Free Radical Biology and Medicine 75 (2014) 149–155

Contents lists available at ScienceDirect

Free Radical Biology and Medicine

journal homepage: www.elsevier.com/locate/freeradbiomed

Original Contribution

Antioxidative effects and percutaneous absorption of five polyphenols

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ARTICLE INFO

Available online 18 July 2014

Keywords:
Polyphenols
In vitro
Percutaneous absorption
Franz cells
Antioxidant activity
Free radicals

ABSTRACT

A new strategy was designed to evaluate the antioxidant effectiveness of five topically applied polyphenols following skin penetration profiles. The antioxidants were the following polyphenol derivatives: epicatechin, resveratrol, rutin, quercetin, and trolox, which was used as the reference antioxidant. The hydrophilic/lipophilic character of these compounds was evaluated, and their antioxidant activity was measured by the DPPH• method. The percutaneous absorption of these polyphenols was obtained by an in vitro methodology using porcine skin biopsies. This methodology involves the quantification of the antioxidants present in each specific skin layer to evaluate antioxidant effectiveness. The antioxidant activity in each skin layer was also determined by the DPPH• method. The results indicated that lipophilic antioxidants (epicatechin, resveratrol, quercetin, and trolox) penetrated deeper into the skin layers, whereas a more hydrophilic compound, rutin, remained on the skin surface. The antioxidant evaluation of each skin compartment suggested that resveratrol and rutin were the most effective topically applied compounds in view of their antioxidant activity and their skin penetration profile.

The skin is the outermost barrier of the body and is directly exposed to solar radiation and pollutants in a pro-oxidant environment [1]. Cumulative and prolonged exposures to ultraviolet radiation may induce deleterious reactions in skin, including cutaneous aging, immunosuppression, photocarcinogenesis, and various inflammatory skin disorders [2–6]. UVB and UVA radiation induces DNA damage directly and indirectly through oxidative stress by increasing the level of reactive oxygen species [7,8]. Irradiation of the visible and near-infrared regions has recently been demonstrated to penetrate the skin, causing damage in the inner layers of the skin [9,10].

Beings maintain complex systems of multiple types of antioxidants such as glutathione, vitamin C, vitamin A, and vitamin E as well as enzymes. The major constituents of the enzymatic system are manganese superoxide dismutase [11], glutathione peroxidase [12], and members of the thioredoxin family [13].

Research has shown that compounds such as polyphenolic agents can scavenge oxidative radicals and reduce skin damage. Some studies on topically applied antioxidant drugs have confirmed the above findings and have demonstrated that photodamage decreases when the skin is supplemented with antioxidants [14–16].

The most suitable candidates for antioxidative improvement could be plant antioxidants because plants defend themselves in a pro-oxidant environment. Topical administration of natural antioxidants from plants could prove useful in increasing the endogenous cutaneous protection system, replenishing cutaneous stores, and reducing/preventing UV-induced skin damage.

Polyphenolic agents are compounds that are common in fruits and vegetables [17] and can act as antioxidants under certain conditions [18]. The mechanism of nutritional phenolic antioxidants has been studied in vivo [19]. Polyphenols at low concentrations have been shown to provide protection. Moreover, they have been reported to have antioxidant properties in vitro [20,21]. Noteworthy examples are green tea catechins, which possess anticancer properties [22] that protect the brain [23]. Berry anthocyanins enhance memory in rats and humans [24,25]. The polyphenols used in this work were resveratrol, (−)-epicatechin, rutin, quercetin, and trolox (Fig. 1). For our study, these compounds were selected because of their interesting biological, pharmacological, and medicinal properties [26–28]. Two conditions are necessary for a compound to be a promising candidate as a protective agent against oxidative damage to the skin: (1) the candidate should permeate the stratum corneum to reach the deeper cutaneous layers without significant leakage into the systemic circulation and (2) the candidate must possess a considerable level of antioxidant activity.

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http://dx.doi.org/10.1016/j.freeradbiomed.2014.07.014
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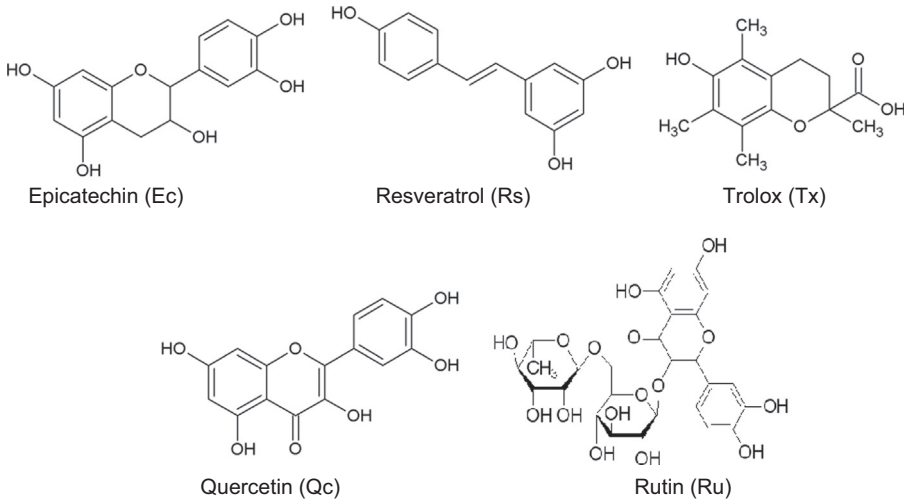

Fig. 1. Chemical structures of the polyphenols investigated.

