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ScienceDirect
International Journal of Pharmaceutics 336 (2007) 257–262
INTERNATIONAL JOURNAL OF PHARMACEUTICS
www.elsevier.com/locate/ijpharm

In vitro evaluation of quercetin-3-*O*-acyl esters as topical prodrugs

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Received 13 July 2006; received in revised form 1 December 2006; accepted 3 December 2006
Available online 6 December 2006

Abstract

Quercetin-3-*O*-acyl esters (I–VI) were synthesized and their usefulness as quercetin topical prodrugs was evaluated. Quercetin esters were assayed to determine their water stability and solubility, their susceptibility to undergoing enzymatic hydrolysis and their permeation through excised human skin. Quercetin ethyl (I) and hexyl (IV) esters proved poorly stable in aqueous media and they were not assayed further. Among the derivatives tested, quercetin propyl (II) and butyl (III) esters were more water-soluble than the parent drug. Esters II, III and V were readily hydrolyzed by human plasma and esters II and III penetrated excised human skin better than quercetin from aqueous saturated solutions. On the basis of the results obtained, esters II and III could be regarded as promising quercetin topical prodrugs.

Keywords: Quercetin esters; Topical prodrugs; In vitro skin permeation; Human skin

1. Introduction

Flavonoids, a group of naturally available plant phenolics, have been shown to possess several biological properties, such as antioxidant, hepatoprotective, antithrombotic, antihypertensive, anti-inflammatory, antiallergic, antitumor, bactericidal and antiviral activities (Morel et al., 1993; Middleton and Kandswami, 1994; Van Acker et al., 1996).

Due to their properties, flavonoids are widely used in pharmaceutical and cosmetic field beyond that in food industry. Many of their activities may be related, partially at least, to flavonoid ability to penetrate into the cell membrane thus affecting membrane-dependent processes, such as arachidonic acid metabolism, exocytotic histamine release, cyclic AMP phosphodiesterase activity and free radical-initiated lipoperoxidation (Baumann et al., 1980; Formica and Regelson, 1995).

Topical administration of flavonoids has met with considerable interest due to their wide pharmacological profile (Hormann and Korting, 1994). Among the flavonoids proposed for topical administration, quercetin has been reported to possess high antiradical and anti-inflammatory activities (Formica and Regelson, 1995), which were attributed to the presence of a cathecol moiety, a C_2–C_3 double bond and a 3-OH group in its structure (Van Acker et al., 1996; Katsarou et al., 2000).

A major problem in quercetin topical delivery is its low skin permeability, which could be due to its poor lipophilicity. Previous works (Bonina et al., 1996) on the in vitro topical antioxidant activity and skin permeation of three flavonoids (quercetin, hesperetin and naringenin) showed that the effectiveness in inhibiting UV-induced lipid peroxidation decreased in the order quercetin > hesperetin > narigenin but quercetin skin permeation was significantly lower than that of the other two flavonoids tested.

Among the different methods, which could be investigated to increase quercetin topical delivery, the prodrug approach represents one of the most promising. Topical prodrugs obtained by chemical modification of a drug into a bioreversible form are designed in order to improve drug bioavailability and hence therapeutic efficacy. Regeneration of the parent drug occurs in vivo by either enzymatic or chemical processes. Since the skin shows a high enzymatic activity, mainly due to esterase activity (Pannatier et al., 1978; Bickers and Kappas, 1980), many

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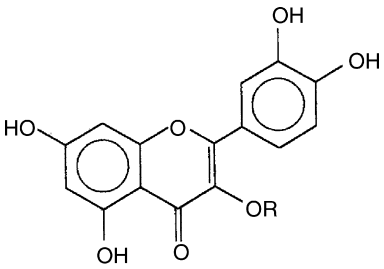
doi:10.1016/j.ijpharm.2006.12.003



I R = -$COCH_3$

II R = -$COCH_2CH_3$

III R = -$CO(CH_2)_2CH_3$

IV R = -$CO(CH_2)_4CH_3$

V R = -$CO(CH_2)_{10}CH_3$

VI R = -$CO(CH_2)_{12}CH_3$

Fig. 1. Chemical structure of esters I–VI.

ester prodrugs have been designed and thoroughly investigated to increase drug topical delivery (Chan and Li Wan Po, 1989; Sloan, 1989).

