pISSN 1229-1153 / eISSN 2465-9223 *J. Food Hyg. Saf.*
Vol. 39, No. 4, pp. 299~303 (2024)
https://doi.org/10.13103/JFHS.2024.39.4.299

Journal of Food Hygiene and Safety
Available online at http://www.foodhygiene.or.kr

Comparing Molecular Weight Dependent Absorption Rates of Collagen in Oral Mucosal and Epidermis/dermis Tissue Models

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(Received June 24, 2024/Revised July 4, 2024/Accepted July 5, 2024)

ABSTRACT - Collagen, as an indicator of skin health, has been developed and used for various purposes. The development of an optimized collagen product suitable for use has become an important research field as the consumption of collagen increases. In particular, various efforts are being made to increase its absorption rate. In this study, the transdermal and oral epithelial cell permeabilities of various molecular weight collagen products sold in Korea were compared using a Franz diffusion cell system. The collagen absorption rate of oral mucosal tissue compared to skin epidermis/dermis tissue was significantly higher than that of collagen at M.W. 500 and 1,000 (approximately 10 times and 2 times higher, respectively). Additionally, collagen with a molecular weight of 500 Da increased the absorption rates by 2-3 times compared with products with a molecular weight of 1,000. Collagen with a molecular weight of 500 Da showed the highest C*max* and AUC*t* values, and all parameters in the oral mucosal cell test group were higher than those in the skin epidermis/dermis cells. Our findings suggest an increased absorption rate through oral mucosal cells rather than skin absorption, confirming that low molecular weight collagen is a major factor increasing the absorption rate.

Key words: Collagen, Molecular weight, Oral mucosa cell, Franz Cell system, Absorption

Collagen is the main component of connective tissue and is mainly found in bones and skin, but it is a component distributed throughout our body, including joints, membranes of each organ, and hair. It is the most abundant protein in mammals[1)], accounting for 25-35% of the total protein component. It exists as a fibrous solid, and it can be seen that it has a complex horizontal pattern structure when viewed with a transmission electron microscope[2)]. According to the levels of mineralization, collagen tissues may be categorized by rigid (bone) and compliant (tendon). Collagen is also abundant in organs such as corneas, blood vessels, the gut, and the dentin in teeth[3)]. In muscle tissue, collagen constitutes 1-2% of muscle tissue and accounts for 6% of the weight of strong, tendinous muscles[4)]. The fibroblast is well-known cell that consist of collagen. Gelatin, one of collagen derivatives is also used in food and industry[5)].

As an indicator of skin health, such collagen has been developed and used for various purposes. In particular, as the aging population increases due to an aging society, the demand for their health and skin care is increasing. As the consumption of collagen increases, the development of an optimized collagen product suitable for use is an important research field, and various efforts are being made to increase the absorption rate of collagen in particular. Collagen can be manufactured with various molecular weights, and the absorption rates of skin dermis and oral epithelial cells are different according to their molecular weight, so it is important to distinguish them more clearly and develop a formulation suitable for the purpose.

These collagens have skin moisturizing power[6)], wrinkle improvement[7)], skin elasticity increase[8)], and protein supplementation[9)]. Various studies are being conducted for various purposes, and product development is being actively

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carried out accordingly. However, since too many types of collagen products and collagen raw materials with different molecular weights are used, verification of actual absorption and utilization rates during intake has not been properly confirmed. Therefore, the difference in absorption according to intake methods such as oral or skin application of collagen and the identification of absorption rates for skin epidermis/dermis and oral mucosal cells according to the molecular weight of collagen molecules are essential for the manufacture of products with improved bioavailability.

Therefore, the objective of this study is to compare the epidermis/dermis and oral mucosal cell absorption rates of various molecular weight collagen products using the Franz Cell system. To evaluate the absorption rates of various molecular weight collagen products, the changes in absorption rates of various molecular weight collagen were determined in the Franz Cell system with epidermis/dermis and oral mucosal cells. Furthermore, to prove the efficacy of absorption rates of various molecular weight collagen, pharmacokinetic parameters were investigated in these cell models.

