Skin Permeability of Porcine Placenta Extracts and Its Physiological Activities

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Abstract

We investigated the skin permeability and various biological activities of porcine homogenate of placenta (HP) with the highest protein contents (452.89 µg/mg). The content of protein in subcritical extract of HP (SPE) was decreased from the initial content of 452.9 µg/mg to 262.7 µg/mg at 3 h subcritical extract. The contents of amino type nitrogen (A-N) were sharply increased from 35.1 µg/mg of initial content to 305.9 µg/mg at 3 h subcritical extract. The HP showed a noticeable activity in terms of antioxidant capacity for ferric reducing antioxidant power (FRAP) assay and especially for 2,2'-Azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) method. HP, SPE-0.5, SPE-2 and SPE-3 showed inhibitory effect on elastase activities with an IC₅₀ of 46.1, 42.9, 31.6 and 34.7 µg/mL, respectively. SPEs showed more significantly inhibitory effect than HP (p<0.05). The skin permeability of the SPEs was higher than that of the HP. SPE-3 showed highest skin permeation and the permeability was significantly higher than that of HP. SPE-2 also showed significantly higher permeation than HP after 4 h. As expected, increase of extraction time significantly increased skin permeability in the subcritical extract of HP (SPE). From these results, in terms of cost and source availability, porcine placenta extracted with subcritical extraction has advantages over untreated PE and have potential as a cosmetic ingredient.

Key words: placenta extract, radical scavenging activity, tyrosinase, elastase

Introduction

The placenta is a temporary organ that is present in females during gestation and supplies oxygen and nutrients to the developing fetus. The placenta is discharged from the mother’s body when the fetus is born. The nutritional substances including bioactive compounds therein, known as the placenta extract (PE), can be extracted. The placenta is considered as a reservoir of cytokines, hormones, bioactive peptides, enzymes, growth factors, vitamins and minerals (Togashi et al., 2002). PE also contains many valuable bioactive compounds that have various bio-capabilities, including inhibition of aging, inflammation, sunburn, gene mutation, anaphylacticity and oxidation (Kim et al., 2003). PE has been used as a biomedicine for wound healing in Korean folk medicine (Hong et al., 2010; Nath and Bhattacharyya, 2007), and the immunomodulatory effects of human PE have been demonstrated in multiple studies (Fang et al., 2007; Lee et al., 2013).

Placenta extracts (PEs) have been used to cosmetic and pharmaceutical products for whitening effect and oxidative stress related diseases (Togashi et al., 2002). When it comes to the sources of placenta tissues, human placenta extracts have been the most highly favored source of placenta extract. However, the use of human placenta extract is limited for the ethical issue in collecting the human placenta. Alternatively, sheep placenta extracts have been used to replace human placenta extracts. But, the use of sheep placenta extracts carries the risk of spongiform encephalopathy (Wrathall, 1997). To avoid these problems, porcine placenta are emerging as a new industrial source of placenta extracts. Porcine placenta extract is regarded as a suitable alternative of human placenta extract due to high genetical homogeneity between human and porcine placenta (McGregor et al., 2005; Pruitt et al., 1994).

Recently, benefits of the topical use of PE on chronic and non-healing wounds have been reported (Tiwary et al., 2006). PE also features as a component of various skin ointments and was used for skin vitalizing, nourishment, melanocyte growth and pigment inducing activities (Pal et al., 2002) as well as for the treatment of skin...
hypersensitivity like dermatitis and psoriasis. Although porcine PE-containing cosmetic materials have been claimed to provide enhanced skin permeability and to exert biological activities, few studies have been carried out on the skin permeability and biological activities.

For the use of porcine PE as a cosmeceutical, dermal absorption is an important factor for cosmetic ingredients. In this study, we investigated the skin permeability and various biological activities of porcine PE as cosmeceutical ingredients.

Materials and Methods

Preparation of porcine placenta extract (PPE)
The pig placentas obtained from normal delivery were washed thoroughly with sodium hydrate solution (NaOH) and chopped into small pieces (<1 cm), and the sliced tissues were resuspended in PBS and freeze-dried to eliminate any residual liquid (Georgieva et al., 1995). Sliced porcine placenta was homogenized by blender (HR-2084, Philips Electronics N.V., Netherland) for 10 min and centrifuged. Precipitate (water: 95%, protein: 5%) mixed with water (700 mL) and subcritical extracted by subcritical extractor system (DIONEX ASE 100, Dionex corporation, USA). During extraction, the pressure was maintained 375 bar. Extractions were performed for 30 min, 2 h and 3 h. The extract was centrifuged at 2,800 g for 20 min. The supernatants were concentrated using a vacuum evaporator at 40°C and lyophilized to produce the PPE.

Stimulated gastrointestinal digestion procedures
A gastrointestinal digestion study was performed by using the method developed by Gil-Izquierdo et al. (Gil-Izquierdo et al., 2002). The first part was simulating gastric digestion in vitro, based on the principle of equilibrium dialysis. Briefly, tri-distilled water (80 mL) was added to dried sample and the pH was adjusted 2.0 by HCl. The total volume was adjusted to 85 mL by tri-distilled water if less than 85 mL. Then, 3.0 mL freshly prepared pepsin (Sigma Chemical Co., USA) solution was added. The mixture was then incubated in shaking water bath at 40°C and lyophilized to produce the PPE.

Skin permeability test across Franz-type diffusion cell models
Skin permeation was determined by the method of Sonavane et al. (2008), with certain modifications. Male Sprague-Dawley (SD) rats, weighing 250 to 300 g (Nara Bio Animal Center, Korea), were used for the study. The hair was removed from rats with an electric clipper and an electric razor 1 day before the study. Rats were anaesthetized with ether anesthesia and decapitated. The skin was exercised immediately. The skin was cut into in to small pieces (3×3 cm). A small piece of skin put between donor cell and receptor cell of Franz-type diffusion cell. Then, 4.9 mL of 0.1 M sodium phosphate buffer (pH 7.4) was used as a receptor medium, and 100 µL of HP and SPE were placed on the donor side. The receptor medium was kept at 37°C and stirred with a magnetic stirrer at 400 rpm. The protein content of the transports was determined by using the bicinchoninic acid (BCA) method, according to the manufacturer’s instructions (Pierce Chemicals Ltd, USA). Bovine serum albumin was used as a standard.

Bioavailability of porcine placenta by intestinal sac
Everted intestinal sac experiments were performed according to the method described by Tandon and Prakash (1972) with some modifications. Male Sparague-Dawley rats weighting 220-250 g (about 7 wk) fasted overnight with free access of water. The jejunum was collected after urethane anesthesia. Collected jejunum was flushed with ice-cold Krebs-Henseleit into a bicarbonate (KHB) buffer to remove intestinal contents. The jejunum was gently stretched and cut into segments (10 cm long each). Each of the sacs was carefully everted with a glass rod. The serosal fluid transfer was reflected to the increase in the volume of fluid in the gut. Total amount of transported extract sample was expressed as total protein contents. Each sac ligated at one end. 1 mL KHB buffer was added to the sacs, after which the other end was ligated to seal the sac. The sacs were contained to conical tubes with continuous supply of 5% CO₂ and 95% O₂ and the other for removal and addition of the serosal fluid. The conical tubes were incubated in a water bath at 37°C for an hour. Intestinal transport of the sample was expressed as mg of protein/g tissue dry weight. The protein contents of the transports were determined according to the BCA method.