectiveness of the penetration enhancers tested. Each experiment was run in duplicate on three different skin donors; results are expressed as mean ± S.D.
* $p < 0.05$ versus water; ** $p < 0.05$ versus ethanol.

3. Results and discussion

UV radiation penetrates deeply into the skin and topically applied drugs can afford a satisfactory photoprotection only if they are able to permeate through the stratum corneum and, thus, to reach deeper skin layers. In the first part of the present study the in vitro permeation profiles of naringenin, hesperetin and quercetin through excised human skin were evaluated.

As clearly shown by drug Q_{24} values (Table 1), hesperetin, naringenin and, at a very low degree, quercetin, were able to permeate through SCE membranes. In vivo and in vitro studies have demonstrated that the flavonoid apigenin is able to penetrate into deeper layers of mouse skin (Li and Birt, 1996; Li et al., 1996).

Although lipid-soluble substances are usually considered to penetrate the stratum corneum fairly rapidly, lipophilicity does not appear, in the present study, to be the key parameter in determining flavonoid skin permeation, since no relationship was observed between skin permeation values and liposolubility of the drugs tested. In fact, in a previous paper (Bonina et al., 1996), we calculated, by means of reverse-phase chromatographic retention times on octadecyl silica columns, log K' values, a parameter known to be well correlated to log octanol/water partition coefficients; the log K' values for naringenin, quercetin and hesperetin were 0.458, 0.510 and 0.530, respectively.

One way to improve low drug permeability through human skin is to use penetration enhancers (which include lipophilic solvents, surfactants of various structures, fatty acids and terpenes), which reversibly modify the barrier resistance of the skin (Barry, 1988, 1991). For this reason we also evaluated, in the same experimental system, flavonoid permeation after skin pretreatment with D-limonene and lecithin. These two penetration enhancers are already established as possessing low toxicity and being able to improve cutaneous permeation, and are accepted for cosmetic use (Bonina and Montenegro, 1994).

As may be noted from ER values (Table 1), D-limonene and lecithin increased, to different degrees, cutaneous permeation of hesperetin and naringenin. However they were unable to increase quercetin permeation through the skin.

Generally, the modes of action of skin penetration enhancers involve increased drug diffusivity through the skin by affecting the intercellular lipids or the intracellular proteins, or both, and/or

increasing the partitioning of the drug into stratum corneum (Barry, 1988, 1991). Enhancement effect of D-limonene and lecithin is, very likely, mainly due to the reduction of skin resistance to drug permeation and a consequent increase in permeant diffusion coefficient (Mahjour et al., 1990; Bonina and Montenegro, 1994; Yamane et al., 1995).

Thus the better permeation of hesperetin and naringenin achieved following skin pretreatment with D-limonene and lecithin should be due to increased diffusion coefficients of these highly lipophilic drugs (Saija et al., 1995b).

As to quercetin, its poor capability to permeate through excised human skin, also following pretreatment with penetration enhancers, might be explained by its absolute water insolubility. In fact, skin absorption of a drug is determined by its physicochemical properties; in particular, water solubility may play a role as important as lipophilicity in the skin permeation process, especially for very lipophilic drugs (Guy and Hadgraft, 1992).

Thus, on the basis of these in vitro findings, the successive in vivo experimental phase was carried out to evaluate the efficacy of naringenin and hesperetin from gel formulations containing D-limonene or lecithin. With the aim of determining if the penetration enhancers employed in our study had a significant photoprotective effect, some preliminary experiments with gels containing only D-limonene and lecithin (applied to the skin under the same experimental conditions described above) were run. However, on their own, both penetration enhancers proved to be ineffective in inhibiting UV-B-induced erythema (data not shown).

To assess the protective effect of naringenin and hesperetin against UV-B-induced skin erythema, the extent of erythema in human volunteers was monitored by means of reflectance spectrophotometry.