Materials and Methods

Materials

Oral mucosa (Neoderm®-OD) and skin epidermal (Neoderm®-ED) tissues derived from human were purchased from Tego Science Inc. (Seoul, Korea). Film-type of collagen products classified by molecular weight (CH.V.; M.W. 500 and M.W. 1,000) were donated by EVERIT Co., Ltd. (Daejeon, Korea). The standard collagen product, collagen peptide-SP series (the average molecular weight of collagen, 500 and 1,000 Dalton) were purchased from G.M.P. Co., Ltd. (Seoul, Korea). Tailor-made Franz diffusion cell system was donated by B&C Tech Inc. (Daejeon, Korea) *O*-phthaldialdehyde and *N*-acetyl-L-cysteine were purchased from Sigma-Aldrich Co. (St. Louis, MO, USA). Unless noted, all chemicals were purchased from Sigma-Aldrich Co. (St. Louis, MO, USA).

Experimental Protocol for Permeability Test using Franz Cell System

The collagen permeability test was conducted using the Franz Diffusion Cell system following a method[10]. The purchased oral and epidermal tissues were cut to fit the Franz cell size and divided into 1.5 cm×1.5 cm squares for use. The cut oral and epidermal tissues (1.5 cm×1.5 cm) were washed in PBS (0.1 M, phosphate buffered saline, pH 7.0) for 30 minutes, and each human-derived tissue cell was placed on the upper part of the receptor chamber. After filling the receptor phase (0.1 M phosphate buffered saline, pH 7.0), stabilizing the skin for 30 minutes and drying the surface, cut the film-type collagen samples (500 and 1,000 molecular weight, respectively) to fit the receiving compartment, fix it, and reflux from 0 to 8 hours to react. Quantitative analysis of collagen content was performed through *o*-phthal-dialdehyde (OPA) assay[11] by retaking each aqueous solution in receptor chambers. Using the OPA method[11], standard collagen products with an average molecular weight of 500 and 1,000 Dalton of were reacted at different concentrations using the OPA method, and the equation was obtained through the quantitative curve as follows. The linearity of the standard substance (STD) was examined in the range of 6 concentrations (0.1 to 1.0 mg/mL). OPA analysis was repeated three times for each concentration to confirm the absorbance value (Abs 335 nm) for each STD concentration. The slope of the calibration curve is 0.9018, the Y-intercept is -0.0020, and the correlation coefficient (R^2) is 0.9999, verifying linearity by concentration of the standard substance. The detection limits of each sample were 0.021 and 0.033 mg/ml, respectively, and the recovery rates were confirmed to be over 95.7%, and the collagen content for each molecular weight was quantified according to the equation.

Statistical analysis

All data are presented as mean±SD. Statistical analyses were carried out using the statistical package SPSS 11 (Statistical Package for Social Science, SPSS Inc., Chicago, IL, USA) program and significance of each group was verified with the analysis of One-way ANOVA followed by the Student's *t*-test for comparison of means.

Results and Discussion

Effect of Collagen Products on Permeability and pharmacokinetics parameters

The pharmacokinetics (PK) of various molecular weight (M.W.) collagen products was evaluated using the Franz Diffusion Cell system with epidermis/dermis and oral mucosal cells. The total permeated collagen-time curve (AUC*t*) and maximum permeated collagen levels (C*max*) seem to be dose-dependent in all treatments (Table 1, 2). For the 500 M.W. collagen we observed a significantly higher absorption with oral mucosal cells, compared to epidermis/dermis cells in all tested doses (Table 1). The observed AUC*t* and C*max* were almost ten times higher with the oral mucosal cells (Table 1). When we evaluated the PK with 1,000 M.W. collagen, a similar trend was observed, since both AUC*t* and C*max* were higher in oral mucosal cells (Table 2). However, the difference was not as pronounced as it was with the 500 M.W. collagen experiment. More

specifically, the observed absorption rate with oral mucosal cells was only three times higher, when compared to epidermis/dermis cells (Table 2). When we compared the AUC*t* and C*max* values between same cell-lines and different collagen M.W., we also observed dramatic differences (Table 1, 2). More specifically, in oral mucosal cells we observed that the 500 M.W. collagen resulted to higher AUC and C*max* when compared to the 1,000 M.W.

