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Effects of Collagen Ingestion and their Biological Significance

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Abstract

Collagen is the most abundant extracellular matrix protein in animal tissues, and heat-denatured collagen (gelatin) and its hydrolysate (collagen peptide) are frequently used as food supplements. Clinical trials revealed that supplemental ingestion of 5-10 g collagen peptide daily improved the properties of facial skin, suppressed ultraviolet-induced skin erythema, and improved the T-cell-related immune status of Japanese people who ingest an average of 1.9 g collagen from their daily diet. Ingested collagen is digested and absorbed partly as prolylhydroxyproline (Pro-Hyp) and hydroxyprolylglycine (Hyp-Gly), resulting in their rather high concentrations in blood. The beneficial effects of collagen peptide ingestion appear to be mediated at least partly by these collagen-derived dipeptides. Collagen-derived Pro-Hyp exhibits physiological activity in the *in vitro* differentiation processes of skin fibroblasts, chondrocytic cells, and pre-adipocytes; in addition, the expression of oligopeptide transporter genes has been confirmed in pre-adipocytes. Thus, it is tempting to speculate that the target cells of Pro-Hyp are precursor cells. Pro-Hyp is generated endogenously when collagen turnover is enhanced, such as in an ear inflamed by contact dermatitis, and ingested Pro-Hyp appears in the same dermatitic ear when ingested orally. Thus, endogenous and food-derived Pro-Hyp co-localizes in the same tissue and may co-interact. Further studies on Pro-Hyp may reveal novel interactions between homeostasis of the tissue and this animal-derived nutrient.

Keywords: Collagen; Collagen peptide; Prolylhydroxyproline; Hydroxyprolylglycine

Abbreviations

CP: Collagen Peptide; Gly: Glycine; Pro: Proline; Hyp: Hydroxyproline: Pro-Hyp: Prolylhydroxyproline; Hyp-Gly: Hydroxyprolylglycine; DNFB: 2,4-Dinitrofluorobenzene; V: Vehicle; HIF-1: Hypoxia-Inducible Factor 1; DAMPs: Damage-Associated Molecular Patterns; PAMPs: Pathogen-Associated Molecular Patterns

Effects of Collagen Ingestion

Collagen is the most abundant extracellular matrix protein in animal tissues, and genes encoding for multiple types of collagen has been identified in human and animals. Collagen can be extracted from collagen-rich materials such as skin, bone, and fish scales as gelatin using hot water, and gelatin or its hydrolysate (collagen peptide: CP) is frequently used as a food supplement. CP ingestion has been reported to have beneficial effects on the skin such as reduced skin wrinkles and increased collagen synthesis [1,2]. Although collagen synthesis is increased with CP ingestion, deterioration of a fibrotic disease with CP ingestion was not observed in a study by Dr. Kenji Sato using choline deficiency-induced animal model of liver cirrhosis [3]. Beneficial effects were also observed for bone [4] and joints [5]. However, the effective dose of a supplement will change depending on the amount of collagen obtained from the daily diet; the adequate ingestion of a nutrient from the diet results in supplemental ingestion of this nutrient having little or no beneficial health effect. Therefore, the effective dose of a nutrient in clinical trials could change depending on the dietary habits, as well as the genetic background and life style, of the trial participants.

Type I collagen is the most abundant type of collagen in animal-derived foods. The content of collagen in food varies considerably depending on its origin; it is very high in beef tendon, pork chitterlings, chicken sternum cartilage, and fish skin (Table 1) [6].

Using the values of collagen content reported by Noguchi et al. [6], we estimated that Japanese people ingest on average 1.9 g collagen from their daily diet [6].

Clinical trials to investigate the effects of CP supplemental ingestion by Japanese people have been published; placebo-controlled double-blind trials revealed that daily ingestion of 5 g CP improved the properties of facial skin [7] and suppressed ultraviolet-induced skin erythema [8]. It was also reported that the T-cell-related immune status of Japanese suffering from chronic tiredness was improved by the intake of 10 g CP per day for 8 weeks [9]. Thus, supplemental ingestion of 5 g to 10 g CP by Japanese people, in addition to their daily intake of 1.9 g collagen, delivers beneficial health effects.

Digestion and Absorption of Collagen

The amino acid sequence of collagen in the triple-helical region is represented as $-(Gly\text{-}X\text{-}Y)_n-$. The X and Y positions are frequently occupied by proline (Pro) and hydroxyproline (Hyp), respectively, and thus the di-peptide sequences Pro-Hyp and Hyp-Gly are frequently found in collagen. Hyp can be used as a marker for investigation of the digestion and absorption of collagen, because Hyp is not found in other proteins, with for a few exceptions such as elastin.

