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Research Paper

Enhanced skin delivery of quercetin by microemulsion

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Abstract

Objectives For topical application of quercetin it is necessary to improve the low efficiency of its intradermal delivery as well as its low solubility in aqueous and organic vesicles. The aim of this study was to determine the usefulness of a microemulsion for that purpose.

Methods A microemulsion consisting of isopropyl myristate, 150 mM NaCl solution, Tween 80 and ethanol was prepared. The skin delivery of quercetin by microemulsion using excised guinea-pig and Yucatan micropig skin in Franz diffusion cells was examined. Lipid peroxidation in skin was also tested using iron(II) and citrate.

Key findings Using a w/o microemulsion as a vehicle, intradermal delivery of quercetin was significantly increased, as was its solubility. Quercetin penetrated deep into the skin, but no transfer was observed into the receptor compartment. It was confirmed that quercetin retained in the skin dose-dependently inhibited lipid peroxidation.

Conclusions The findings indicate the potential use of microemulsions for the skin delivery of quercetin, where it exerts antioxidative effects.

Keywords intradermal delivery; microemulsion; quercetin; skin

Introduction

Polyphenols, such as flavonoids, are known as antioxidants and their application has been used for topical purposes, such as photoprotection against UV-induced skin damage (photoageing), skin cancer prevention and skin care.[1–3] Among the polyphenols, quercetin has been reported to have antiradical and anti-inflammatory activities,[4] and its topical application has been examined.[5] However, as with many polyphenols, intradermal delivery of quercetin is inefficient, due to its low skin permeability as well as its low solubility in both aqueous and organic media. Systems should therefore be improved to enable its efficient incorporation. To this end, the use of prodrugs,[5] chemical enhancers,[6] and liposomes[7] has been examined for the skin delivery of quercetin and other flavonoids.

In this study, we used a microemulsion as an enhancement system, which consisted of an aqueous phase, an organic phase, a surfactant and a co-surfactant component. The microemulsion is thermodynamically stable and has been shown to have high solubilisation capacity and to facilitate the skin permeation of both lipophilic and hydrophilic drugs;[8,9] however, it has not been widely used for skin absorption of quercetin or other polyphenols. In this study, we aimed to determine the usefulness of a microemulsion for intradermal delivery of quercetin to prevent peroxidation of skin lipids in an in-vitro study. Since antioxidative effects of quercetin in the epidermis and dermis are expected after permeating the stratum corneum, the concentration of quercetin in the deeper skin layers was determined.

Materials and Methods

Materials

Quercetin (guaranteed grade), polyoxyethylene sorbitan monooleate (Tween 80) and isopropyl myristate (IPM) were obtained from Nacalai Tesque (Kyoto, Japan). The impurity of quercetin was less than 1% as determined by high-performance liquid chromatography (HPLC). Resveratrol and *trans*-ferulic acid were from Tokyo Kasei Kogyo Co. (Tokyo, Japan). Ethanol and all other reagents were purchased from Wako Pure Chemical Industries (Osaka, Japan). Dorsal skin was excised from Hartley strain male guinea-pigs following the protocol approved by the Animal Experimentation Committee of Kobe Pharmaceutical

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University. Pentobarbital sodium was used for anaesthesia. Subcutaneous fat and other extraneous tissues were trimmed. Yucatan micropig skin (YMP skin set) was purchased from Charles River Japan (Yokohama, Japan). The fat and subdermal tissue were removed following Fujii *et al*.[10]

Preparation of the microemulsions

Microemulsions A (o/w type) and D (w/o type), composition as shown in Table 1, were prepared using 150 mM NaCl solution as an aqueous phase and IPM as an oil phase, Tween 80 as a surfactant and ethanol as a co-surfactant, by modifying the method of Lee *et al*.[11] Ethanol also works as an ingredient in the aqueous phase. Microemulsion S, in which oleic acid was used as the oil phase and Span 20 as well as Tween 80 as surfactants, was prepared by modifying the method of Peltola *et al*.[12] Each microemulsion was obtained by vortex mixing for a few minutes. The particle size of the microemulsion was determined with quasi-elastic light scattering using a particle analyser FPAR-1000 (Otsuka Electronics Co., Hiratsuka, Japan). Quercetin was added to the pre-formed microemulsion.

