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Department of Pharmaceutical Botany, Medical University of Lublin

DANIEL ZAŁUSKI, HELENA DANUTA SMOLARZ

Plant inhibitors of metalloproteinases and the possibility of their aplication in the prevention of photoaging

Roślinne inhibitory metaloproteinaz i możliwość ich zastosowania w prewencji fotostarzenia

CHARACTERISTICS OF PHOTOAGING

For many years it has been fashionable to expose oneself to the sun and solarium. UV irradiation causes premature skin aging called photoaging [3]. Photoaging (extrinsic skin aging) is a complex biological process affecting various layers of the skin with the major damage seen in the connective tissue of the dermis [35]. A major cause of photoaging is sunlight, and is composed of ultraviolet (UV) and infrared (IR) radiation. Histological studies revealed that the major changes in aged skin are localized in the dermis, which is composed predominantly of type I and II collagens, elastin, fibronectin [17], glycosaminoglycans [2]. Clinically, photoaging is characterized by loss of elasticity, increased roughness and dryness, irregular pigmentation, deep wrinkling, leathery appearance, blister formation and impaired wound healing [35].

MATRIX METALLOPROTEINASES

UV irradiation leads to photodestruction, phototransformation and photooxidation of collagen. These changes are modified by UV-A and UV-B [30]. UV radiation releases proinflammatory cytokines and matrix metalloproteinases (MMPs), substances that play a key role in photoaging [11]. MMPs are a group of enzymes produced in human skin (fibroblasts and keratinocytes) and other cells (tumor cells and limphocytes) [9].

The main feature of MMPs is degradation of the extracellular matrix (ECM). Their natural substrates are insoluble proteins, complex mixtures of proteins and associate macromolecules, structural components of extracellular matrices [23]. There are 28 of the matrix metalloproteinases. MMPs are divided into six families: the collagenase family, the gelatinase family, the stromelysin family, the matrylisin family, and the membran-type family [40]. They are synthesized as inactive zymogens and must be enzymatically activated for being active [8]. MMPs are Zn dependent proteins, active at neutral pH [19].

In the skin aging a key role is played by MMP-1, MMP-2, MMP-3, MMP-9 and MMP-13. UV – B is known to induce the expression of MMP-1, MMP-3 and MMP-9 in the normal human epidermis *in vivo* and UV – A is known to induce the expression of MMP – 1 by dermal fibroblasts and the expression of MMP-1, MMP-2 and MMP-3 in cultured HDFs (human dermal fibroblasts) [17]. The main MMPs in photoaging and their substrates are listed in Table 1.

Table 1. The main MMPs in photoaging and their substrates

Matrix metalloproteinase (MMP)	Substrates	Induction by	
		UVA	UVB
MMP-1	collagen I, II, III, VII, VIII, X, gelatin, etaktyn, agrecan	+	+
MMP-2	collagen I, IV, V, VII, X, XI, XIV, gelatin, fibronectin, laminin, agrecan, elastin	+	+
MMP-3	collagen III, IV, V, IX, X, XI, gelatin, laminin, fibronectin, elastin, agrecan, cazein, tenascin	+	+
MMP-9	collagen IV, V, VII, X, XIV, gelatin, agrecan, elastin, fibronectin	+	+
MMP-13	collagen I, II, III	+	+

The MMP levels increase as a consequence of the natural aging process, and after exposure to irradiation. The activity of metalloproteinases is regulated at the transcriptional, translation levels and by endogenous inhibitors such as tissue inhibitors of metalloproteinases [34]. Moreover, expression and activity of MMP-1 and other MMPs are induced *in vivo* and *in vitro* upon UVA and UVB irradiation [40]. Presently, known are four natural tissue inhibitors of metalloproteases: TIMP – 1, TIMP – 2, TIMP – 3 and TIMP – 4. The main function of TIMPs is participation in controlling the local activities of MMPs in tissues. Besides TIMPs, in human body there are other inhibitors of metalloproteases: macroglobulins [39], interferon gamma (IFN - γ), interleukine – 4 (IL-4), [34]. The external inhibitors of MMPs are antibiotics (tetracyclines and antracycline), batimastat and marimastat [34], carboxyalkyl, hydroxamates agents [19]. TIMPs controlle the breakdown of extracellular matrix (ECM) by metalloproteinases. UV-irradiation increases the activity of metalloproteinases. As a result human skin loses its elasticity, it is thinner, dry and rough [38].