Meat/Fish Food products	Collagen content (mg/g)	Meat/Fish Food products	Collagen content (mg/g)
Beef	7.5	Salmon	8.2[a]
Beef tendon	49.8		24.1[b]
Pork	11.9	Sierra	10.4[a]
Pork chitterlings	30.8		12.8[b]
Chicken thigh	15.6	Yellowtail	9.7[a]
Chicken wing tip	15.5		16.2[b]
Chicken drumstick	19.9	Broiled eel	55.3
Chicken liver	8.6	Daggertooth pike conger skin	76.6
Chicken gizzard	23.2	Squid	13.8
Chicken sternum cartilage	40	Shrimp	11.5
Ham	11.2	Boiled and dried baby sardines	19.2
Tuna	5.7	Sand eel	12.9
Short-neck clam	11	-	-

Table 1: Collagen content in animal-derived food materials [a) without skin, b) with skin. Modified from [6]].

It is believed that proteins are hydrolyzed into amino acids prior to the absorption of the amino acids from the gastrointestinal tract, and that little peptide absorption occurs during protein digestion [10]. However, it was reported that there is a considerable amount of Hyp-containing peptide in the plasma following the ingestion of gelatin [11]; however, the peptide's identity remained unknown until 2005. Iwai et al. [12] identified six collagen-derived Hyp-containing oligopeptides in human blood after oral ingestion of CP; the peptide-form of Hyp increased and reached a maximal level of 20-60 nmol/mL of plasma after ingestion of 9.4 g-23 g of CP, and was primarily in the form of the dipeptide Pro-Hyp. Another collagen-derived dipeptide, Hyp-Gly, was identified in the blood after ingestion of CP, but its ratio to Pro-Hyp varied extensively between subjects [13]. Recently, 13 oligopeptides, including Pro-Hyp and Hyp-Gly, were accurately measured in the blood of individuals following ingestion of CP by using stable isotope-labeled collagen oligopeptides as internal standards [14]. Hyp-containing oligopeptides likely appear in blood because they are highly resistant to blood proteases and are stable in serum [12,13,15].

These studies indicate that collagen-derived oligopeptides appear in blood at fairly high concentrations and suggest that the beneficial effects of collagen ingestion are mediated by these oligopeptides. Shigemura et al. [16] reported that Pro-Hyp increased the number of fibroblasts that migrated from explanted skin *in vitro* and that Pro-Hyp enhanced the growth of fibroblasts on collagen gel in a dose-dependent manner. Hyp-Gly was more effective than Pro-Hyp at stimulating murine fibroblast proliferation on collagen gel [13]. *In vitro* treatment of human skin fibroblasts with Pro-Hyp resulted in increased cell proliferation and hyaluronic acid synthesis, probably by elevating hyaluronan synthase 2 mRNA levels [17]. Treatment of murine chondrocyte ATDC5 with Pro-Hyp inhibited differentiation into mineralized chondrocytes *in vitro* [18], and hyaluronic acid production by the synovium cell line HIG-82 was enhanced by treatment with Pro-Hyp [19]. Furthermore, the size of oil droplets decreased significantly upon treatment with Pro-Hyp during the differentiation of murine pre-adipocyte 3T3-L1 into mature adipocytes *in vitro* [20]. These *in vitro* studies can be related with several *in vivo* animal studies of CP ingestion, including the promotion of cutaneous wound healing in a rat model of pressure ulcer [21], protection from articular cartilage damage induced by excess ingestion of phosphorus in mice [18], and lowering of blood lipids following the ingestion of oil in rats [22].