Measurement of intradermal delivery

The in-vitro study on the skin incorporation of quercetin was performed as described previously.[13] Guinea-pig dorsal skin or Yucatan micropig skin was mounted in a Franz cell with a water jacket (37°C). The available diffusion area was approximately 0.71 cm^2 and the receptor cell had a capacity of about 4.7 ml. After 12 h pretreatment of the skin and washing both donor and receptor compartments, 1 ml microemulsion containing quercetin was added to the donor compartment while phosphate-buffered saline (pH 7.4; PBS) was added to the receptor compartment. After 20 h treatment (40 h treatment for Yucatan micropig skin), at which time the amount of quercetin penetrating the skin was close to the maximum level, the skin was removed from the cell, and the treated skin area was punched out and washed with ice-cold methanol. After drying at ambient temperature, the skin was weighed (~0.09 g), minced and placed in 10 ml methanol, and then homogenised using a tissue homogeniser. The samples were then centrifuged and the supernatant layer was used to determine the concentration of quercetin by HPLC (L-6000; Hitachi, Tokyo, Japan). Separation was performed on a reversed-phase column (Mightysil RP-18 GP, 4.6 mm i.d., 150 mm) using a mobile phase consisting of methanol, water and phosphoric acid at a ratio of 100 : 100 : 1. The detection wavelength was 360 nm, and *trans*-ferulic acid was used as the internal standard.

Table 1 Composition of microemulsions

Microemulsion	Isopropyl myristate	Oleic acid	NaCl solution	Tween 80	Span 20	Ethanol
A	8	–	25	20	–	47
D	33	–	7	30	–	30
S	–	40	7	20	15	18

Composition is give as %, w/w.

The concentration of quercetin in the deeper skin layers was determined after freezing the skin rapidly with dry ice and methanol, transferring it into a cryomicrotome, cutting the whole piece of skin into surface parallel sections and homogenising as described above.

Measurement of skin permeation

In-vitro skin permeation of quercetin and other polyphenols, which were examined for comparison, was performed as described previously.[14] Microemulsion D, containing quercetin or other polyphenols, was added to the donor compartment while PBS was introduced into the receptor compartment. The concentration of quercetin and other polyphenols was set at 20 mM. Sodium ascorbate was added at 10 mM in both aqueous phases in the donor compartment and receptor compartment to prevent the oxidation of quercetin. Bovine serum albumin (1%) was also added to the receptor compartment to increase the solubility of quercetin and other polyphenols in the receptor fluids. Samples (150 μl) were taken from the receptor cells periodically over 30 h. The concentration of quercetin was determined by HPLC as described above. The concentration of other polyphenols was analysed using a mobile phase consisting of methanol, water and phosphoric acid at a ratio of 100 : 100 : 1 for *trans*-ferulic acid, and 75 : 125 : 1 for resveratrol. The detection wavelength was 323 nm for *trans*-ferulic acid and 305 nm for resveratrol. As internal standards, quercetin was used for *trans*-ferulic acid and *trans*-ferulic acid was used for resveratrol.

Apparent permeability coefficients (K_p) of quercetin and other polyphenols were obtained according to Equation 1 from the initial straight portion of the permeation curve dM_R/dt, while the concentration gradient between compartments was almost completely maintained.

$$K_p = dM_R/dt \times 1/AC_D \quad (1)$$

M_R is the amount of each polyphenol permeating the receptor compartment, A is the diffusion area and C_D is the concentration of each polyphenol in the donor compartment.