The composition of the ECM depends on a coordinated balance between matrix degrading proteinases and their inhibitors. The high activity of MMPs is a reason for many cosmetological and medical defects, e.g., angiogenesis leading to telangiectasias, inflammation [7], apoptosis and tumor evolution [37].

PLANT INHIBITORS OF MATRIX METALLOPROTEINASES

Cosmetology as one of the medicine sciences uses diffrent active ingredients for the purpose of inhibition of skin aging. In cosmetology the natural inhibitors of MMPs find their application in diffrent cosmetic formulations as compounds protecting skin from wrong external agents and as potential agents inhibiting skin aging. In cosmetology the matrix metalloproteinases are used as skin anti-aging factors [38].

Many researchers from all over the world search for chemical substances as a potential source of new therapeutic agents. Their natural source are plants. A number of plants are basic components of facial and body creams, salves and shaving creams [20].

In cosmetic formulations are used, e. g., *Macrocystis pyrifera* (L.), *Camellia sinensis* (L.) and *Eucommia ulmoides* (Oliv.), [20]. Other plants containing inhibitors of MMPs are listed in Table 2.

Table 2. Examples of plant inhibitors of MMPs

Plant	Compound	Ihibition of MMP forms	References
Macrocystis pyrifera	uronic and fatty acids	MMP-2	[25]
Camellia sinensis	Polyphenols	MMP-1, 2, 3, 7, 9	[18]
Emblica officinalis	Phenolic compounds	MMP-1	[10]
Eucomia ulmoides	aucubin	MMP-1	[14]
Curcuma longa	curcumin	MMP-2, 14	[24]
Doliocarpus verruculosus	betulinic acid	stromelysin collagenase	[33]
Evodia officinalis	alkaloids, flavonoids	MMP-1	[10], [14]
Ferula persica	pericasulphide B umbelliprenin	MMP-2, 9	[31]
Glycine max	genistein	MMP-2	[15]
Passiflora edulis *Passiflora foetida*	glycosides, phenols, alkaloids, flavonoids	MMP-2, 9	[29]
Viola ibukiana	flavonoids	MMP-1	[26]
Viola hondoensis	flavonoids	MMP-1	[27]

MACROCYSTIS PYRIFERA (L.)

One of the first vegetable inhibitors of MMPs was isolated from algae *Macrocystis pyrifera* (L.) [Fig. 1]. *Macrocystis pyrifera* belongs to the *Laminariaceae* family.

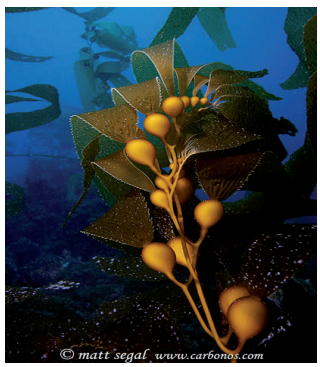

Fig. 1. *Macrocystis pyrifera*

Algal products are used in the food, cosmetic and pharmaceutical industries. In short, algae are a promising group to provide new biochemically active substances [5].

The extract from *Macrocystis pyrifera* contains active ingredients inhibiting matrix metalloproteases, such as: amino acids, minerals [5], uronic acids: L-guluronic and D-mannuronic [12], and fatty acids, which include: ω - 3: α-linolenic acid (ALA, C18:3), eicosapentaenoic acid (EPA, C20:5), docosahexaenoic acid (DHA, C22:6) ω - 6: linoleic acid (LA, C18:2), arachidonic acid (AA, C20:4) [6],

According to Kim et al. [17], eicosapentaenoic acid (EPA) can slow down UV-induced MMP expression by inhibiting the MEK1/ERK/c-Fos and SEK/JNK/c-Jun pathways. UV radiation leads to increasing MMP-1 expression by activation of two types of enzymes MEK1 and SEK1 kinase. The pretreatment of HDFs (human dermal fibroblasts) with EPA decreased UV-induced MMP-1

expression by inhibiting ERK kinase (MEK1) and JNK kinase (SEK1). It resulted in the decrease of c-Fos expression and c-Jun expression induced by UV, which led to the inhibition of UV-induced activator protein – 1 DNA binding activity (AP-1) and MMP – 1 respectively. EPA can be a potential agent for the prevention and treatment of skin aging. EPA inhibits UV-induced MMP-1 expression in human dermal fibroblasts. Moreover, the researchers suggested that the inhibitory effects of PUFAs (polyunsaturated fatty acids) on UV-induced MMP-1 expression were dependent on their structure. N-3 and n-9 PUFAs inhibited UV-induced MMP-1 expression, but n-6 PUFAs did not. The mechanism of the inhibition of UV-induced MMP-1 expression by EPA is introduced below (Fig.2.) [17].