It therefore seems probable that the biological activities of ingested collagen are mediated, at least partly, by collagen-derived oligopeptides such as Pro-Hyp and/or Hyp-Gly. Transportation of an oligopeptide into a cell can be mediated by proton-coupled oligopeptide transporters PEPT1, PEPT2, PHT1, and PHT2 (Ci1 in mice). In this context, it is noteworthy that the genes encoding these oligopeptide transporters are expressed in Pro-Hyp-responsive pre-adipocytes and their expression profiles change when they differentiate into mature adipocytes [20]. It is not clear at present whether Pro-Hyp exhibits activity in the extracellular space by binding to an unidentified receptor, or by modulating signal transduction pathways after entering a cell. However, it is known that collagen-derived oligopeptides modulate the catalyzing activity of enzymes including angiotensin I-converting enzyme [23,24] and dipeptidyl peptidase IV [25]. Hypoxia-inducible factor 1 (HIF-1) is a ubiquitously expressed key regulator responsible for the induction of multiple genes that facilitate adaptation to low oxygen tension (hypoxia). The stability and subsequent transcriptional function of HIF-1α (a subunit of HIF-1) is controlled partly through hydroxylation of two specific proline residues by a family of 2-oxoglutarate-dependent dioxygenases resulting in the formation of Hyp [26]. Therefore, the working hypothesis of this study is that collagen-derived oligopeptides, after being transported into the cytoplasm, modulate the enzymatic activity of a family of 2-oxoglutarate-dependent dioxygenases. Studies of the activity of 2-oxoglutarate-dependent dioxygenases in Pro-Hyp-responsive skin fibroblasts [16,17], chondrocytes [18], synovium cells

[19] and adipocytes [20] may reveal novel aspects of collagen-derived oligopeptide functions.

Possible Target Cells of Pro-Hyp

As described above, cells that respond to Pro-Hyp include 1) skin fibroblasts that migrated from explanted skin and proliferated [13,16], 2) chondrocytic cells that differentiate into mineralized chondrocytes *in vitro* [18], and 3) pre-adipocytes that mature into adipocytes *in vitro* [20]. Explant culture of a skin sample is an *in vitro* model of cutaneous wound healing, and differentiation of stem cells plays a pivotal role in the healing process [27]. In the latter two cases, chondrocytic cells and pre-adipocytes respond to Pro-Hyp when they are undergoing differentiation processes. Although information is limited with regard to what cell types respond to Pro-Hyp, it is tempting to speculate that the target cells of Pro-Hyp are precursor cells that differentiate or mature into more differentiated states, since differentiation processes are involved in all three cases, though embryonic stem cells seem not to respond to Pro-Hyp and Hyp-Gly [28].

Co-Localization of Endogenous and Food-derived Pro-Hyp

Peptide-form Hyp has been identified in the urine of growing children [29] and patients with bone tumors [30] or rheumatoid arthritis [31]. These studies suggest that collagen oligopeptides are generated endogenously by extensive degradation of collagen when the turnover of collagen is enhanced. Therefore, it seems likely that endogenously generated oligopeptides and food-derived oligopeptides co-localize in the same tissue and interact with each other. We studied this possibility by using Pro-Hyp and Pro double-labeled with the stable isotopes $^{13}C_5$ and ^{15}N [32]. Endogenous Pro-Hyp was identified in inflammatory ear tissue caused by allergic contact dermatitis in mice fed a Hyp-free diet (Figure 1).

When labeled Pro-Hyp was administered orally to these mice, labeled Pro was detected after 4 h in the ear with chronic inflammation but not in the control ear of the same mouse (Figure 2A). This suggests that ingested Pro-Hyp was cleaved by an enzyme that is expressed or activated in chronically inflamed tissue, generating free Pro that can be incorporated into newly synthesized collagen. On the other hand, when labeled Pro was administered, labeled Pro-Hyp was identified at 4 h in the inflamed ear but not in the control ear, suggesting that orally ingested Pro was incorporated into newly synthesized collagen and that this collagen was degraded, generating Pro-Hyp in the chronically inflamed tissue (Figure 2B). Thus, turnover of tissue collagen and digestion of food-derived collagen may interact with each other in the same tissue through generation of the common dipeptide Pro-Hyp. Accumulation of food-derived Pro-Hyp in the inflammatory tissue may explain the promotion of skin wound healing by ingestion of CP [21].

It is noteworthy that the concentration of Hyp-Gly was very low in the ear subjected to contact dermatitis, despite the generation of Pro-Hyp at this site (Figure 1). In contrast, Hyp-Gly was found in the blood of individuals who ingested CP [13]. Hyp-Gly might therefore be produced differentially between two processes: endogenous collagen turnover and digestive cleavage of food-derived collagen.