Measurement of solubility

Quercetin solubility was measured after incubation in an excess amount of microemulsion, IPM or 150 mM NaCl solution at 37°C for about 20 h. After centrifugation at 12 000*g* for 1 min, the concentration of the supernatant was determined by HPLC as described above.

Measurement of partition coefficients

Partition coefficients of polyphenols between *n*-octanol and PBS (pH 7.4) were measured as described previously.[15] PBS solution (3 ml) of polyphenols (0.1–1.0 mM) was mixed with 3 ml *n*-octanol in test tubes with glass stoppers. PBS and *n*-octanol solutions were pre-saturated with either *n*-octanol or PBS and deoxygenised with a nitrogen stream. The test tubes were set at 37°C for 18 h in a shaking water bath. After shaking, incubation continued for another 1 h. The concentration of polyphenols in the PBS phase and *n*-octanol phase was determined by HPLC as described above.

Measurement of lipid peroxidation

The anti-lipoperoxidative activity of quercetin retained in the skin was estimated by the formation of malondialdehyde (MDA) tested using iron(II) and citrate.[16] Skin was homogenised in 1.15% KCl solution. To 1.0 ml of each sample containing about 1 mg protein, ammonium iron(II) sulfate and sodium citrate were added to a final concentration of 50 μM and 2 mM, respectively, and kept for 30 min at 37°C. To determine MDA formation, 0.20 ml of 8.1% sodium dodecyl sulfate solution, 1.5 ml acetate buffer adjusted to pH 3.5, 50 μl of 0.8% butylhydroxytoluene in glacial acetic acid, 1.5 ml of 0.8% thiobarbituric acid (TBA) solution, and 0.7 ml of 5 mM iron(III) chloride solution were further added in that order, followed by 60 min incubation at 60°C. After cooling, the MDA–TBA complex was extracted with 5 ml *n*-butanol and pyridine mixed solution, centrifuged at 1660*g* for 10 min, and the absorbance of the supernatants was read at 532 nm.

Statistical analysis

Data were analysed using the Kruskl–Wallis test. Individual differences between medians were examined using Dunn's multiple comparison test.

Results

Delivery of quercetin to skin by microemulsion

We examined the effects of microemulsions on the skin accumulation of quercetin. The mean particle diameter of microemulsions A, D and S was 39.8 ± 22.8 nm, 42.5 ± 21.5 nm, and 25.1 ± 12.2 nm, respectively. We examined the effect of a microemulsion on the solubility of quercetin. As shown in Table 2, the microemulsion significantly improved the solubility. Since the improvement by microemulsion D was particularly marked, we attempted the skin delivery of quercetin using microemulsion D.

As shown in Figure 1, for the results on excised guinea-pig skin, the intradermal delivery of quercetin increased dose-dependently when using a microemulsion as a vehicle. At a saturated concentration in microemulsion D, the incorporation of quercetin into the skin markedly increased by about 50 times compared with when applied as a suspension in NaCl solution, and by about 13 times compared with when applied as a suspension in IPM. About 0.4% quercetin added to the donor component was incorporated into skin in this condition. The enhancement effects were smaller than with microemulsion D under saturated concentrations when either Tween 80 or ethanol, components of the microemulsion, was added to the NaCl solution.

Table 2 Effects of microemulsions on quercetin solubility

Vehicle	Solubility (mM)
NaCl solution	0.31 ± 0.15
Isopropyl myristate	0.64 ± 0.14
Microemulsion A	29.2 ± 3.10[a]
Microemulsion D	78.6 ± 8.9[a,b]
Microemulsion S	22.3 ± 2.4[a]

Data are the means ± SD of three experiments. [a]$P < 0.001$, significantly different compared with the values in NaCl solution and isopropyl myristate. [b]$P < 0.001$, significantly different compared with the values in microemulsion A and microemulsion S.

