Life Sciences 174 (2017) 77–82

Contents lists available at ScienceDirect

Life Sciences

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

Effect of orally ingested diosgenin into diet on skin collagen content in a low collagen skin mouse model and its mechanism of action

Akinori Haratake, Daisuke Watase *, Shuichi Setoguchi, Nami Nagata-Akaho, Kazuhisa Matsunaga, Jiro Takata

Laboratory of Drug Design and Drug Delivery, Faculty of Pharmaceutical Sciences, Fukuoka University, 8-19-1 Nanakuma, Jonan-ku, Fukuoka 814-0180, Japan

ARTICLE INFO

Article history:
Received 6 December 2016
Received in revised form 25 February 2017
Accepted 27 February 2017
Available online 1 March 2017

Keywords:
Skin collagen content
Diosgenin
Oral ingestion
Low collagen skin mouse model
Primary cultured fibroblast

ABSTRACT

Aims: Influence on collagen content with oral ingestion of diosgenin (Dios) was investigated in established low collagen skin mouse model. And its mechanism of action was investigated using primary cultured fibroblasts.
Main methods: Hairless mice were fed a low protein diet with Dios for 8 weeks and the contents of collagen in skin were determined by measuring the content of hydroxyproline (Hyp). In primary cultured fibroblasts, the numbers of fibroblast were determined by incubating with Dios for 120 h; the contents of Hyp were determined by incubating with Dios for 24 or 72 h using fibroblasts of confluent state; the expressions of messenger ribonucleic acid (mRNA) were determined by incubating with Dios for 24 h.
Key findings: Oral ingestion of Dios in the diet for 8 weeks led to a dose-dependent increase in the Hyp content as collagen content of skin. In proliferating of primary cultured fibroblasts, Dios treatment led to a decrease of adenosine 5′-triphosphate content indicating decrease of the cell number. In the cells reached to confluent, although increase of Hyp content in the control indicating progress of fibroblasts differentiation were observed, the content of Hyp remained unchanged with Dios treatment. Finally, addition of Dios led to a decrease the α-tubulin and c-*fos* mRNA expressions relating to the cell cycle.
Significance: It is concluded that Dios can improve skin collagen content by shifting the dynamics of the fibroblasts from proliferation to differentiation *via* cell cycle arrest.

© 2017 Elsevier Inc. All rights reserved.

1. Introduction

Skin appearance is a primary indicator of age, with skin becoming unevenly colored, rough, lax, and wrinkled as it ages [1,2]. A common feature of aged skin is the fragmentation of the dermal collagen matrix. With this in mind, the development of supplements with the improvement of the dermal collagen matrix would be highly desirable.

Diosgenin (Dios), which was originally extracted from wild yam roots (*Dioscorea composite* or *Dioscorea villosa*), is a steroidal saponin that can be found in a wide variety of plants [3]. It is noteworthy that extracts from these plants have been used in traditional medicines for the treatment of diabetes [4–6], hypercholesterolemia [7,8], and gastrointestinal ailments [9,10]. Furthermore, Dios increased the secretion of collagen from human skin fibroblastic cells [11]. In contrast, collagen messenger ribonucleic acid (mRNA) expression was higher in ovariectomized rats compared with sham rats; the administration of Dios led to a decrease in the expression of collagen mRNA in the ovariectomized group [12]. The results of previous studies have shown that Dios did not exhibit any activity towards breast cancer-burdened mice, while 17β-estradiol accelerated tumor growth [13]. In addition, Dios enhanced deoxyribonucleic acid (DNA) synthesis in a three-dimensional human skin model, which used restructured keratinocytes derived from foreskin specimens [13].