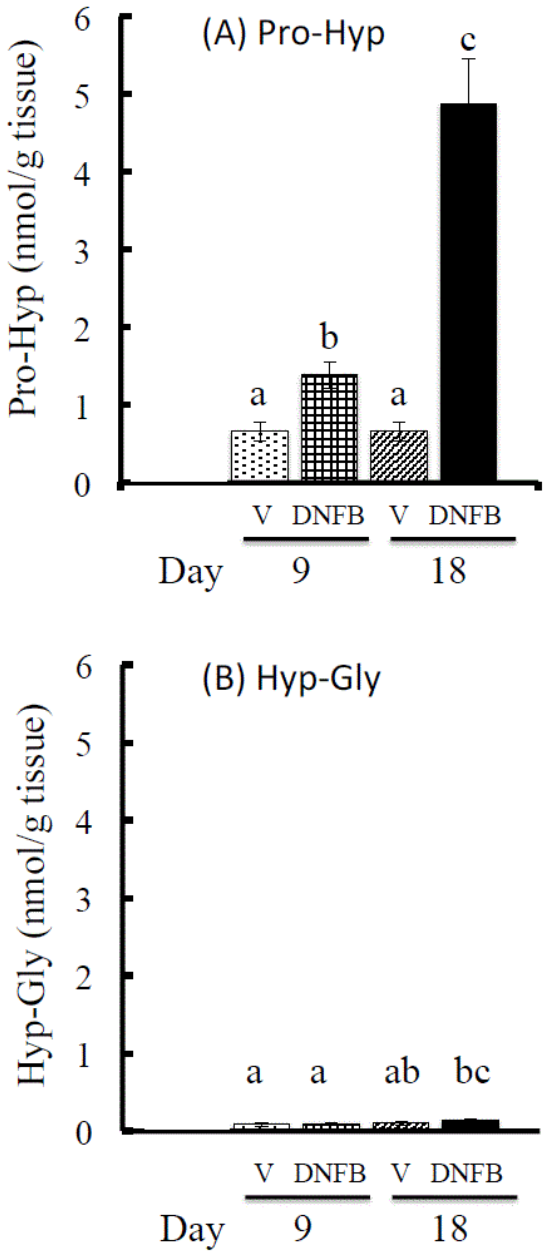

Figure 1: Concentrations of Pro-Hyp (A) and Hyp-Gly (B) in the ear of a mouse fed a Hyp-free diet at 9 and 18 days following the induction of contact dermatitis. Values in the same figure not sharing a common letter above the bar are significantly different from each other at $p<0.05$. Modified from [32]. DNFB: 2,4-dinitrofluorobenzene.

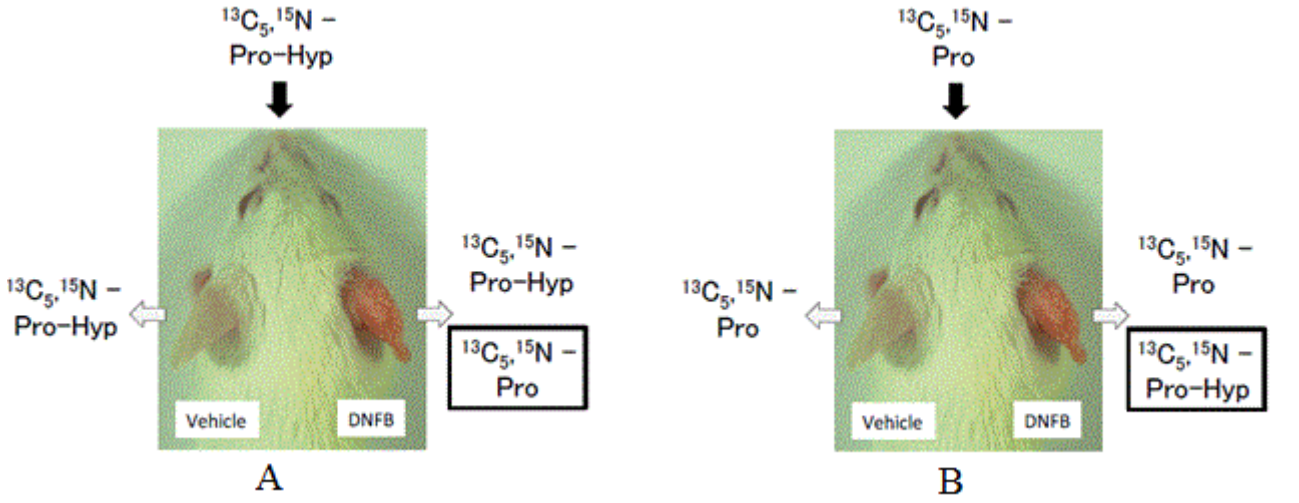

Figure 2: Identification of $^{13}C_5$, ^{15}N-Pro and $^{13}C_5$,^{15}N-Pro-Hyp in the ear of a mouse at 4 h after oral administration of $^{13}C_5$,^{15}N-Pro-Hyp (A) or $^{13}C_5$,^{15}N-Pro (B). Modified from [32]. DNFB: 2,4-dinitrofluorobenzene.