Recently, Saija et al. (2003) reported that quercetin-3-*O*-acyl esters with short acyl chains (quercetin-3-*O*-acetate and querctein-3-*O*-propionate) possessed in vitro antioxidant and photoprotective activities and were able to interact with phospholipid bilayers. The authors suggested that such quercetin derivatives could be considered good candidates as photoprotective agents. Further studies on quercetin esters showed that quercetin-3-*O*-palmitate was able to interact with phospholipid membranes mainly at level of the lipid domains of the bilayers (Sardone et al., 2004).

In this work, 3-*O*-acyl esters of quercetin (Fig. 1) were investigated in order to evaluate their usefulness as quercetin topical prodrugs. With this aim, quercetin-3-*O*-acyl esters were assayed to determine their water stability, their enzymatic hydrolysis rates and their permeation through excised human skin.

2. Materials and methods

2.1. Materials

Quercetin was obtained from Extrasyntase (Lyon, France). Acetonitrile and water used in the HPLC procedures were of LC grade and were obtained from Merck (Darmstadt, Germany). All other chemicals or solvents were of reagent grade.

2.2. Synthesis of quercetin-3-O-acyl esters

Quercetin-3-*O*-acyl-esters were synthesized as previously reported (Lambusta et al., 1993). Briefly, a two-step method combining both chemical and biological procedures was used. In the first step, quercetin was chemically transformed in a pentaester, which was selectively hydrolysed, in the second step, by means of a lipase to obtain a 3-*O*-acyl ester. Esters containing an acylic group ranging from C_2 to C_{14} were prepared (Fig. 1) and characterized as previously reported (Lambusta et al., 1993).

2.3. Chemical and enzymatic hydrolysis of quercetin-3-O-acyl esters

The chemical hydrolysis rate of quercetin 3-O-acyl esters was studied in solution of isotonic phosphate buffer, pH 7.4, at 37 °C. The disappearance of the ester was monitored by the HPLC method described below.

The enzymatic hydrolysis of quercetin 3-O-acyl esters was evaluated in human plasma at 37 °C as described elsewhere (Pignatello et al., 2005). Stock solutions were prepared by dissolving a weighed amount of each compound in methanol to give a concentration 3 mg/ml. A volume of 0.04 ml of this solution was added with 3.96 ml of prewarmed (37 °C) human plasma previously diluted to 80% with 50 mM phosphate buffer, pH 7.4, pre-thermostated at 37 °C (3.2 ml of human plasma contained about 175 mg of total protein as assesses by the dye binding method using bovine serum albumin).

The resulting solution was kept at 37 °C and 0.2 ml samples were withdrawn at intervals and added to 0.4 ml of cold (4 °C) acetonitrile to precipitate serum proteins. After centrifugation for 10 min at 10,000 rpm and at 5 °C, the supernatant was assayed by the HPLC method described below in order to determine the amount of quercetin and its acyl esters.

Pseudo-first-order hydrolysis rate constants for the chemical and enzymatic hydrolysis were calculated from the slopes of linear plots of the logarithm of residual quercetin esters against time.

2.4. Solubility

Quercetin and its esters water solubility was determined by stirring an excess of each derivative in water with a magnetic stirrer for 24 h at room temperature. Then the mixture was filtered using a Millex HV13 filter (0.22 μm, Waters-Millipore Corporation, Milford, MA, USA) and the concentration of each compound in its saturated solutions was determined by HPLC analysis.

2.5. Quercetin and esters I–VI lipophilicity

As previously reported (Bonina et al., 1996), oil/water partition coefficients can be estimated using reverse-phase chromatographic retention times (RT) due to the good relationship between log octanol/water partition coefficients and log capacity factor ($\log K$) values determined using octadecyl silica columns.

Each compound was dissolved in methanol (concentration 10 μg/ml). Aliquots of each solution were filtered using Millex HV13 filters (0.22 μm, Waters-Millipore Corporation) and analyzed by the HPLC method described below.

log K values were calculated according to the following equation:

$$\log K = \log\left(\frac{t_r - t_0}{t_0}\right)$$

where t_r was the retention time of the sample peak and t_0 was the retention time of a non-retained solvent peak.

