p-Coumaric acid as a skin whitening agent
Novel findings on its ability to attenuate melanin synthesis

Abstract

p-Coumaric acid (p-CA) is a naturally occurring secondary metabolite of plants and its potential as a skin whitening agent attracts attention. p-CA was found to be a highly potent and selective inhibitor of human tyrosinase (TYR) compared to other well known TYR inhibitors such as arbutin and kojic acid. p-CA inhibited melanin synthesis in murine melanoma cells, human epidermal melanocytes and cultured 3-dimensional human skin equivalents. It also attenuated UVB-induced cytotoxicity. p-CA formulated in a cream permeated skin tissues ex vivo and its topical application mitigated UVB-induced inflammatory erythema and skin pigmentation in vivo in human skin. These findings support that p-CA has biochemical properties that make it suitable as a skin whitening ingredient in cosmetics.

INTRODUCTION

Melanin is a major contributor to skin pigmentation and prevents UV-induced skin damage. However, its abnormal accumulation due to pathological or environmental factors causes aesthetic problems. In addition, many people prefer white or clean skin, and seek cosmetic products to decrease pigmentation. Thus, various ingredients such as arbutin and kojic acid have been incorporated into cosmetics in purpose of controlling unwanted skin pigmentation (1,2). However, their actual efficacies remain controversial and efforts to develop safer and more effective skin whitening ingredients continue. Here, we review recent studies on p-coumaric acid (p-CA) as a potential skin whitening agent for cosmetic use.

SKIN AND MELANIN

The skin is a vast organ that provides a barrier against mechanical, chemical and biological factors that have potentially harmful effects (3). The skin is composed of three major layers, which from outside to inside are the epidermis, dermis and hypodermis. Keratinocytes constitute most of the epidermal cells and melanocytes, Merkel cells and Langerhans cells make up the remainder. Skin color contributes to human beauty and attractiveness (4). The color of skin is determined by the distribution of various chromophores such as melanin, hemoglobin, bilirubin, and carotenoids. Melanin is the dark pigment responsible for coloration of the skin, hair and eyes. Melanocytes, which lie at the stratum basale of the epidermis, synthesize melanin within membrane-bound organelles called melanosomes (5).

Melanogenic enzymes act in a cascade to synthesize melanin in melanosomes (6). Tyrosinase (TYR, monophenol, dihydroxyphenylalanine:oxygen oxidoreductase, EC 1.14.18.1) initiates melanin synthesis by catalyzing the oxidations of L-tyrosine and L-3,4-dihydroxyphenylalanine (DOPA) to DOPA quinone. The conjugations of DOPA quinone with cysteine and glutathione yield 5-S-cysteinyl DOPA and glutathionyl DOPA, respectively, which are progressively polymerized to the reddish-yellow pheomelanin. The spontaneous oxidation of DOPA quinone to DOPAchrome in the absence of thiol compounds leads to the synthesis of the brownish-black eumelanin. Melanin-containing melanosomes are then transferred via dendrites to keratinocytes, which results in an even distribution of melanin throughout the epidermis (7).

NEED OF SKIN WHITENING AGENTS

Although genetic background is the major determinant of skin pigmentation, other non-genetic factors such as hormonal changes, chronic inflammation, ageing and ultraviolet light also affect it by stimulating melanin synthesis. Of course, melanin provides a “shield” against harmful ultraviolet radiation (UV) that...
can induce photoageing and photocarcinogenesis (8). In fact, enhanced melanin synthesis by forskolin has been shown to reduce the incidence of skin cancer in mice (9). Furthermore, the frequency of malignant melanoma is reported to be significantly lower in dark-skinned people (10). However, abnormally excessive melanin deposition triggered by pathophysiological or environmental factors can cause aesthetic skin problems as observed in cases of melasma, freckles, and senile lentigines.