Biological Significance of Collagen-Derived Oligopeptides

Collagen-derived oligopeptides are generated in small amounts in normal tissue because of slow collagen turnover. However, invasion by a pathogen or a tissue wound evokes inflammatory responses, and the extracellular matrix, including collagen, is rapidly reorganized. Abundant Pro-Hyp appears in the inflamed tissue, as was shown in the case of chronic inflammation of the skin [32]. This implies that collagen-derived oligopeptides such as Pro-Hyp could provide damage- or pathogen-associated molecular patterns (DAMPs, PAMPs) [33] representing damage to the extracellular matrix. Therefore, the appearance of Pro-Hyp in a tissue could be an extracellular matrix-associated signal to initiate tissue repair by stimulating stem cells or immune cells. This protective response could be a common system throughout the body because collagen is a ubiquitous extracellular matrix component. Further studies on Pro-Hyp may reveal novel aspects of the interactions between tissue homeostasis and this animal-derived nutrient.

References

1. Proksch E, Segger D, Degwert J, Schunck M, Zague V, et al. (2014) Oral supplementation of specific collagen peptides has beneficial effects on human skin physiology: a double-blind, placebo-controlled study. Skin Pharmacol Physiol 27: 47-55.
2. Proksch E, Schunck M, Zague V, Segger D, Degwert J, et al. (2014) Oral intake of specific bioactive collagen peptides reduces skin wrinkles and increases dermal matrix synthesis. Skin Pharmacol Physiol 27: 113-119.
3. Dr. Kenji Sato (2016) Kyoto University, Japan.
4. Moskowitz RW (2000) Role of collagen hydrolysate in bone and joint disease. Semin Arthr Rheumat 30: 87-99.
5. Clark KL, Sebastianelli W, Flechsenhar KR, Aukermann DF, Meza F, et al. (2008) 24-Week study on the use of collagen hydrolysate as a dietary supplement in athletes with activity related joint pain. Curr Med Res Opin 24: 1485-1496.
6. Noguchi C, Kobayashi M, Koyama Y (2012) Amount of collagen ingested by Japanese adult women from their diet. Jpn J Nutr Diet 70: 120-128.
7. Kuwaba K, Koyama Y, Koikeda T, Tsukada Y (2014) Effects of collagen peptide ingestion on skin properties. Placebo-controlled double-blind trial. Jpn Pharmacol Ther 42: 995-1004.
8. Koyama Y, Kuwaba K, Kondo S, Tsukada Y (2014) Supplemental ingestion of collagen peptide suppresses ultraviolet-induced erythema. A randomized double-blind placebo-controlled study. Jpn Pharmacol Ther 42: 781-790.
9. Koyama Y, Kuwaba K, Kusubata M, Hayashida O, Takara T, et al. (2015) Supplemental ingestion of collagen peptide improves T-cell-related human immune status. Jpn Pharmacol Ther 43: 51-56.
10. Newey H, Smyth DH (1959) The intestinal absorption of some dipeptides. J Physiol 145: 48-56.
11. Prockop DJ, Keiser HR, Sjoerdsma A (1962) Gastrointestinal absorption and renal excretion of hydroxyproline peptides. Lancet 2: 527-528.
12. Iwai K, Hasegawa T, Taguchi Y, Morimatsu F, Sato K, et al. (2005) Identification of food-derived collagen peptides in human blood after oral ingestion of gelatin hydrolysates. J Agric Food Chem 53: 6531-6536.
13. Shigemura Y, Akaba S, Kawashima E, Park EY, Nakamura Y, et al. (2011) Identification of a novel food-derived collagen peptide, hydroxyprolyl-glycine, in human peripheral blood by pre-column derivatisation with phenyl isothiocyanate. Food Chem 129: 1019-1024.
14. Taga Y, Kusubata M, Ogawa-Goto K, Hattori S (2014) Highly accurate quantification of hydroxyproline-containing peptides in blood using a protease digest of stable isotopelebeled collagen. J Agric Food Chem 62: 12096-12102.
15. Weiss PH, Klein L (1969) The quantitative relationship of urinary peptide hydroxyproline excretion to collagen degradation. J Clin Invest 48: 1-10.
16. Shigemura Y, Iwai K, Morimatsu F, Iwamoto T, Mori T, et al. (2009) Effect of prolyl-hydroxyproline (Pro-Hyp), a food-derived collagen peptide in human blood, on growth of fibroblasts from mouse skin. J Agric Food Chem 57: 444-449.
17. Ohara H, Ichikawa S, Matsumoto H, Akiyama M, Fujimoto N, et al. (2010) Collagen-derived dipeptide, proline-hydroxyproline, stimulates cell proliferation and hyaluronic acid synthesis in cultured human dermal fibroblasts. J Dermatol 37: 330-338.