The lipophilicity of quercetin and its esters was also calculated using the ACD Log P software package, version 5.15 (Advanced Chemistry Development Inc., Toronto, Canada) and the OSIRIS Property Explorer (www.actelion.com).

2.6. In vitro skin permeability of quercetin and its 3-O-acyl esters

Epidermal membranes were prepared according to the procedure described by Kligman and Christophers (1963). Briefly, subcutaneous fat was carefully trimmed from samples of adult human skin (obtained from abdominal plastic surgery; mean age 39 ± 7 years) and the skin was immersed in distilled water at 60 ± 1 °C for 2 min, after which the epidermis was removed from the dermis using a scalpel blade. Epidermal membranes were dried in a desiccator at approximately 25% RH. Samples of dried epidermis were rehydrated by immersion in distilled water for 1 h prior to the experiment. Skin samples were mounted in Franz type diffusion cells whose exposed skin surface area was 0.75 cm^2 and receptor volume was 4.5 ml. The receiving compartment was filled with ethanol/water 50/50 for ensuring pseudo-sink conditions by increasing the solubility of quercetin and its derivatives in the receiving phase. The use of such a solution as receptor phase has been already described in the literature to ensure solubility of poor water-soluble compounds in in vitro percutaneous absorption studies (Mueller, 1988; Touitou and Fabin, 1988; Bonina et al., 1991). Four hundred microliters of saturated water solution of quercetin and its derivatives II, III and V were applied to the skin surface and the experiments were run for 22 h. Samples of the receiving solution were withdrawn at 22 h and analyzed with the HPLC method described below in order to determine the amount of quercetin and its esters permeated.

Results are expressed as the mean ± S.D. and Student's t-test was used to evaluate the significance of the difference between mean values. Values of $P < 0.05$ were considered statistically significant. The enhancement factor (E.F.) was calculated as follows:

$$\text{E.F.} = \frac{\text{cumulative amount permeated applying the prodrug}}{\text{cumulative amount permeated applying the drug}}$$

2.7. HPLC analysis

The HPLC system consisted of a Varian ProStar model 230 (Varian, Milan, Italy) with autosampler Varian model 410 and a Galaxie software for data elaboration.

Quercetin and its derivatives I–IV were determined by HPLC using a Waters C_{18} Simmetry 4.6 mm × 25 cm reverse phase column and a mobile phase consisting of acetonitrile/acetic acid 0.17 M 35:65, under isocratic conditions. A similar HPLC analytical method has been reported for quercetin by Crozier et al. (1997). The analyses of derivatives V and VI were performed using different linear gradients. The introduction of promoieties with longer acyl chains in the structure of quercetin required the use of elution gradients in order to obtain acceptable RT values. The same gradients were applied also to quercetin to be sure that its peak did not overlap upon other eluting component peak.

For derivative V, the gradient started with acetonitrile/acetic acid 0.17 M 35:65, changed to acetonitrile/acetic acid 0.17 M 90:10 over 15 min and returned to the initial conditions over 10 min (gradient 1). For derivative VI, the gradient started with acetonitrile/acetic acid 0.17 M 35:65, changed to acetonitrile/acetic acid 0.17 M 100:0 over 15 min and then returned to the initial conditions over 15 min (gradient 2). All the analyses were carried out at room temperature at a flow rate of 1.0 ml/min. Twenty microliters of each samples were injected and the column effluent was monitored continuously at 375 nm for quercetin and 350 nm for its derivatives. The detection limit was 0.1 μg/ml. The retention times were: quercetin 3.7 min, I 4.3 min, II 5.9, III 10.7 min, IV 28.1 min, V (gradient 1) 15.6,

Table 1
Half-life in phosphate buffer and in human plasma ($t_{1/2}$), log capacity factor (log K), calculated partition coefficient (log P), water solubility (S_w), cumulative amount permeated through excised human skin after 22 h (Q_{22}) and enhancement factor (E.F.) for quercetin and its esters I–VI

