Effects of Poly-L-arginine on the Permeation of Hydrophilic Compounds through Surface Ocular Tissues

Eiichi NEMOTO,a Hirokazu TAKAHASHI,a Daisuke KOBAYASHI,a,b HideoUEDA,a and Yasunori MORIMOTO*a,b

*a Department of Pharmaceutics, Faculty of Pharmaceutical Sciences, Josai University; 1–1 Keyakidai, Sakado, Saitama 350–0295, Japan; and b Research Institute of TTS Technology; 1–1 Keyakidai, Sakado, Saitama 350–0295, Japan.

Received September 14, 2005; accepted November 5, 2005; published online November 8, 2005

We investigated whether poly-L-arginine (PLA) could improve permeation of the hydrophilic compounds, FITC-labeled dextran (MW 3800, FD-4) and pyridoxamine, through ocular surface tissues. Samples of cornea, conjunctiva, or conjunctiva/sclera composite from Japanese white rabbits were mounted in Ussing chambers to measure FD-4 and pyridoxamine permeation and transepithelial electric resistance (TEER). The integrity and viability of the conjunctiva were assessed by chronological TEER monitoring and MTT assay, respectively. The permeability coefficient (Papp) of FD-4 in the cornea, conjunctiva, and conjunctiva/sclera composite was increased by the addition of PLA (MW 38 kDa, PLA (50)) at 0.1 mg/ml by 6.81-, 9.78-, and 7.91-fold, respectively. The Papp of pyridoxamine was also increased in the presence of PLA (50) by 7.98-, 4.67-, 8.31-fold, respectively. A corresponding reduction in TEER was observed in all tissues. However, the reduced TEER in the case of the conjunctiva had recovered to ca. 70% 120 min after replacing the mucosal fluid with fresh bicarbonated Ringer’s solution. MTT assay results indicated that treatment of the conjunctiva with 0.1 mg/ml PLA (50) did not change the production of formazan compared to that without PLA (50), indicating that the conjunctival viability is not significantly affected by PLA (50). Our findings suggest that PLA may be useful in promoting the ocular delivery of hydrophilic drugs without producing significant epithelial damage.

Key words poly-L-arginine; cornea; conjunctiva; permeation enhancer; MTT assay

Drugs that are topically applied to the cul-de-sac enter the intraocular area via both corneal and noncorneal pathways which are composed of cornea and conjunctiva–sclera, respectively.1) Although the corneal pathway is thought to be the primary route of intraocular entry for most drugs, penetration via the noncorneal pathway can also be important for the penetration of drugs which are poorly absorbed across the cornea.2,3) In addition, the noncorneal pathway could be more important than the corneal pathway for the delivery of drugs to the posterior segment of the eye.2,3)

The first barrier to intraocular entry via the corneal and noncorneal pathways is the cornea and conjunctiva, respectively. These epithelial tissues are known to have tight junctions that act as a barrier in the paracellular spaces.4,5) Because of this barrier structure in the epithelia, insufficient drug may be absorbed after instillation. Thus, subconjunctival and intravitreous injections are generally used in ocular pharmacotherapy.1,6) These invasive methods may not be acceptable to many patients and could potentially increase the risk of infection.6) In order to obtain a simpler and more acceptable form of application, the development of effective instilled formulations would be a major improvement. A number of ocular penetration enhancers, including calcium chelator (EDTA) and bile salts, have already been investigated, and it was found that those enhancers increased the apparent permeability coefficient (Papp) of FITC-dextran (MW: 4000, FD-4) by 2.9- to 15.5-fold in excised cornea and conjunctiva.7) However, these enhancers might not be acceptable from a safety point of view, because the following were observed: severe epithelial damage, such as a change in cellular morphology produced by EDTA and deoxycholate in the cornea,9) leakage of cytosolic proteins produced by EDTA in the rectum,9) and permeabilization caused by dissolution of the plasma membrane produced by bile salts.10)

Recently, a novel type of penetration enhancer has been developed for mucosal drug delivery. Illum et al.11) showed that the polycationic compound, chitosan, greatly enhanced the absorption of insulin across the nasal mucosa in rats and sheep. Basic amino acid polymers such as poly-L-arginine (PLA) and poly-L-lysine (PLL) are also shown to effectively increase permeability of various compounds in nasal, tracheal, and kidney epithelia.12–14) Although it is reported that PLL induces potential epithelial damages in tracheal epithelium,13) PLA appears to enhance the absorption of hydrophilic molecules in mucosal tissues without producing any obvious epithelial changes, such as morphological alteration, leakage of cytosolic proteins, and release of phospholipids from the plasma membrane.15,16) Thus, PLA is considered to be an ideal permeation enhancer for epithelial drug delivery including ocular delivery.