18. Nakatani S, Mano H, Sampei C, Shimizu J, Wada M (2009) Chondroprotective effect of the bioactive peptide prolyl-hydroxyproline in mouse articular cartilage in vitro and in vivo. Osteoarth Cart 17: 1620-1627.
19. Ohara H, Iida H, Ito K, Takeuchi Y, Nomura Y (2010) Effects of Pro-Hyp, a collagen hydrolysate-derived peptide, on hyaluronic acid synthesis using in vitro cultured synovium cells and oral ingestion of collagen hydrolysates in a guinea pig model of osteoarthritis. Biosci Biotechnol Biochem 74: 2096-2099.
20. Minaguchi J, Tometsuka C, Koyama Y, Kusubata M, Nagayasu A, et al. (2012) Effects of collagen-derived oligopeptide prolylhydroxyproline on differentiation of mouse 3T3-L1 preadipocytes. Food Sci Technol Res 18: 593-599.
21. Nakao K, Kusubata M, Hara K, Igarashi M, Yamazaki N, et al. Effects of collagen peptide ingestion on healing skin wound in a rat model of pressure ulcer. Jpn Pharmacol Ther 41: 587-596.
22. Saito M, Kiyose C, Higuchi T, Uchida N, Suzuki H (2009) Effect of collagen hydrolysates from salmon and trout skins on the lipid profile in rats. J Agric Food Chem 57: 10477-10482.
23. Kim SK, Byun HG, Park PJ, Shahidi F (2001) Angiotensin I converting enzyme inhibitory peptides purified from bovine skin gelatin hydrolysate. J Agric Food Chem 49: 2992-2997.
24. Herregods G, Camp JV, Morel N, Ghesquiere B, Gevaert K, et al. (2011) Angiotensin I-converting enzyme inhibitory activity of gelatin hydrolusates and identification of bioactive peptides. J Agric Food Chem 59: 552-558.
25. Ohno M, Ito K, Lan VT, Kusubata M, Tometsuka C, et al. (2015) Synergistic inhibition of human dipeptidyl peptidase IV by combinations of peptides. Peptides 69: 115-117.
26. Ke Q, Costa M (2006) Hypoxia-inducible factor-1 (HIF-1). Mol Pharmacol 70: 1469-1480.
27. Ojeh N, Pastar I, Tomic-Canic M, Stojadinovic O (2015) Stem cells in skin regeneration, wound healing, and their clinical applications. Int J Mol Sci 16: 25476-25501.
28. Date Y, Hasegawa S, Yamada T, Inoue Y, Mizutani H, et al. (2013) Major amino acids in collagen hydrolysate regulates the differentiation of mouse embryonic bodies. J Biosci Bioeng 116: 386-390.
29. Ziff M, Kibrick A, Dresner E, Gribetz Hj (1956) Excretion of Hydroxyproline in Patients With Rheumatic and Non-Rheumatic Diseases. J Clin Invest 35: 579-587.
30. Hosley HF, Taft EG, Olson KB, Gates S, Beebe RT (1966) Hydroxyproline excretion in malignant neoplastic disease. Arch Intern Med 118: 565-571.
31. Bienenstock H, Kibrick AC (1969) Urinary excretion of prolylhydroxyproline in rheumatic diseases. Ann Rheum Dis 28: 28-30.
32. Kusubata M, Koyama Y, Tometsuka C, Shigemura Y, Sato K (2015) Detection of endogenous and food-derived collagen dipeptide prolylhydroxyproline (Pro-Hyp) in allergic contact dermatitis-affected mouse ear. Biosci Biotechnol Biochem 79: 1356-1361.
33. Lotze MT, Zeh HJ, Rubartelli A, Sparvero LJ, Amoscato AA, et al. (2007) The grateful dead: damage-associated molecular pattern molecules and reduction/oxidation regulate immunity. Immunol Rev 220: 60-81.