On the other hand, the extract of *Macrocystis pyrifera* inhibited the expression of the metalloproteinases (MMPs) in human keratinocytes. The concentrations of the extract were 4, 10, 20 mg/ml. The application of the extract of *Macrocystis pyrifera* has shown an anti-ageing action with a reduction in the fine lines [4].

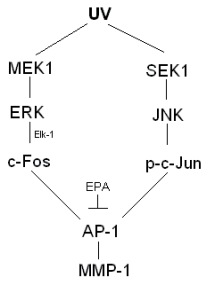

Fig. 2. Mechanism of the inhibition of UV-induced MMP-1 expression by EPA

CAMELLIA SINENSIS (L.)

Another plant with propreties inhibiting MMPs is *Camellia sinensis* (L.) (green tea), [41]. *Camellia sinensis* belongs to the *Theaceae* family [21]. In use are the leaves of this plant (Fig. 3).

Fig. 3. *Camellia sinensis*

Camellia sinensis contains four major polyphenols: (-)-epicatechin (EC), (-)-epicatechin-3-gallate (ECG), (-)-epigallocatechin (EGC) and (-)-epigallocatechin-3-gallate (EGCG). Some other agents that show chemopreventive activities have been found in green tea as well. These include caffeine, flavandiols, flavanoids, phenolic acid as well as alkaloids theobromine and theophylline. EGCG is the major compound of *Camellia sinensis* constituting approximately 40% of the total polyphenols [1]. Kim and et al. studied the influence of EGCG expression of MMP-1 (collagenase 1) in cultured human fibroblasts. The results showed that EGGC blocked UVA-induced collagenase expression. According to researchers, the inhibitory mechanism of collagenase by EGCG is related to transcription factors, AP-1 and NF-κB. The activity of MMPs is dependent on these factors. The level of MMPs is higher after exposure to the sunlight. The obtained results suggest that EGCG has a potential anti-photoaging effect [10].

Other studies have shown similar results. A purified extract of *Camellia sinensis* (0.2%, wt/vol) blocked the expression of MMP in the photoaged model of mouse skin. A decrease of the expression of MMP-2 (67%), MMP-3 (63%), MMP-7 (62%) and MMP-9 (60%) was noticed. This is another piece of evidence in favour of using green tea polyphenols in prevention of solar UV light photoaging in human skin [36].

EUCOMMIA ULMOIDES (OLIV.)

Eucommia ulmoides (Oliv.) belongs to the *Eucommiaceae* family (Fig.4). *Eucommia ulmoides* is known as the Gutta Percha Tee, the Rubber Bark Tree and Du-Zhong [28].

Fig. 4. *Eucommia ulmoides*

The main bioactive components of *Eucommia ulmoides* are lignans and iridoids. Eucommia bark is rich in bicyclooctane and tetrahydrofuran type lignans, e.g.: pinoresinol, syringaresinol, medioresinol, olivil, 1-hydroksypinoresinol and their mono-and diglucosides. Two of the major constituents of both Eucommia bark and leaf are iridoids geniposidic acid and aucubin. Moreover, *Eucommia ulmoides* contains terpens (eucommiol, eucommioside I, β-sitosterol glucoside) and phenols (caffeic acid, methyl chlorogate, *erythro* – guaiacylglycerol, *threo* – guaiacylglycerol, guaiacylglycerol β-coniferyl aldehyde ether, dihydrodehydrodiconiferyl alcohol, *erythro* - dihydrodehydrodiconiferyl alcohol, *threo* - dihydrodehydrodiconiferyl alcohol) [8].

The other chemical constituents isolated from bark of this plant are: rutin, chlorogenic acid [32], β-amyrin, squalen, ulmoprenol, dulcit, ajugosid, reptosid, ulmosid and eucomiosid [13].