Table 1. Pharmacokinetics (PK) parameters of collagen (M.W. 500) in skin epidermis/dermis and oral mucosal tissues

500 (M.W.)	Groups	PD parameters	
		AUC_t (hr·mg/dL)	C_{max} (mg/dL)
Epidermis/ dermis tissue	0.1 g/mL	$0.018\pm0.003^{c,F}$	$0.006\pm0.003^{b,E}$
	0.5 g/mL	$0.024\pm0.003^{b,E}$	$0.008\pm0.003^{b,E}$
	1.0 g/mL	$0.031\pm0.004^{a,D}$	$0.010\pm0.004^{a,D}$
Mucosal tissue	0.1 g/mL	$0.101\pm0.012^{c,C}$	$0.032\pm0.012^{c,C}$
	0.5 g/mL	$0.260\pm0.022^{b,B}$	$0.076\pm0.022^{b,B}$
	1.0 g/mL	$0.305\pm0.035^{a,A}$	$0.116\pm0.035^{a,A}$

Total permeated collagen-time curve (AUCt) and maximum permeated collagen levels (Cmax). Different corresponding letters indicate significant differences at $P<0.05$ by Duncan's test. [a-e]Different letters indicate statistically significant differences within each tissue group, [A-F]Different letters indicate statistically significant differences between two different tissue groups one-way ANOVA followed by Duncan's test of $P<0.05$.

Table 2. Pharmacokinetics (PK) parameters of collagen (M.W. 1,000) in skin epidermis/dermis and oral mucosal tissues

1,000 (M.W.)	Groups	PD parameters	
		AUC_t (hr·mg/dL)	C_{max} (mg/dL)
Epidermis/ dermis tissue	0.1 g/mL	$0.047\pm0.003^{b,C}$	$0.011\pm0.000^{c,D}$
	0.5 g/mL	$0.048\pm0.004^{b,C}$	$0.013\pm0.001^{b,D}$
	1.0 g/mL	$0.076\pm0.004^{a,B}$	$0.018\pm0.001^{a,C}$
Mucosal tissue	0.1 g/mL	$0.052\pm0.017^{b,C}$	$0.011\pm0.003^{c,D}$
	0.5 g/mL	$0.100\pm0.018^{a,A}$	$0.024\pm0.003^{b,B}$
	1.0 g/mL	$0.137\pm0.020^{a,A}$	$0.031\pm0.004^{a,A}$

Total permeated collagen-time curve (AUCt) and maximum permeated collagen levels (Cmax). Different corresponding letters indicate significant differences at $P<0.05$ by Duncan's test. [a-c]Different letters indicate statistically significant differences within each tissue group, [A-D]Different letters indicate statistically significant differences between two different tissue groups one-way ANOVA followed by Duncan's test of $P<0.05$.