Compound	$t_{1/2}$ (h)[a]		log K	log P[b]		S_w (μg/ml)	Q_{22} ± S.D.[c] (μg/cm^2)	E.F.
	Buffer pH 7.4	Human plasma		A	B			
Quercetin	N.D.[d]	N.D.	0.433	1.82	1.80	3.60	0.45 ± 0.12	1.0
I	34	N.D.	0.517	2.51	2.29	N.D.	N.D.	–
II	65	0.52	0.691	3.04	2.76	32.00	1.77 ± 0.19	3.9
III	142	1.05	0.985	3.57	3.22	231.00	5.37 ± 0.29	11.9
IV	24	N.D.	1.433	4.64	4.15	N.D.	N.D.	–
V	90	1.22	N.D.	7.82	6.93	2.55	N.DE[e]	–
VI	144	N.D.	N.D.	8.89	7.86	0.60	N.D.	–

[a] $t_{1/2}$ was calculated from the equation: $t_{1/2} = \ln 0.5 / k$, k being the pseudo-first-order rate constant.
[b] log P was calculated A, using the ACD Log P software version 5.11; B, using the OSIRIS Property explorer.
[c] Each experiment was run in duplicate on three different skin donors ($n = 3$).
[d] N.D., not determined.
[e] N.DE, not detectable.

VI (gradient 2) 16.5 min. The compounds were quantified by measuring the peak areas in relation to those of standards chromatographed under the same conditions.

3. Results and discussion

3.1. Chemical and enzymatic hydrolysis of quercetin-3-O-acyl esters

As reported in Table 1, esters I and IV showed a poor stability in water and no relationship was observed between length of the acyl chain and hydrolysis rate in water of the esters. Therefore, the different chemical stability of esters I–VI could be attributed to molecular spatial arrangements and/or intra/intermolecular interactions that could differently affect esters I–VI lability in aqueous medium.

Since ester' reconversion into the parent drug within the skin is essential for their successful use as topical prodrugs we evaluated the enzymatic cleavage of esters I–VI in human plasma. Other authors have reported the feasibility of using human plasma as a model to assess the hydrolysis rate of ester prodrugs for topical delivery (Johansen et al., 1986; Bundgaard et al., 1989). As shown in Table 1, esters II, III and V were readily hydrolyzed by human plasma and no significant difference of their half-lives were observed. From these results, a rapid enzymatic hydrolysis of esters II, III and V within the viable skin could be expected. Esters I, IV and VI enzymatic hydrolysis rates were not evaluated since these derivatives did not possess suitable physico-chemical properties to be regarded as quercetin topical prodrugs. As mentioned above, esters I and IV did not show sufficient water stability, while ester VI was too lipophilic (see below for details) to be expected to permeate through the skin better than the parent drug.

3.2. Solubility

Quercetin and its 3-*O*-acyl esters water solubility are reported in Table 1. Quercetin derivatives with shorter acyl chains (esters II and III) were more water soluble than quercetin, while the solubility of esters V and VI was lower than that of the parent drug. Water solubility of esters I and IV was not determined due to their poor stability in aqueous medium.

A parabolic relationship seems to exist between length of the promoiety acyl chain and water solubility with compound III exhibiting the maximum solubility (Fig. 2).

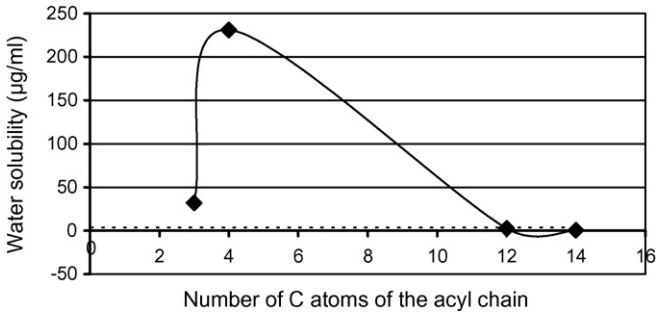


Fig. 2. Relationship between water solubility of esters II, III, V and VI and the length of their acyl chain. Quercetin water solubility is shown as a horizontal dashed line.