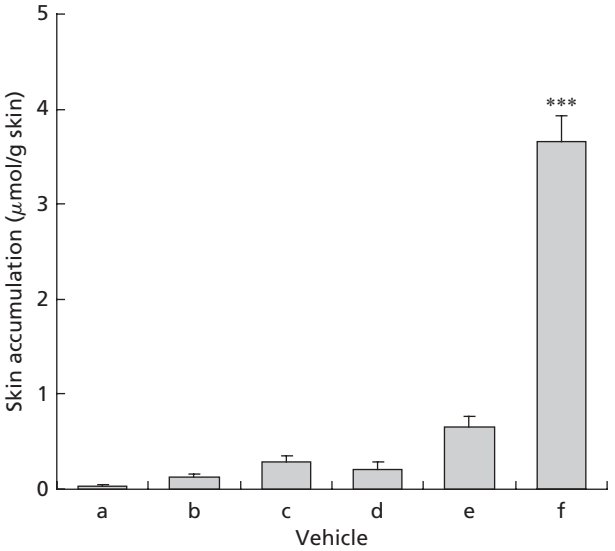

Figure 1 Effects of microemulsions on skin accumulation of quercetin in guinea-pig skin. Microemulsions were applied as a suspension in either 150 mM NaCl solution (a), or isopropyl myristate (b), in a vehicle consisting of Tween 80 and 150 mM NaCl solution (30 : 70) (c), in a vehicle consisting of ethanol and 150 mM NaCl solution (30 : 70) (d), at 20 mM in microemulsion D (e), at saturated concentration (78.6 mM) in microemulsion D (f). Data are the means ± SD of four experiments. ***$P < 0.001$, significantly different compared with the values in other conditions.

Enhanced delivery by microemulsion was also confirmed when Yucatan micropig skin, which is hairless and has been suggested to show physiological similarity to human skin,[17] was used, with quercetin applied with microemulsion D (Figure 2). We performed a further study using guinea-pig skin.

Since there was a possibility that quercetin was only adsorbed into the skin surface, we examined the concentration–depth profiles of quercetin by cutting the skin into surface parallel sections using a cryomicrotome. As shown in Figure 3, the distribution of quercetin in deep skin layers was confirmed, although the concentration near the surface was higher.

Skin permeation of quercetin

Transport of quercetin into the receptor compartment was examined in addition to other polyphenols for comparison; the flux and permeability coefficients are given in Table 3. No transfer into the receptor compartment was observed for quercetin. The molecular weights of quercetin, *trans*-ferulic acid and resveratrol were 302, 194 and 228, respectively. Logarithm values of the partition coefficients between *n*-octanol and PBS of the same polyphenols were 2.74,[15] –0.88 and 2.97, respectively. *Trans*-ferulic acid, which has the lowest molecular weight and is the most hydrophilic among the polyphenols tested, showed good permeability. Resveratrol, which has a lower molecular weight and is more hydrophobic than quercetin, showed very limited permeation.

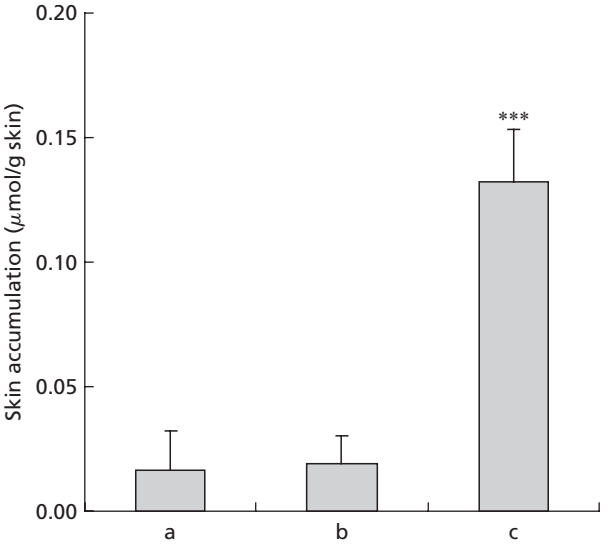

Figure 2 Skin accumulation of quercetin in Yucatan micropig skin. Microemulsions were applied as a suspension in either 150 mM NaCl solution (a) or isopropyl myristate (b), as microemulsion D at saturated concentration (78.6 mM) (c). Data are the means ± SD of four experiments. ***$P < 0.001$, significantly different compared with the values in NaCl and isopropyl myristate.