A brief methodological comment is needed before discussing the results. This reflectance method provides skin reflectance spectra, generally in the range of 400–700 nm, from which the values of different color space systems (CIELab, Lch, etc.) can be obtained using different CIE illuminants (C, D_{65}, D_{50}, A, etc.) and 2 or 10° illuminant observer. From spectral data it is possible to calculate, at different wavelengths, the relative reflectance or the logarithm of inverse reflectance (LIR), which indicates the absorption of skin chromophores (hemoglobin, melanin, etc.). The use of erythema index (*EI*) obtained from skin reflectance spectral values has been suggested for more accurate and reliable evaluations of skin erythema (Dawson et al., 1980; Andersen et al., 1991). Since skin erythema is due to increased hemoglobin content in skin vessels, *EI* values are calculated (Eq. (1)) by subtracting LIR values at 510 and 610 nm (mainly due to melanin absorption) from the sum of hemoglobin LIR values at 540, 560 and 580 nm, which represent the wavelengths of hemoglobin absorption peak (Dawson et al., 1980).

A typical time-course of erythema for skin sites exposed to UV-B radiation and then treated with gels containing flavonoids, with or without penetration enhancers, for one subject, is shown in Fig. 1. From ΔEI versus time plots, the area under the response-time curve (AUC_{0-72}) was computed using the trapezoidal rule; AUC_{0-72} values are reported in Table 2. As may be noted, hesperetin and naringenin from formulations containing the flavonoid alone were completely ineffective in decreasing UV-B-induced erythema, since no significant difference was observed in comparison with controls (sites treated with gel without active compounds). The presence of penetration enhancers in the formulation markedly modulates flavonoid capability to reduce skin erythema; in fact, both D-limonene and lecithin enhanced, to a significant extent, the photoprotective activity of naringenin and hesperetin.

These two flavonoids were previously shown to protect efficiently phosphatidylcholine liposomal membranes against UV radiation-induced peroxidation (Bonina et al., 1996). The lack of in vivo photoprotective activity observed in the present study may be attributed to an insufficient skin permeation of these drugs. Consistent with this hypothesis, naringenin and hesperetin elicit a significant protective effect against UV-B-induced skin erythema when administered together with