3.3. Quercetin and esters I–VI lipophilicity

Results derived from HPLC analyses showed a progressive increase of RT values by increasing the length of the acyl chain of esters I–VI. The extremely long RT of esters V and VI under isocratic conditions did not allow us to determine their log *K* values. In order to determine the lipophilicty of all the compounds tested we calculated log *P* values of quercetin and its derivatives I–VI using two different softwares available on the market. Partition experiments between octanol and water were not performed due to esters I and IV poor stability in aqueous medium.

log *K* and log *P* values of quercetin and derivatives I–VI are shown in Table 1. As expected, all the derivatives were more lipophilic than the parent drug and a linear relationship between the length of the acyl chain of the derivatives and their lipophilicity was observed taking into account experimentally determined log *K* values ($r^2 = 0.995$) and log *P* values calculated using the ACD software version 5.15 ($r^2 = 0.999$) or the OSIRIS Property Explorer ($r^2 = 0.999$) (Fig. 3a–c, respectively). Similar relationships have already been reported for topical prodrugs obtained using promoieties with an increasing number of $-CH_2-$ units (Bonina et al., 1991; Kasting et al., 1992). The average methylene π-value, which is a measure of the fragment effect of a methylene group on partitioning between a lipid and an aqueous phase, was 0.53 using the ACD software version 5.15 and 0.46 using the OSIRIS Property Explorer. These values were consistent with those determined for other series of prodrugs (Wasdo and Sloan, 2004).

3.4. In vitro skin permeability of quercetin and its 3-O-acyl esters

In vitro skin permeation results are reported in Table 1. Results were expressed as cumulative amount permeated after 22 h because the sensitivity of the analytical method did not allow us to detect quercetin and its esters in the receptor phase before 16–18 h from the beginning of the experiment and no flux could be calculated in these conditions.

Only esters II, III and V were assessed for their skin permeation ability since the other derivatives did not show a sufficient chemical stability (esters I and IV) or they were too lipophilic (ester VI).

Since the dermis in vitro can act as a significant additional barrier to the absorption of lipophilic compounds (Bronaugh and Stewart, 1984), in this work, epidermal membranes were used to assess in vitro skin permeation of quercetin and its 3-*O*-acyl esters. Each compound was applied to the skin as saturated aqueous solution so as to ensure a constant driving force while providing the maximum thermodynamic activity. After 22 h, no quercetin could be detected in the receptor phase following the application to the skin of quercetin esters. Other authors (Bundgaard et al., 1989) studying in vitro permeation