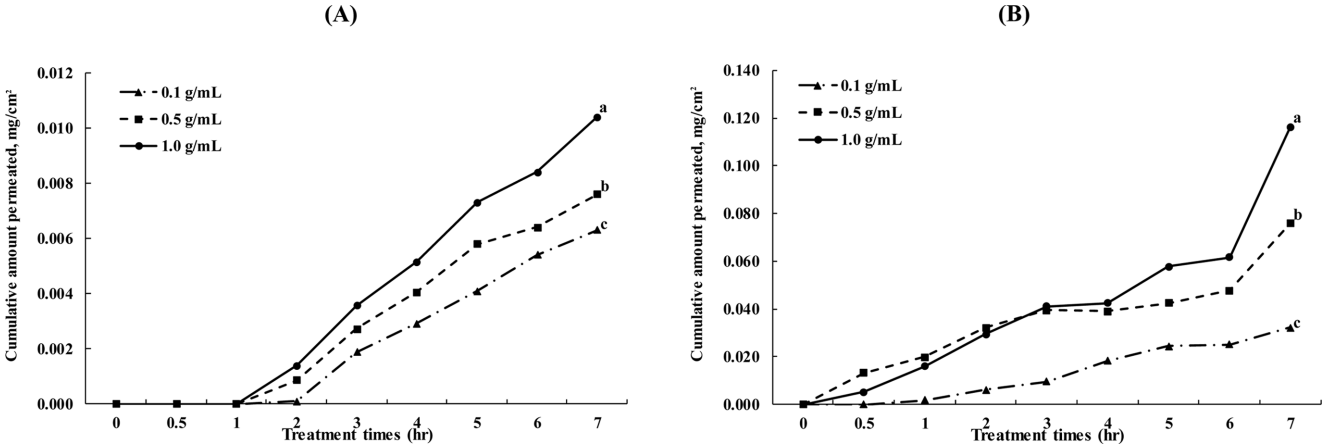

Fig. 1. Comparison of accumulative amount of permeated collagen (M.W. 500) in skin epidermis/dermis (A) and oral mucosal (B) tissues. [a-c]Different letters indicate statistically significant differences within each group one-way ANOVA followed by Duncan's test of $P<0.05$.

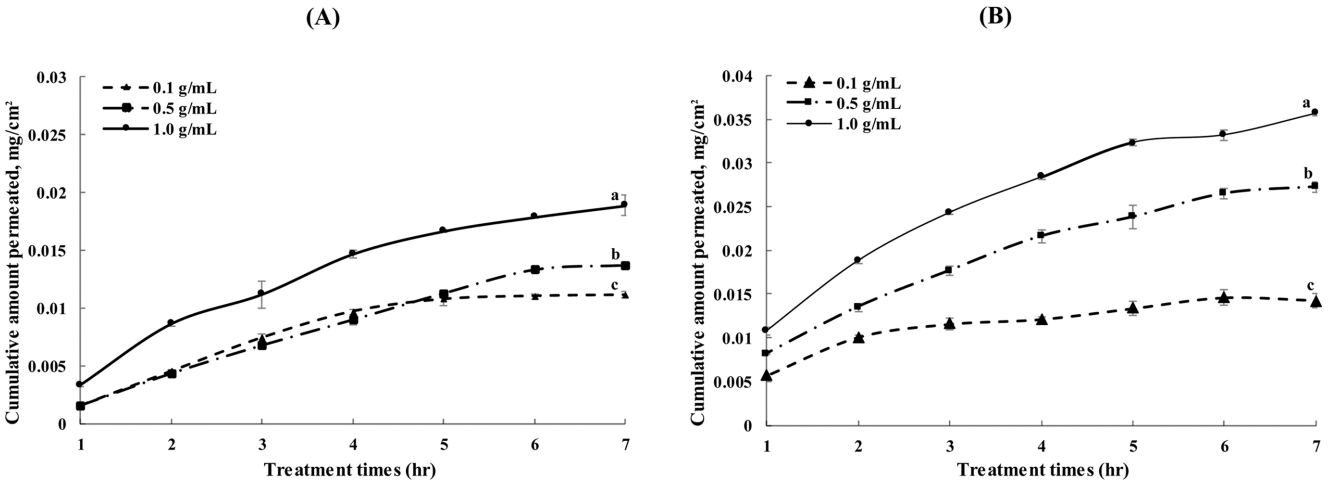

Fig. 2. Comparison of accumulative amount of permeated collagen (M.W. 1,000) in skin epidermis/dermis (A) and oral mucosal (B) tissues. [a-c]Different letters indicate statistically significant differences within each group one-way ANOVA followed by Duncan's test of $P<0.05$.

collagen (Table 1, 2). More specifically, 500 MW collagen resulted to almost 3 times higher AUC and C*max* than those observed with 1,000 MW collagen, in oral mucosal cells. A different trend was observed in the epidermis/dermis cell line. The AUC*t* and C*max* with the 1,000 M.W. collagen was almost two times higher than the values observed with the 500 M.W. collagen (Table 1, 2).