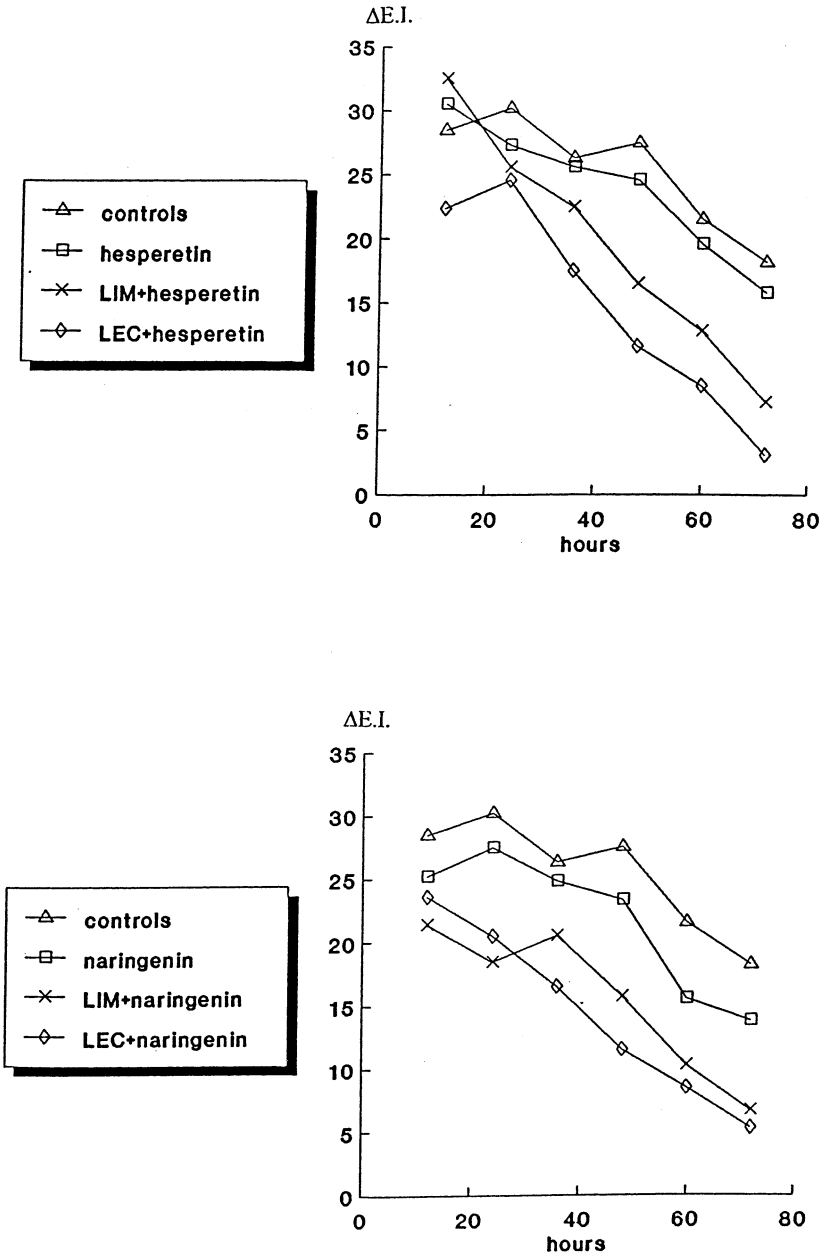

Fig. 1. Typical trend of erythema index variation (ΔEI) versus time for one subject. Gel formulations were applied on skin sites after exposure to UV-B radiation and left for 6 h.

D-limonene or lecithin, which have proved to be able to increase flavonoid in vitro skin permeation (Table 1). However, as shown in Table 2, there is no significant difference among AUC values calculated for formulations containing both flavonoids and penetration enhancers. The different order of skin permeation enhancement observed between in vitro and in vivo experiments may be explained by several hypotheses. Firstly, microcirculation may favour drug resorption at

Table 2
AUC_{0-72} values obtained treating, after skin exposure to UV-B radiation, the skin sites with gel formulations containing flavonoids, with or without penetration enhancers

Subject	AUC^{a}_{0-72}						
	Control	Hesperetin	Hesperetin + D-limonene	Hesperetin + lecithin	Naringenin	Naringenin + D-limonene	Naringenin + lecithin
A	1855	1732	1402	1079	1585	1138	1029
B	1626	1540	1171	793	1418	934	836
C	1475	1385	1059	832	1271	1053	974
D	1361	1462	983	690	1075	872	703
E	1715	1360	847	1022	1122	1121	1058
F	1659	1265	1213	624	1553	793	765
Mean	1615	1457	1112*§	840*§	1337	985*^	894*^
S.D.	160	149	177	164	197	128	134
PIE	–	9.8	31.1	48.0	17.2	39.0	44.6

[a] Each value represents the mean of two different sites in the same subject.
* $p<0.05$ compared with controls; § $p<0.05$ compared with hesperetin; ^ $p<0.05$ compared with naringenin.

the dermis. Secondly, drug protein binding and metabolism can occur within viable skin layers. Finally, biological responses are often observed after times not comparable with the in vitro lag-time of the drug tested.

Since the receptor fluid may be rate-limiting in the diffusion process and so have influenced the results obtained from in vitro experiments (Zatz, 1985), we carried out a series of preliminary experiments to evaluate the photoprotective activity of quercetin. The drug proved to be ineffective in reducing UV-B-induced erythema, also in presence of lecithin or D-limonene (data not shown). However quercetin was previously shown to possess strong effectiveness against UV-induced peroxidative degradation of liposomal membranes (Bonina et al., 1996) and appeared able to protect neurovascular structures in skin from oxidative damage (Skaper et al., 1997). Furthermore oral intake of quercetin prevents the UV-B-induced suppression of skin contact hypersensitivity in the mouse (Steerenberg et al., 1997). Thus other strategies known to increase drug penetration into the skin need to be taken into consideration so that topically applied quercetin might be employed to prevent oxidative skin damage.

In conclusion we have demonstrated, by means of in vitro and in vivo experiments, that hesperetin and naringenin may be successfully employed as topical photoprotective agents; however their topical activity needs to be optimized by using a suitable formulation.

References

Andersen, P.H., Abrams, K., Bjerring, P., Maibach, H.A., 1991. A time-correlation study of ultraviolet B-induced erythema measured by reflectance spectroscopy and laser Doppler flowmetry. Photodermatol. Photoimmunol. Photomed. 8, 123–128.

Barry, B.W., 1988. Action of skin penetration enhancers—the lipid protein partitioning theory. Int. J. Cosmet. Sci. 10, 281–293.

Barry, B.W., 1991. Lipid-protein-partitioning theory of skin penetration enhancement. J. Control. Release 15, 237–248.