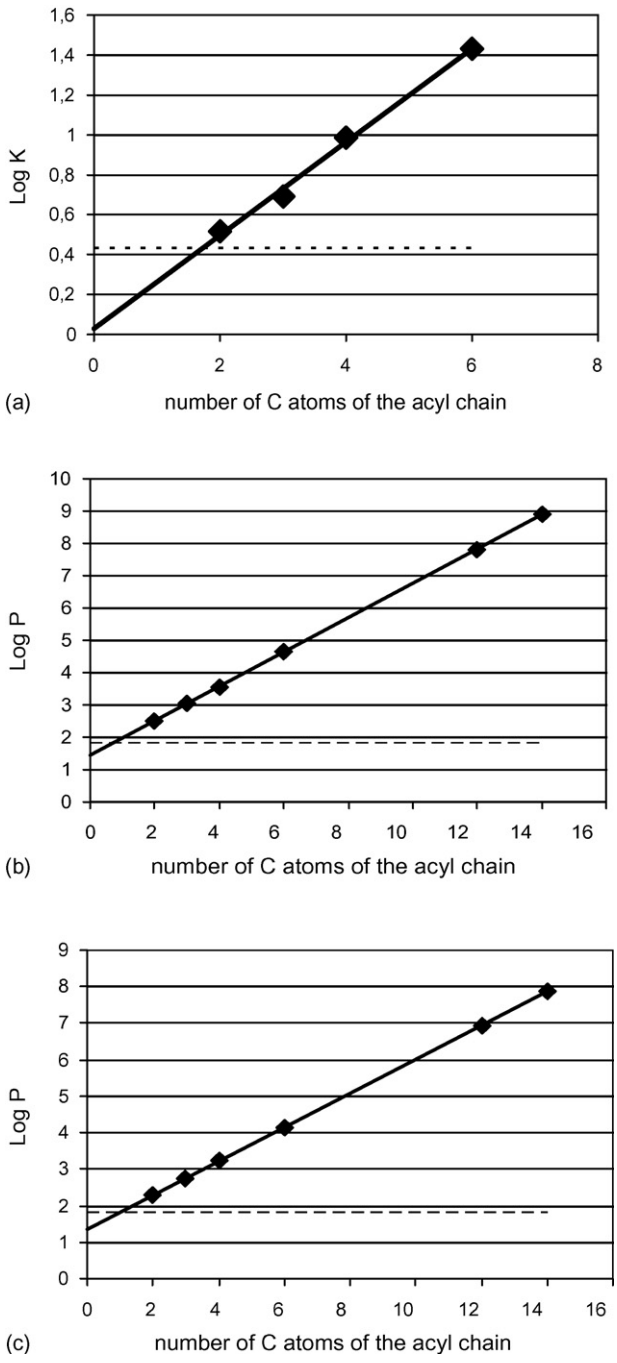


Fig. 3. Relationship between the lipophilicity of esters I–VI and the length of their acyl chain. (a) log K values; (b) log P values calculated using the ACD software version 5.15; (c) log P values calculated using the OSIRIS Property Explorer. Quercetin log K and log P values are shown as a horizontal dashed line.

of prodrugs through full-thickness human skin reported high enzymatic hydrolysis. The lack of enzymatic activity observed in our experiments could be due to the use of epidermal membranes obtained by means of thermal separation since heating is known to damage enzymatic systems. Preliminary in vitro skin permeation experiments performed using full thickness human skin (freshly excised) showed almost undetectable fluxes applying both quercetin and its esters on the skin (unpublished data).

As shown in Table 1, esters II and III significantly increased the cumulative amount of quercetin permeated through the skin while ester V showed values lower than that of the parent drug. The enhancement factor (E.F.) was about 4 and 12 for esters II and III, respectively.

As widely reported in the literature (Scheuplein and Blank, 1971; Kligman, 1983), the outermost layer of the skin, the horny layer, is regarded as the main barrier to skin permeation. This layer is a complex, heterogeneous membrane composed of distinct lipid and protein domains. Depending on the physicochemical properties of the permeant, polar and lipid transport pathways may be involved. The effect of lipophilicity on skin permeation of very hydrophilic permeants from aqueous solutions is negligible, suggesting that their permeation may occur through a polar pathway similar to water with regards to its selectivity to permeant structure. On the other hand, skin permeation of more lipophilic compounds increases in proportion to their octanol/water partition coefficients, suggesting that a lipid transport pathway may be involved (Anderson, 1993). However, very lipophilic drugs proved to be poor skin permeants due to their unfavorable partitioning from the stratum corneum to the viable epidermis. In vivo skin permeation studies performed on different non-steroidal anti-inflammatory drugs showed that the maximum permeation was observed for drugs having octanol/water partition coefficient values of about 2 (Yano et al., 1986). Sloan (1989) pointed out that for a homologous series of topical prodrugs an increase of lipophilicity results in an enhanced delivery of the parent drug when associated to a greater water solubility compared to the parent drug. The results of our study are in agreement with the theory proposed by Sloan (1989), since the highest permeation was obtained from derivative III, which showed the highest solubility in water. A bell shape profile in water solubility has already been reported for other homologous series of prodrugs (Bonina et al., 1991). The increase of water solubility of esters II and III compared to quercetin could be attributed to a decrease of intermolecular bonding interactions and to the introduction of a polar group due to the esterification of the 3-OH group of quercetin. However, the increase of the acyl chain length (esters V and VI) resulted in a increase of the lipophilic character with a corresponding decrease of water solubility. Therefore, in our study the propionyl group seems to be the best promoiety to improve quercetin skin permeation since the resulting increase of parent drug lipophilicity was associated with a notable improvement of water solubility, likely due to a better ability of the propionyl group to decrease intermolecular bonding interactions.