Collagen peptide (CP) from gelatin hydrolysate is used in several foods and dietary supplements, and the ingestion of CP has been shown to induce numerous biological processes. The results of several animal experiments and preclinical trials have provided evidence to support the beneficial effects of CP [14–19]. The oral ingestion of CP exhibited beneficial effects on the synthesis of the dermal matrix during clinical trials in humans [20,21] and animal experiment [22]. Furthermore, it has been reported previously that a low collagen skin mouse model has been established using male hairless mice and that the administration of CP and glycine, alanine, and proline mixture had a significant influence on the collagen content in the model [23]. Moreover, the effects of dipeptides and amino acids derived from CP towards primary cultured fibroblasts have shown that the CP induces changes in the state of the fibroblasts [23]. These findings therefore indicate the possibility that the Dios plays an important role in determining the collagen content of the skin.

In the present study, the effects of the oral ingestion of Dios on skin collagen content in a low collagen skin model have been investigated. Furthermore, to explain the action mechanism of Dios influence on proliferation and differentiation in primary cultured murine fibroblasts and influence on mRNA expression relating cell cycle have been investigated.

* Corresponding author.
E-mail address: watase@fukuoka-u.ac.jp (D. Watase).

http://dx.doi.org/10.1016/j.lfs.2017.02.013
0024-3205/© 2017 Elsevier Inc. All rights reserved.

Table 1
Composition (g/kg) of the normal (AIN-93M) and low protein diets used in this study.

Ingredient	Diet groups	
	Normal	Low protein
Milk casein	140.000	100.000
L-Cysteine	1.800	1.500
Corn starch	465.692	465.692
alpha-Corn starch	155.000	155.000
Sucrose	100.000	140.300
Soybean oil	40.000	40.000
Cellulose powder	50.000	50.000
AIN-93M mineral mix	35.000	35.000
AIN-93M vitamin mix	10.000	10.000
Choline bitartrate	2.500	2.500
TBHQ[a]	0.008	0.008
Total	1000.000	1000.000

[a] Tertiary butylhydroquinone.

2. Materials and methods

2.1. Animals

Male hairless mice (Hos:HR-1) at 5 or 6 weeks old were purchased from Japan SLC (Shizuoka, Japan), and housed in standard cages (345 × 403 × 177 mm) under controlled conditions (*i.e.*, ambient temperature, 23 ± 2 °C; relative humidity, 60 ± 10%; 12-hour light/dark cycle), where they were given commercial diet (Table 1, AIN-93M, Oriental Yeast, Tokyo, Japan) and water *ad libitum*. All procedures regarding the care and use of the animals were conducted in accordance with the regulations dictated by the Experimental Animal Care and Use Committee of Fukuoka University.

2.2. Chemicals

A low protein diet, where the protein content of the AIN-93M was reduced from 14 to 10%, was purchased from Oriental Yeast (Table 1). To match the total number of calories consumed in the normal and low protein diets, the low protein diet was supplemented sucrose of the amount meeting the calories of protein reduction. Dios was purchased from Sigma (St. Louis, MO, USA). All of the other chemicals used in this study were purchased from Wako Pure Chemical Industries (Osaka, Japan), unless noted otherwise, and used as supplied without further purification.

2.3. Influence of oral Dios on a low collagen skin mouse model

Because a low collagen skin mouse model used for this study was not able to find remarkable differences in the histologic observation in the sections of whole skin between normal diet and low protein diet groups, Hyp contents as the collagen contents were determined and were compared those of each group.

Nine-week-old male hairless mice were randomly divided into groups (n = 10). The normal diet group was fed a normal diet (AIN-93M), whereas the other groups of low collagen skin mouse model were fed a low protein diet containing 0 (control), 0.05, 0.1, or 0.2% (w/w) Dios with *ad libitum* access to water for 8 weeks. The total food intake for each group was recorded five times a week and the body weight of each mouse was recorded once a week. After 8 weeks of consuming the diet, the mice were euthanized without suffering by cervical dislocation, and dorsal skin specimens (8 mm in diameter) were collected and stored at −30 °C prior to being analyzed.