Ho et al. [14] investigated the inhibitory effects of *Eucommia ulmoides* on the activity of MMP-1. The methanol extract of *Eucommia ulmoides* showed a potent inhibitory effect on the matrix metalloproteinase – 1 (MMP-1) production in ultraviolet B irradiated human fibroblasts. The most active compound was aucubin, which is a common iridoid glucoside, isolated from the cortex and leaves. Aucubin suppressed the production of MMP-1 at a concentration of 0.01 µg/ml. The MMP-1 inhibitory effect of aucubin at this concentration was 57.3%. According to the authors, aucubin can play a role in preventing the photoaging, which is attributable to MMP-1 mediated dermal damage [14]. On the other hand, a methanol extract of *Eucommia ulmoides* increased collagen synthesis [22].

Plant drugs play an important role in the health care systems. The use of plants in cosmetology is a great promise for new cosmetic discoveries based on their active compounds. In fact, it is largely thanks to plant drugs that the cosmetic industry is growing today. Hopefully, future research will lead to the discovery of new biologically active compounds, which will act on different biological levels.

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SUMMARY

Photoaging is a process of skin aging caused by long-term exposure to the ultraviolet radiations of the sun. Photoaging is characterized in part by wrinkles, altered pigmentation, loss of skin elasticity, increased roughness and dryness. The UV radiation releases enzymes called matrix metalloproteineases (MMPs) in human body. MMPs degrade the extracellular matrix (ECM). Skin ECM includes structural proteins such as: collagens, elastin, fibronectin, laminin, gelatin and agrecan. The destruction of ECM can lead to premature skin aging, angiogenesis, inflammation, apoptosis and tumor evolution. There are five types of MMPs: collagenases, gelatinases, stromelysins, matrylisins and membran-type MMPs. In photoaging a key role is played by MMP - 1, MMP - 2, MMP - 3, MMP - 9 and MMP - 13. The activity of all MMPs is dependent on present inhibitors, both natural and synthetic. Known are four types of tissue inhibitors: TIMP – 1, TIMP - 2, TIMP - 3 and TIMP-4. Besides, many researchers look for chemical substances as sources of new therapeutic agents. Their natural source are plants. Several plants, e.g., *Macrocystis pyrifera* (L.), *Camellia sinensis* (L.) and *Eucommia ulmoides* (Oliv.), are known to possess extracts able to inhibit MMPs.

STRESZCZENIE

Fotostarzenie się jest procesem starzenia się skóry, którego główną przyczyną jest długotrwała ekspozycja skóry na promieniowanie ultrafioletowe. Skutkiem tego procesu jest przedwczesne pojawianie się zmarszczek, nadmierna melanogeneza, utrata elastyczności, zwiększona szorstkość i suchość skóry. W organizmie człowieka promieniowanie UV powoduje uwalnianie enzymów zwanych metaloproteinazami macierzy zewnątrzkomórkowej (MMPs). MMPs powodują rozkład mecierzy zewnatrzkomórkowej (ECM). Macierz zewnątrzkomórkowa zbudowana jest z białek strukturalnych takich jak: kolagen, elastyna, fibronektyna, laminina, żelatyna i agrekan. Rozkład ECM może być przyczyną wcześniejszego starzenia się skóry, angiogenezy, stanów zapalnych, apoptozy i rozwoju nowotworów. Obecnie znanych jest pięć rodzajów MMPs: kolagenazy, żelatynazy, stromielizyny, matrylizyny i błonowe MMPs. W fotostarzeniu największy udział przypisuje się MMP-1, MMP-2, MMP-3, MMP-9 i MMP-13. Aktywność MMPs jest regulowana przez inhibitory pochodzenia naturalnego jak i syntetyczne. Znane są cztery rodzaje tkankowych inhibitorów metalloproteinaz: TIMP-1, TIMP-2, TIMP-3 i TIMP-4. Wielu naukowców poszukuje biologicznie aktywnych związków chemicznych w celu zastosowania ich jako inhibitory MMPs. Ich naturalnym zródłem są rośliny. Do znanych roślin posiadających takie właściwości należą: *Macrocystis pyrifera* (L.), *Camellia sinensis* (L.) and *Eucommia ulmoides* (Oliv.).