Bond, J.R., Barry, B.W., 1988. Damaging effect of acetone on the permeability barrier of hairless mouse skin compared with that of human skin. Int. J. Pharm. 41, 91–93.

Bonina, F.P., Montenegro, L., 1994. Effects of some non-toxic penetration enhancers on in vitro heparin skin permeation from gel vehicles. Int. J. Pharm. 111, 191–196.

Bonina, F., Montenegro, L., 1996. In vivo evaluation of radical scavenger compounds in cosmetic formulations by means of skin reflectance spectrophotometry. SÖFW J. 122, 1–3.

Bonina, F., Lanza, M., Montenegro, L., Puglisi, C., Tomaino, A., Trombetta, D., Castelli, F., Saija, A., 1996. Flavonoids as potential protective agents against photo-oxidative skin damage. Int. J. Pharm. 145, 87–94.

Bronaugh, R.L., Stewart, R.F., 1984. Methods for in vitro percutaneous absorption studies: III. Hydrophobic compounds. J. Pharm. Sci. 73, 1255–1258.

Bronaugh, R.L., Stewart, R.F., Simon, M., 1986. Methods for in vitro percutaneous absorption studies: VII. use of excised human skin. J. Pharm. Sci. 75, 1094–1107.

Casley-Smith, J.R., Curri, S., Foldi, M., 1993. Topical treatment of acute hindlimb lymphedema of the rat using a troxerutin-phosphatidylcholine complex in liposomal-like microdispersion. Lymphology 26, 25–27.

Dalle Carbonare, M., Pathak, M.A., 1992. Skin photosensitizing agents and the role of reactive oxygen species in photoaging. J. Photochem. Photobiol. B. 14, 105–124.

Darr, D., Fridovich, I., 1994. Free radicals in cutaneous biology. J. Invest. Dermatol. 102, 671–675.

Dawson, J.B., Barker, D.J., Ellis, D.J., Grassam, E., Catterill, J.A., Fisher, G.W., Feather, J.W., 1980. A theoretical and experimental study of light absorption and scattering by in vivo skin. Phys. Med. Biol. 25, 696–709.

Della Loggia, R., Tubaro, A., Dri, P., Zilli, C., Del Negro, P., 1986. The role of flavonoids in the anti-inflammatory action of *Chamomilla recutita*. In: Cody, V., Middleton, E. Jr., Harborne, J.B. (Eds.), Plant Flavonoids in Biology and Medicine: Biochemical, Pharmacological and Structure-Activity Relationships. Alan R. Liss, New York, pp. 481–484.

Fuchs, J., Packar, L., 1991. Photooxidative stress in the skin. In: Sies, H. (Ed.), Oxidative Stress: Oxidants and Antioxidants. Academic Press, London, pp. 559–583.

Guy, R.H., Hadgraft, J., 1992. Percutaneous penetration enhancement: physico-chemical considerations and implications for prodrug design. In: Sloan, K.B. (Ed.), Prodrugs—Topical and Ocular Drug Delivery. Marcel Dekker, New York, pp. 1–16.

Iurkiewicz, B.A., Bissett, D.L., Buettner, G.R., 1995. Effect of topically applied tocopherol on ultraviolet radiation-mediated free radical damage in skin. J. Invest. Dermatol. 104, 484–488.

Jager, A., Wälti, M., Neftel, K., 1988. Side-effects of flavonoids in medical practice. In: Cody, V., Middleton, E. Jr., Harborne, J.B., Beretz, A. (Eds.), Plant Flavonoids in Biology and Medicine II: Biochemical, Cellular, and Medicinal Properties. Alan R. Liss, New York, pp. 379–394.

Kootstra, A., 1994. Protection from UV-B-induced DNA damage by flavonoids. Plant Mol. Biol. 26, 771–774.

Li, B., Birt, D.F., 1996. In vivo and in vitro percutaneous absorption of cancer preventive flavonoid apigen in different vehicles in mouse skin. Pharma. Res. 13, 1710–1715.

Li, B., Pinch, H., Birt, D.F., 1996. Influence of vehicle, distant topical delivery, and biotransformation on the chemopreventive activity of apigenin, a plant flavonoid, in mouse skin. Pharma. Res. 13, 1530–1534.