Also, it is interesting to note that the uptake mechanism seemed to be affected both by M.W. of collagen and cell-line tested (Fig. 1, 2). More specifically, we observed that the 500 M.W. collagen was not readily absorbed in epidermis/dermis cells, since after 1 hr there was no detectable collagen (Fig. 1A). On the other hand, we observed that the 1,000 M.W. collagen were readily absorbed in epidermis/dermis cells and collagen was detected after 1 hr (Fig. 2A). Both 500 M.W. and 1,000 M.W. collagen were readily available from the beginning in oral mucosal cells and collagen was detected after 1 hour (Fig. 1B, 2B).

Our findings demonstrate that the absorption rate of collagen depends on the M.W. and the cells that it is applied to. From all observations it clearly demonstrated that collagen, regardless of MW is more bioavailable through oral mucosal cells. Also, we observed that maximum absorption rate occurs with the 500 M.W. collagen through the oral mucosal cells. However, if collagen is applied through epidermis/dermis cells, the 1,000 M.W. collagen resulted in a higher absorption rate when compared to the 500 M.W.

Therefore, our results provide an interesting rationale that can be used for the design of more efficacious collagen products. Based on our findings, the best method of application of collagen is through the mouth and lower M.W. collagen seems to be much more bioavailable. However, if the desired method of application is through the skin, then the higher M.W. collagen seems to be more appropriate. As above results, developing highly absorbable collagen products, it is more effective to use a film-dissolved formulation for absorption by oral epithelial cells than to apply it to the skin. Meanwhile, in the case of developing a skin-applying product low-molecular-weight collagen peptide with a molecular weight of 1,000 Daltons or less might be used since there was low significant difference in epithelial cell permeability between collagen molecular weights of 500 and 1,000 Daltons.

More research is necessary to better understand the different mechanisms involved and to confirm our interesting findings.

Acknowledgments

This research was financially supported by the Ministry of SMEs and Startup, Korea, under the "Regional Specialized Industry Development Program (Regional Star Industry)" supervised by the Korea Institute for Advancement of Technology (KIAT) (S2906741).

국문요약

최근 국내외 화장품과 식품산업에서 다양하게 사용되어지고 있는 콜라겐 단백질 제품은 점차 그 용도와 특성에 따라 보다 고도화, 기능화 되어 가고 있다. 피부 건강의 지표인 콜라겐은 다양한 용도로 개발되어 사용되고 있으며, 콜라겐의 소비가 증가함에 따라 용도에 적합한 최적화된 콜라겐 제품의 개발이 중요한 연구 분야이다. 특히

여러 기업과 연구기관들에 의해서 콜라겐의 흡수율을 높이기 위한 다양한 노력이 이루어지고 있다. 따라서 본 연구에서는 프란즈(Franz) 세포 시스템을 이용하여 국내에서 판매되는 다양한 분자량별 콜라겐 제품의 경피 및 구강 상피세포 투과성을 비교하였다. 그 결과, 피부 표피/진피 조직과 비교하여 구강점막 조직의 콜라겐 흡수율이 분자량 500달톤과 1,000달톤의 경우 모두 통계적으로 유의하게(각각 약 10배, 2배) 높은 것으로 확인되었다. 또한, 분자량별 구강점막 조직 흡수율을 비교한 결과, 분자량 500달톤의 콜라겐이 분자량 1,000달톤 제품에 비해 흡수율이 2-3배 증가함을 확인하였다. 분자량 500달톤의 경우 Cmax와 AUCt 값이 가장 높게 나타났으며, 피부 표피/진피 세포에 비해 구강점막세포 시험군의 모든 지표가 높은 것으로 나타났다. 본 연구 결과는 피부 흡수보다는 구강 점막세포를 통한 콜라겐의 흡수방법이 콜라겐 체내 흡수증가에 유효한 수단임을 시사하며, 분자량 1,000달톤 이하에서도 보다 더 작은 500달톤의 저분자 콜라겐의 흡수율이 증가되는 것으로 보아 콜라겐의 분자량이 흡수율 증가의 주요한 요소임을 확인할 수 있었다.

Conflict of interests

The authors declare no potential conflict of interest.

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