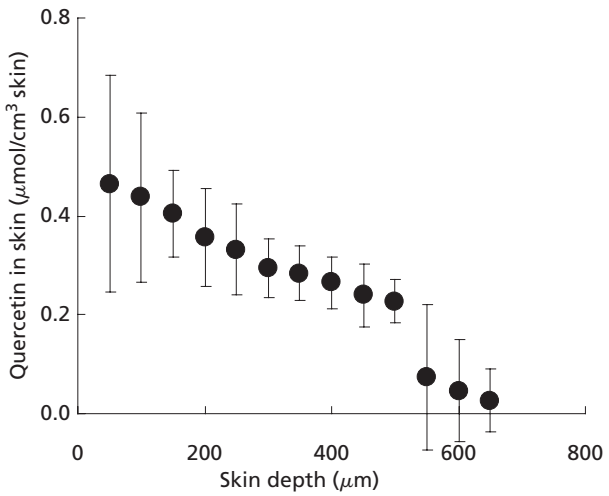

Figure 3 Concentration–depth profile of quercetin in guinea-pig skin. Quercetin was applied with microemulsion D at saturated concentration (78.6 mM). Data are the means ± SD of four experiments.

Effects of incorporated quercetin on lipid peroxidation of skin

To determine the antioxidative effects of polyphenols incorporated into the skin using microemulsion as a vehicle, the antilipoperoxidative activity of quercetin retained in the skin was estimated by the formation of MDA using iron(II) and citrate.[16] As shown in Figure 4, quercetin retained in the skin significantly inhibited lipid peroxidation in a dose-dependent manner.

Table 3 Flux and permeability coefficients of polyphenols applied with microemulsion D

Polyphenol	Flux ($\times 10^{-2}$ μmol/cm per h)	K_p ($\times 10^{-3}$ cm/h)
Trans-ferulic acid	10.2 ± 1.2	4.67 ± 0.55
Resveratrol	0.0664 ± 0.0109	0.0283 ± 0.0046
Quercetin	Not detected	Not detected

K_p, permeability coefficient. Data are the means ± SD of four experiments.

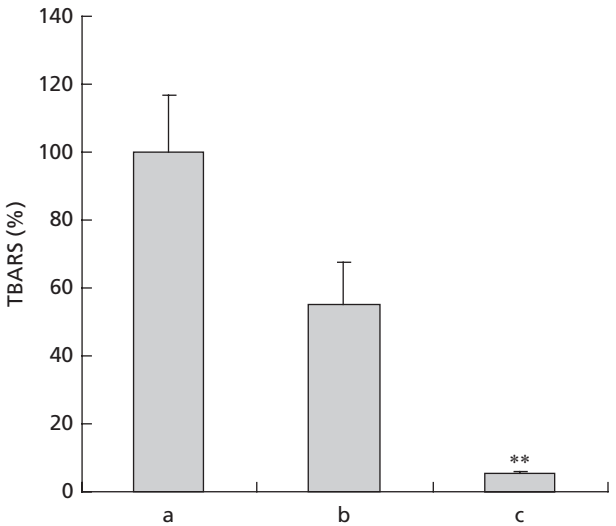

Figure 4 Effect of quercetin on lipid peroxidation. The effect of quercetin was tested by using ammonium iron(II) sulfate and sodium citrate when applied with microemulsion D in guinea-pig skin at 0 mM (a), 20 mM (b) or saturated concentration (78.6 mM) (c). Data are the means ± SD of four experiments. TBARS, thiobarbituric acid reactive substances. **$P < 0.01$, significantly different compared with the value in the absence of quercetin.