From the data obtained by in vitro experiments on homologous series of prodrugs, a mathematical model that correlates prodrugs' water and lipid solubility with their fluxes through the skin from a lipid vehicle has been developed (Roberts and Sloan, 1999, 2000). This mathematical model proved to be successful to predict the in vitro flux through the skin of homologous series of 6-mercaptopurine and 5-fluorouracil prodrugs from aqueous vehicles (Sloan et al., 2003). These mathematical models have pointed out that prodrugs' lipophilicity alone is not predictive of their delivery through the skin since the skin barrier shows a lipid-aqueous biphasic nature due to the multilamellar

bilayered structure of the intercellular components of the horny layer. Therefore, a suitable balance between water solubility and lipophilicity has to be regarded as a key parameter in determining prodrug ability to improve drug skin permeation (Guy and Hadgraf, 1992; Sloan, 1989; Sloan et al., 2003).

In conclusion, esters II and III showed the main requirements needed to be regarded as quercetin topical prodrugs since they were fairly stable in water, they underwent a rapid enzymatic hydrolysis and they improved in vitro quercetin skin permeation.

Further in vivo studies are planned in order to evaluate the potential use of esters II and III as topical prodrugs of quercetin.

Acknowledgment

We thank for financial support "Fondi di Ricerca Ateneo 2002" from University of Catania, Italy.

References

Anderson, B.D., 1993. Prodrugs and their topical use. In: Shah, V.P., Maibach, H.I. (Eds.), Topical Drug Bioavailability, Bioequivalence and Penetration. Plenum press, New York, pp. 69–89.

Baumann, J., Bruchhausen, F.V., Wurm, G., 1980. Flavonoids and related compounds as inhibitors of arachidonic acid peroxidation. Prostaglandins 20, 627–639.

Bickers, D.R., Kappas, A., 1980. The skin as a site of chemical metabolism. In: Gram, T.E. (Ed.), Extrahepatic Metabolism of Drugs and other Foreign Compounds. MTP, Lancaster, pp. 295–318.

Bonina, F.P., Montenegro, L., De Capraris, P., Bousquet, E., Tirendi, S., 1991. 1-Alkylazacycloalkan-2one esters as prodrugs of indomethacin for improved delivery through human skin. Int. J. Pharm. 77, 21–29.

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.

Bundgaard, H., Mork, N., Hoelgaard, A., 1989. Enhanced delivery of nalidixic acid through human skin via acyloxymethyl ester prodrugs. Int. J. Pharm. 55, 91–97.

Chan, S.Y., Li Wan Po, A., 1989. Prodrugs for dermal delivery. Int. J. Pharm. 55, 1–16.

Crozier, A., Jensen, E., Lean, M.E.J., McDonald, M.S., 1997. Quantitative analysis of flavonoids by reversed-phase high performance liquid chromatography. J. Chromatogr. 761, 315–321.

Formica, J.V., Regelson, W., 1995. Review of the biology of quercetin and related flavonoids. Fd. Chem. Toxic. 33, 1061–1080.

Guy, R.H., Hadgraf, J., 1992. Percutaneous penetration enhancement: physicochemical considerations and implications for prodrug design. In: Sloan, K.B. (Ed.), Prodrugs: Topical and Ocular Delivery. Dekker, New York, pp. 1–16.

Hormann, H.P., Korting, H.C., 1994. Evidence for the efficacy and safety of topical herbal drugs in dermatology: part I: anti-inflammatory agents. Phytomedicine 1, 161–171.

Johansen, M., Mollgaard, B., Wotton, P.K., Larsen, C., Hoelggard, A., 1986. *In vitro* evaluation of dermal prodrug delivery-transport and bioconversion of a series of aliphatic esters of metronidazole. Int. J. Pharm. 32, 199–206.

Kasting, G.B., Smith, R.L., Anderson, B.D., 1992. Prodrug for dermal delivery: solubility, molecular size and functional group effects. In: Sloan, K.B. (Ed.), Prodrugs: Topical and Ocular Delivery. Dekker, New York, pp. 117–161.