Mahjour, M., Mauser, B., Rashidbaigi, Z., Fawzi, M.B., 1990. Effect of egg yolk lecithins and commercial soybean lecithin on in vitro skin permeation of drugs. J. Control. Release 14, 243–252.

Mefferth, H., Diezel, W., Sonnichesen, N., 1976. Stable lipid peroxidation products in human skin: detection, ultraviolet light-induced increase, pathogenic importance. Experientia 32, 1937–1938.

Middleton, E. Jr., Kandswami, C., 1994. The impact of plant flavonoids on mammalian biology: implications for immunity, inflammation and cancer. In: Harborne, J.B. (Ed.), The Flavonoids. Advances in Research Since 1986. Chapman and Hall, Cambridge, pp. 619–652.

Montenegro, L., Bonina, F., Dederen, J.C., 1996. In vivo photoprotective effect of β-bis(carboxyethyl)germanium sesquioxide. J. Soc. Cosmet. Chem. 47, 307–313.

Montenegro, L., Bonina, F., Rigano, L., Giogilli, S., Sirigu, S., 1995. Protective effect evaluation of free radical scavengers on UVB induced human cutaneous erythema by skin reflectance spectrophotometry. Int. J. Cosmet. Sci. 17, 91–103.

Mortimer, P.S., 1997. Therapy approaches for lymphedema. Angiology 48, 87–91.

Saija, A., Scalese, M., Lanza, M., Marzullo, D., Bonina, F., Castelli, F., 1995a. Flavonoids as antioxidant agents: importance of their interaction with biomembranes. Free Rad. Biol. Med. 19, 481–486.

Saija, A., Bonina, F., Trombetta, D., Tomaino, A., Montenegro, L., Smeriglio, P., Castelli, F., 1995b. Flavonoid-biomembrane interactions: a calorimetric study on dipalmitoylphosphatidylcholine vesicles. Int. J. Pharm. 124, 1–8.

Schoemaker, J.H., Schoemaker, M.T., Zijlstra, H., van der Horst, F.A., 1995. Treatment of erythropoietic protoporphyria with hydroxyethylrutosides. Dermatology 191, 36–38.

Shindo, Y., Witt, E., Han, D., Packer, L., 1994. Dose-response effects of acute ultraviolet irradiation on antioxidants and molecular markers of oxidation in murine epidermis and dermis. J. Invest. Dermatol. 102, 470–475.

Skaper, S.D., Fabris, M., Ferrari, V., Dalle Carbonare, M., Leon, A., 1997. Quercetin protects cutaneous tissue-associated cell types including sensory neurons from oxidative stress induced by glutathione depletion: cooperative effects of ascorbic acid. Free Rad. Biol. Med. 22, 669–678.

Steerenberg, P.A., Garssen, J., Dortant, P.M., van der Vliet, H., Geerse, E., Verlaan, A.P., Goettsch, W.G., Sontag, Y., Bueno-de-Mesquita, H.B., Van Loveren, H., 1997. The effect of oral quercetin on UVB-induced tumor growth and local immunosuppression in SKH-1. Cancer Lett. 114, 187–189.

Touitou, E., Fabin, B., 1988. Altered skin permeation of highly lipophilic molecule: tetrahydrocannabinol. Int. J. Pharm. 43, 17–22.

van Acker, S.A.B.E., van den Berg, D.-J., Tromp, M.N.J.L., Griffoen, D.H., van Bennekom, W.P., van der Vijgh, W.J.F., Bast, A., 1996. Structural aspects of antioxidant activity of flavonoids. Free Rad. Biol. Med. 20, 31–42.

Weber, C., Podda, M., Rallis, M., Thiele, J.J., Trabber, M.J., Packer, L., 1997. Efficacy of topical applied tocoferols and tocotrienols in protection of murine skin from oxidative damage induced by UV-irradiation. Free Rad. Biol. Med. 22, 761–769.

Yamane, M.A., Williams, A.C., Barry, B.W., 1995. Effects of terpenes and oleic acid as skin penetration enhancers to-

wards 5-fluorouracil as assessed with time; permeation, partitioning and differential scanning calorimetry. Int. J. Pharm. 116, 237–251.

Zatz, J., 1985. Percutaneous absorption. In: Smolen, V.F., Ball, L.A. (Eds.), Controlled Drugs Bioavailability, vol. 3. Wiley, New York, pp. 185–239.