The cosmetic relevance of abnormal melanin deposition has prompted the research and development of skin whitening agents that inhibit melanin synthesis. As an enzyme involved in the rate-limiting steps of the melanogenic pathway, TYR has been a major therapeutic target for the control of unwanted skin pigmentation. This strategies adopted include targeting TYR expression at the transcriptional and translational levels, TYR maturation via asparagine-linked oligosaccharide processing, TYR degradation and TYR catalytic activity. Numerous agents have been shown to inhibit TYR expression or activity (1,2,11-13).

p-CA INDUCES CELLULAR MELANIN SYNTHESIS

p-CA is a common secondary metabolite produced in many plants. Its anti-oxidant activity has been demonstrated in cultured cells (14) and animal models (15,16). In 2008, two independent studies reported that p-CA derived from Sasa quelparaetens and Rhodiola sachalinensis had inhibitory effects on cellular melanin synthesis in murine melanocytes cells stimulated by α-melanocyte stimulating hormone (17,18). Its inhibitory effect on cellular melanogenesis was found to be stronger than other structurally related compounds such as 3-(4-hydroxyphenyl)propionic acid, cinnamic acid and caffeic acid, suggesting p-CA has an optimal structure for the inhibition of cellular melanogenesis. The anti-melanogenic effects of p-CA have been verified in human epidermal melanocytes (HEMs) (17,19,20) and cultured 3-dimensional human skin equivalents (21).

![Diagram of melanin synthesis and inhibition by p-CA](image)

Figure 1. p-Coumaric acid (p-CA) has advantageous biochemical properties as a skin whitening agent. UVB stimulates tyrosinase (TYR) which is involved in melanin synthesis from L-tyrosine. The melanin thus formed acts as a ‘shield’ against harmful UVB but its abnormal accumulation can cause aesthetic skin problems. p-CA is a natural constituent of plants like Sasa quelpaerensis and Vaccinium bracteatum. p-CA has a chemical structure similar to L-tyrosine but it lacks the amine group and has a double bond on its side chain. p-CA is a potent inhibitor of TYR. In addition, it absorbs UVB strongly. Due to these properties, we are of the opinion that p-CA provides the anti-melanogenic and UVB-shielding effects required of ideal skin whitening agents.

p-CA IS A POTENT INHIBITOR OF HUMAN TYR

Many previous studies have used mushroom TYR as a substitute for human TYR in screening assays for skin whitening agents, probably because the former is readily available unlike the latter. However, the use of mushroom TYR for this purpose is inappropriate because the amino acid sequences and substrate specificities of human and mushroom TYRs are quite different (22, 23). Although p-CA had been previously identified as an moderate inhibitor of mushroom TYR (24), a systemic assay of p-CA and other reference compounds against TYRs of mushroom, murine and human origin revealed that p-CA is a highly potent and selective inhibitor of human TYR (19). p-CA inhibited murine and human TYRs ~10 and ~100 times more strongly than kojic acid although its effects against mushroom TYR were similar. Enzyme kinetics studies showed that p-CA acted as a mixed type or competitive inhibitor of human TYR depending on which of L-tyrosine and DOPA was used as a substrate (19). Because of the structural similarities between p-CA and the endogenous substrates of TYR, p-CA might act as a pseudo-substrate that binds to and blocks the active site of the enzyme. This notion awaits further study.

High demand for human TYR for screening assays of skin whitening agents has prompted to develop a new source of human TYR by the stable transfection of human embryonic kidney (HEK) 293 cells with a human TYR construct (20). The transformed cell line, HEK293-TYR, proliferated rapidly and expressed the active form of human TYR constitutively, and thus was useful in human TYR inhibition assays. Taking advantage of the HEK293-TYR cells as a source of human TYR, various phenylpropanoids were tested against human TYR (20). The strongest inhibitory effect was observed with p-CA (IC50 = 3 μM), followed by 3-(4-hydroxyphenyl)propionic acid (50 μM), 3-(4-hydroxyphenyl)lactic acid (70 μM), p-methoxy cinnamic acid (120 μM), cinnamic acid (200 μM), caffeic acid (250 μM), m-coumaric acid (270 μM), o-coumaric acid, (300 μM), 3-(4-hydroxyphenyl)pyruvic acid (700 μM), ferulic acid (750 μM), 3-phenylacetic acid (>1000 μM) (20). Structure-activity relationships suggest that the monohydroxyl group at the para position of the phenol ring and the double bond on the side chain of phenylpropanoids are important for TYR inhibition. Additional hydroxyl or methoxyl groups on the phenyl moiety and the hydroxyl and carbonyl groups on the side chain had negative effects on TYR inhibition. Thus p-CA was thought to have an optimal structure as a human TYR inhibitor.