Katsarou, A., Davoy, E., Xenos, K., Armenaka, M., Theoharides, T.C., 2000. Effect of an antioxidant (quercetin) on sodium-lauryl-sulfate-induced skin irritation. Contact Dermatitis 42, 85–89.

Kligman, A.M., Christophers, E., 1963. Preparation of isolated sheets of human skin. Arch. Dermatol. 88, 702–705.

Kligman, A.M., 1983. A biological brief on percutaenous absorption. Drug Dev. Ind. Pharm. 70, 52–56.

Lambusta, D., Nicolosi, G., Patti, A., Piattelli, M., 1993. Enzyme-mediated regioprotection deprotection of hydroxyl-groups in (β)-catechin. Synthesis-Stuttgart 11, 1155–1158.

Middleton Jr., E., 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.

Morel, I., Lescoat, G., Cogrel, P., Sergent, O., Pasdeloup, N., Brissot, P., Cillard, P., Cillard, J., 1993. Antioxidant and iron-chelating activities of the flavonoids catechin, quercetin and diosmetin on iron-loaded rat hepatocyte cultures. Biochem. Pharmacol. 4, 13–19.

Mueller, L.G., 1988. Novel anti-inflammatory esters, pharmaceutical compositions and methods for reducing inflammation. UK Patent GB 2 204 869 A, 23 November.

Pannatier, A., Jenner, P., Testa, B., Etter, J.C., 1978. The skin as drug-metabolizing organ. Drug Metab. Rev. 8, 319–343.

Pignatello, R., Montenegro, L., Stancampiano, A.H.S., Puleo, A., Puglisi, G., 2005. Synthesis and *in vitro* skin permeation of naproxen conjugates with α-alkylamino acids. Curr. Drug Deliv. 2, 185–189.

Roberts, W.J., Sloan, K.B., 1999. Correlation of aqueous and lipid solubilities with flux for prodrugs of 5-fluorouracil, theophylline, and 6-mercaptopurine: a Potts-Guy approach. J. Pharm. Sci. 88, 515–532.

Roberts, W.J., Sloan, K.B., 2000. Prediction of transdermal flux of prodrugs of 5-fluorouracil, theophylline, and 6-mercaptopurine with a series/parallel model. J. Pharm. Sci. 89, 1415–1431.

Saija, A., Tomaino, A., Trombetta, D., Pellegrino, M.L., Tita, B., Messina, C., Bonina, F.P., Rocco, C., Nicolosi, G., Castelli, F., 2003. *In vitro* antioxidant and photoprotective properties and interaction with model membranes of three new quercetin esters. Eur. J. Pharm. Biopharm. 56, 167–174.

Sardone, L., Pignataro, B., Castelli, F., Sampietro, M.G., Nicolosi, G., Marletta, G., 2004. Temperature and pressure dependence of quercetin-3-*O*-palmitate interaction with a model phospholipid membrane: film balance and scanning probe microscopy study. J. Colloid Inter. Sci. 271, 329–335.

Scheuplein, R.J., Blank, I.H., 1971. Permeability of the skin. Physiol. Rev. 51, 703–723.

Sloan, K.B., 1989. Prodrugs for dermal delivery. Adv. Drug Del. Rev. 3, 67–101.

Sloan, K.B., Scott, W., Ezike-Mkparu, U., Murray, T., Nickels, D., Singh, S., Shanks, T., Tovar, J., Ulmer, K., Waranis, R., 2003. Topical delivery of 5-fluorouracil and 6-mercaptopurine by their alkylcarbonyloxymethyl prodrugs from water: vehicle effects on design of prodrugs. Pharm. Res. 4, 639–645.

Touitou, E., Fabin, B., 1988. Altered skin permeation of a 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 Radic. Biol. Med. 20, 331–342.

Wasdo, S.C., Sloan, K.B., 2004. Topical delivery of a model phenolic drug: alkyloxycarbonyl prodrugs of acetaminophen. Pharm. Res. 21, 940–946.

Yano, T., Nakagawa, A., Tsuji, M., 1986. Skin permeability of various non-steroidal antiinflammatory drugs. Life Sci. 39, 1043–1050.