Discussion

Microemulsions offer several advantages for pharmaceutical use, such as long-term stability, high solubilisation capacity for both hydrophilic and lipophilic drugs, and improved dermal and transdermal drug delivery.[18] Microemulsions consist of an oil phase and aqueous phase as well as a surfactant and co-surfactant. Various compounds are used as surfactants, such as polysorbates, polyoxyethylene alkyl ethers and phospholipids, and short-chain alcohols are often used as co-surfactants.[18,19] The findings obtained in this study also revealed that the microemulsion which consisted of 150 mM NaCl solution, IPM, Tween 80 and ethanol, markedly improved the solubility of quercetin. A microemulsion is therefore of benefit for the skin delivery of quercetin, which has very low solubility in both aqueous and non-aqueous vehicles. The increase in solubility with the w/o type microemulsion D was most marked, probably related to the hydrophobicity of quercetin, with the logarithm value of the partition coefficient between *n*-octanol and PBS being 2.74.[15]

Using microemulsion D, intradermal delivery of quercetin also increased markedly. These findings are characteristic of microemulsions, because, in general, the increased solubility of a solute in the vehicle increases its thermodynamic stability in the vehicle, which leads to decreased partition in the skin. As a result, increased solubility in the vehicle does not usually lead to increased intradermal or transdermal drug absorption. The enhancement effect of a microemulsion on the intradermal delivery of quercetin is consistent with the recent report by Vicentini *et al.*,[20] in which a microemulsion comprising propylene glycol, water, Span 80, Tween 80 and canola oil was used.

The findings also showed that the enhancement effect by microemulsion D was much greater than when either Tween 80 or ethanol, components of the microemulsion, was added to the NaCl solution. Therefore, the enhancement effect by the microemulsion was much greater than that by ethanol or Tween 80 itself.

Since enhanced intradermal delivery by microemulsion was also confirmed when Yucatan micropig skin was used, enhancement was induced in the penetration through the stratum corneum. Although the mechanism of the enhanced delivery of quercetin by microemulsion is not clear, one possibility is as follows. Microemulsion vesicles break down when they come into contact with the skin, releasing quercetin, which is expected to be mainly present in the interface region of the microemulsion, into the skin and stimulating its delivery into the surface of the stratum corneum.[8] A microemulsion may also enhance the skin delivery of quercetin partly by mixing its components with lipid lamella of the stratum corneum and enhancing the permeation of quercetin through the stratum corneum. In addition, continuously and spontaneously fluctuating interfaces of microemulsions enable high drug mobility and might enhance the drug diffusion process.[21]

The permeation study, which measured receptor compartment fluid, suggested that polyphenols with a relatively high molecular weight, such as quercetin, remained in the skin, and probably bound to proteins of dermal cells via interaction with hydroxyl groups as well as hydrophobic groups. This finding supports the antioxidative action of quercetin in skin.

From these findings, it was revealed that a microemulsion is useful for delivering quercetin into skin.

Conclusions

We examined the effects of a microemulsion on the intradermal delivery of quercetin *in vitro*. Only slight amounts of quercetin were transferred to the skin from either aqueous or organic medium. Using a microemulsion comprising IPM, 150 mM NaCl solution, Tween 80 and ethanol as a vehicle, intradermal delivery of quercetin significantly increased, as did its solubility. Quercetin penetrated deep into the skin, but no transfer was observed into the receptor compartment, which was different from the findings for polyphenols with lower molecular weights. We confirmed that quercetin retained in the skin by a microemulsion dose-dependently inhibited lipid peroxidation as tested using iron(II) and citrate. The findings of this study indicate the potential use of microemulsions for the intradermal delivery of quercetin.

Declarations

Conflict of interest

The Author(s) declare(s) that they have no conflicts of interest to disclose.

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