The assay method using the HEK293-TYR cell line was also applied to the screening of various plant extracts (21). Of 30 plant extracts tested, the strongest inhibition of human TYR was shown by the extract of Vaccinium bracteatum, and this extract did not inhibit mushroom TYR significantly. After a series of purification steps, the active constituent was isolated and identified to be p-CA.

SKIN PERMEABILITY OF p-CA

For a TYR inhibitor to inhibit cellular melanogenesis, it must access target enzymes inside cells. Thus skin and membrane permeabilities are important issues. Kubo et al. reported that methyl p-coumarate (MPC) decreased the basal melanin content in...
CONCLUSION

The control of unwanted melanin deposition in skin is a major dermatologic and cosmetic challenge. Although laser-based techniques are used to treat such skin problems, results are not always positive or satisfactory. Furthermore, the efficacies and safety of currently used whitening ingredients are not consistently supported by scientific evidence. In these regards, it is worth noting that p-CA has many advantageous biochemical properties vis-a-vis its use as a skin whitening agent. First, p-CA is a potent and selective inhibitor of human TYR, and is far more active than well-known TYR inhibitors such as arbutin and kojic acid. Second, p-CA has the ability to attenuate cellular melanin synthesis in vitro in murine melanoma cells, HEVs, and cultured 3-dimensional human skin equivalents, without causing cytotoxicity. Third, p-CA is absorbable through skin as demonstrated by ex vivo skin permeation tests using excised porcine skin. Firth, p-CA strongly absorbs UV and prevents UVB-induced cell death. Fifth, p-CA appears to mitigate UVB-induced erythema and subsequent pigmentation in vivo in animal and human skins. Thus, we suggest that p-CA be considered a potential skin whitening agent.

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REFERENCES AND NOTES


UVB-SHIELDING PROPERTY OF p-CA

Because melanin is essentially required for protection against UV-induced skin damage, ideal skin whitening agents need to have UV-shielding effects as well as anti-melanogenic effects. We previously observed that the inhibition of cellular melanin synthesis by small interfering RNA-mediated knockdown of TYR led to a decline of cell viability upon UV exposure (28). This suggests that approaches based on the artificial inhibition of melanin synthesis without the provision of UV protection could be dangerous. In this regard, p-CA is an excellent candidate for hypo-pigmenting agents because it has both anti-melanogenic and UVB-shielding properties. This notion has been verified in HEVs treated with p-CA before and after UVB exposure (19). Cells treated after UVB exposure showed significant cell death like UVB-exposed control cells, whereas cells treated before UVB exposure showed significantly less. Furthermore, increased intracellular melanin synthesis caused by UVB exposure was attenuated by p-CA regardless of whether it was added before or after UVB exposure.

EFFICACY OF p-CA IN VIVO

Skin color can be quantified by measuring skin reflectance using the Commission Internationale de l’Eclairage (CIE) L*a*b* color system. Value a* is related to erythema or blood flow while value L* and b* are related to artificial tanning and pigmentation. The effects of p-CA cream and control cream on skin pigmentation were compared in melanin possessing hairless mice (26). UVB exposure of non-treated mice increased a* values and decreased L* values. The UVB-induced changes of a* and L* values were significantly lower in mice pretreated with p-CA cream as compared to animals pretreated with control cream. In addition, p-CA cream was found to have an inhibitory effect on UVB-induced erythema and subsequent pigmentation in human skin (29). These hypo-pigmentation effects of p-CA cream were attributed to p-CA because control cream lacking p-CA did not have these effects. p-CA cream was also found to have a depigmenting effect when it was applied repeatedly for 8 weeks to human skin fully tanned with UVB (29). Thus, continuous application of p-CA cream products to skin, before or after sun exposure, would be beneficial in terms of maintaining a lighter skin color.
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