The collagen contents were estimated by determining the amount of hydroxyproline (Hyp) in the HCl hydrolysate of the samples. The collagen content can be calculated by multiplying the Hyp contents and the value that divided Hyp contents by total amino acid contents. A sample of the skin was weighed and added to pure water (1 mL) before being treated with 12 mol/L HCl (1 mL) and hydrolyzed at 110 °C for 24 h. The hydrolysates were then cooled to ambient temperature and evaporated under vacuum using a centrifugal concentrator (Tomy Seiko, Tokyo, Japan) to give residues, which were dissolved in 1 mL or of 10 mmol/L HCl. The concentration of Hyp was determined by high performance liquid chromatograph (Model 10AD$_{VP}$, Shimadzu, Kyoto, Japan) using a cation-exchange column (AApak Li 6.0 × 100 mm, JASCO, Tokyo, Japan) for the separation of the amino acids and post-column derivatization with ortho-phthalaldehyde, as described previously [23]. The Hyp content of the skin was expressed as μmol/g of wet skin.

2.4. Effects of Dios on primary cultured murine fibroblasts

The effect of Dios on the proliferation and differentiation of primary cultured murine fibroblasts were investigated to clarify the mechanistic mode of action of Dios in the mouse model.

Fibroblasts were obtained from the skin of hairless mice using a method previously described in the literature [23]. Six-week-old male hairless mice were euthanized and their skin was sterilized with ethanol. The dorsal skin was then stripped and rinsed with phosphate-buffered saline. Pieces of the skin (approximately 3 mm^2) were placed into plastic Petri dishes (100 mm in diameter, Falcon BD, Franklin Lakes, NJ, USA) with 8 mL of Dulbecco's modified Eagle's medium (DMEM) containing 1% penicillin-streptomycin and 10% fetal bovine serum (FBS). The dishes were then placed in a humidified incubator at 37 °C in an atmosphere containing 5% CO_2 for 2 weeks. After incubation, the skin pieces were removed and the fibroblasts recovered.

To evaluate influence on fibroblasts proliferation, fibroblast suspensions of 5×10^4 cells/mL in DMEM containing 1% penicillin-streptomycin, 1% FBS, and several concentrations of Dios were prepared. Dios was dissolved in ethanol heated at 65 °C in an ultrasonic bath, because Dios hardly dissolved for many solvents. And this solution was added to cell suspensions after diluted >100 times using medium. Samples of the fibroblast suspensions (0.1 mL) were dispensed into each well of 96-well plastic plates, and the plates were incubated for 6 days at 37 °C in an atmosphere containing 5% CO_2. After incubation, the quantity of adenosine 5′-triphosphate (ATP) relative to the number of living fibroblasts was determined using a CellTiter-Glo assay (Promega, Madison, WI, USA) according to the protocol described previously. Furthermore, the fibroblast lysate and medium were recovered and stored at −30 °C prior to being analyzed. To determine of collagen content, samples of the fibroblast lysate and medium (0.2 mL) were added to 12 mol/L HCl (0.2 mL), and the resulting mixtures were hydrolyzed at 150 °C for 1 h. The hydrolysates were evaporated and were dissolved in 0.2 mL of 10 mmol/L HCl. The amounts of Hyp as an indication of collagen content were analyzed using the method described above. The Hyp contents of the fibroblast lysate and medium samples were expressed as nmol/10^3 cells.

Confluent cells can undergo differentiation and the amount of collagen synthesis was used as an index of differentiation. Following a 6-day period of incubation in DMEM containing 1% penicillin-streptomycin and 10% FBS, the fibroblasts were incubated in 96-well plastic plates in serum-free DMEM containing different concentrations of Dios for 24 and 72 h at 37 °C in an atmosphere containing 5% CO_2. The differentiation of the fibroblasts was evaluated based on the hydroxyproline (Hyp) contents of the fibroblasts lysate and medium samples (nmol/mL) per 10^3 cells to give an indication of the collagen content. The number of fibroblasts was determined according to the method described above, and samples of the fibroblast lysates and medium were recovered and stored at −30 °C prior to analysis. The Hyp contents were analyzed using to the method described above. The Hyp contents were expressed as nmol/10^3 cells.

Download English Version:

https://daneshyari.com/en/article/5556983

Download Persian Version:

https://daneshyari.com/article/5556983

Daneshyari.com