A wide variety of in vitro methods that assess radical scavenging ability have been developed [29], including the measurement of the capacity of a compound to reduce the free radical 1,1′-diphenyl-2-picrylhydrazyl (DPPH$^{\bullet}$) [30,31]. The DPPH$^{\bullet}$ test is a rapid, simple, and common assay that is used in in vitro and in vivo studies [29] to demonstrate the antioxidant activity of compounds. However, to the best of our knowledge, this methodology has not been used to determine antiradical capacity in skin samples. In this work, the DPPH$^{\bullet}$ method was applied to antioxidant compounds to evaluate the ability of polyphenolic compounds to transfer labile H atoms from radicals. The antioxidant capacity of the compounds investigated was compared with trolox, a hydrosoluble analog of vitamin E, which is regarded as a standard in antioxidant measurement.

The aim of this study was to determine the in vitro percutaneous penetration through excised pig skin of five polyphenolic compounds: resveratrol, (−)-epicatechin, rutin, quercetin, and trolox. The skin profiles obtained were related to the lipophilic character of each compound. We also investigated the radical scavenging ability of these phenolic agents toward DPPH$^{\bullet}$ in various skin compartments after topical application.

Materials and methods

Polyphenols

Resveratrol (Rs), (−)-epicatechin (Ec), and 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (trolox, Tx) were purchased from Sigma (St. Louis, MO, USA). Rutin (Ru) and quercetin (Qc) were obtained from Merck (Darmstadt, Germany). Their chemical structures are shown in Fig. 1.

High-performance liquid chromatography analysis of compounds

The concentration of each compound in all samples was determined by HPLC using an external calibration curve of standard samples for each polyphenol at various concentrations. Briefly, Hitachi-Merck HPLC equipment was equipped with a variable UV–Vis detector (L-4250) and a C18 column (LiChrocart 250-4/LiChrosorb RP-18, 5 μm) with a flow rate of 1 ml/min under isocratic conditions. The injection volume was 20 μl. Elution conditions for detection of compounds are given in the supplementary data (Section 1).

Octanol/water partition coefficient of the antioxidants investigated

The method used was similar to that of Liao and Yin [32] to measure the partition coefficient (P_{ow}) of compounds. A solution of 1 mM polyphenol solution in 5 ml of water was prepared. An aliquot of this sample was analyzed by HPLC under the conditions described above to determine the area in order to obtain the total amount of each compound (A_t). A given volume of this solution was mixed with water:1-octanol (Panreac, Barcelona, Spain) (1:1). The mixture was sonicated for 15 min at 50/60 Hz and was centrifuged at 2500 rpm for 15 min. As a result, the mixture separated into two phases. An aliquot of the aqueous phase was analyzed by HPLC to obtain the area representing the polyphenol amount present in the water portion (A_s).

The partition coefficient of each compound was determined by the equation $(A_t - A_s)/A_s$, where $(A_t - A_s)$ represents its area in 1-octanol and A_s denotes its area in water. Compounds with a partition coefficient >1 are lipophilic because their concentration is higher in the 1-octanol layer.

In vitro percutaneous absorption assay

The skin penetration profile for each antioxidant compound dissolved in ethanol solution was determined using pig skin biopsies placed on Franz static diffusion cells (1.86 cm^2 of exposed area, Lara-Spiral, Courtenon, France). Pig skin is a representative membrane for percutaneous absorption because it has permeation characteristics similar to those of human skin [33]. The density of hair follicles is similar in human and porcine skin [34].

The OECD guidelines and the published opinions of the Scientific Committee on Consumer Safety were closely adhered to during this study [35–37]. A number of other principles of percutaneous absorption were considered [38,39].

Porcine skin (three different donors) was obtained from the unboiled back of freshly killed domestic pigs (Landrace Large White race). The processing of the pig skin and the Franz cell system are described in the supplementary data (Section 2). The in vitro percutaneous absorption scheme is also included in the supplementary data (Section 2, Fig. S1).

A minimum of six diffusion cells were required for each experimental assay. A 10.75 μl/cm^2 sample of each polyphenol solution was applied to the entire surface delimited by the upper cell. A control cell containing 10.75 μl/cm^2 of ethanol (Merck) was also used.