The purpose of the present study was to examine the enhancing effect of PLA on the permeability of hydrophilic compounds through the ocular epithelia. We investigated the permeability of FD-4 and pyridoxamine through the excised rabbit cornea, conjunctiva, and conjunctiva/sclera composite in the presence of different concentrations and molecular weights of PLA (14.0 kDa; PLA (10), 35.5 kDa; PLA (50), 141.4 kDa; PLA (100)). The effect of PLA on the integrity of the conjunctiva was assessed by monitoring the transepithelial electric resistance (TEER) over the experimental period. Furthermore, the effect of PLA on the viability of the conjunctiva and cornea was evaluated by using MTT assay after application of PLA.

MATERIALS AND METHODS

Animals Male Japanese white rabbits, weighing 2.5 to 3.0 kg, were purchased from Tokyo Laboratory Animals...
(Tokyo, Japan). The investigations using rabbits described in this report conformed to the Guiding Principles of Laboratory Animal Care (National Institutes of Health publication #85-23).

**Chemicals** Fluorescein isothiocyanate-dextran (MW ca. 38.0 kDa, FD-4), poly-L-arginine hydrochloride (MW ca. 14.0, 35.5 and 141.4 kDa, PLA (10), PLA (50) and PLA (100), respectively) and 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) were obtained from Sigma Chemical Co., Ltd. (St. Louis, MO, U.S.A.). Pyridoxamine was purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). Sodium taurodihydrofusidate (STDHF) was supplied by Leo Pharmaceuticals (Ballerup, Denmark).

All other reagents were of reagent grade.

**Buffer Solutions** Unless otherwise indicated, all experiments were conducted in bicarbonate Ringer’s solution (BRS) maintained at 37°C and pH 7.4 under 95% air/5% CO2. BRS was composed of 111.5 mM NaCl, 4.8 mM KCl, 29.2 mM NaHCO3, 0.75 mM NaH2PO4, 1.04 mM CaCl2, 0.74 mM MgCl2, and 5 mM D-glucose. The osmolarity of the solution was about 300 mOsm/kg H2O.

**Tissue Preparation** Animals were sacrificed by slowly increasing the CO2 concentration in a CO2 gas animal euthanasia cabinet (KN-750-1, Natsume Co., Ltd., Tokyo, Japan). The entire eyeball was then immediately removed from the orbit, taking care not to damage the cornea, conjunctiva and sclera. The excised tissues were trimmed and mounted carefully onto a tissue adapter, which had a circular aperture of 0.44 cm2. The adapter-tissue assembly was then placed in Ussing chambers maintained at 37°C using a circulating water bath. The bathing solution (5 ml on each side) of the tissue was bubbled with 95% O2/5% CO2 to maintain the pH at 7.4 and to provide adequate agitation of the solution.

**Measurement of Bioelectric Parameters** All experiments were performed under open-circuit conditions with intermittent short-circuit conditions using a short-circuit current amplifier (CEZ-9100, Nippon koden, Tokyo, Japan). The potential difference (PD) was measured with two matched calomel electrodes. Two salt-agar bridges (containing 3% agar in 4% KCl), the tips of which were located near the center of the tissue surfaces, were used to connect electrically the reservoir fluids to the electrode wells. The electrical output of the calomel electrodes was amplified by a voltage clamp unit. The direct current across the tissue was transmitted by way of a pair of matched Ag/AgCl electrodes with the tips positioned away from the tissue surfaces at the far ends of the reservoirs. The short-circuit current (Isc) flowing in the bath-tissue-bath current was monitored at 20 min intervals. Transepithelial electrical resistance (TEER) was calculated as follows: TEER = (PD/Isc) × A according to Ohm’s law, where A is the effective permeation area (0.44 cm2).

**Measurement of FD-4 and Pyridoxamine Fluxes** FD-4 and pyridoxamine fluxes were measured by assaying the fluorescence in 0.5 ml receiver fluid collected at 20 min intervals following the addition of FD-4 (2.5 mg/ml) and pyridoxamine (2.5 mg/ml) to the mucosal side. Immediately after each sampling, the volume removed was replaced with an equal volume of an appropriate fresh buffer. Then, 120 min after the addition of FD-4 and pyridoxamine, the different tissues were exposed to PLA for 120 min. The fluorescence of the samples was measured by an HPLC system (system controller: Shimadzu SIL-10A VP, auto injector: Shimadzu SIL-10AT VP, degasser: Shimadzu DGU-12A, column oven: Shimadzu CTO-10A) connected to a fluorescence spectrophotometer (Shimadzu RF-10AXL). The quantitative measurement of FD-4 was performed as follows: column: TOSOH TSK-GEK octadecylo-NPR, mobile phase A: 10 mM phosphate buffer (pH 8.0), mobile phase B: acetonitrile, flow rate: 1.0 ml/min, elution conditions: isocratic (A:B=90:10), excitation wavelength: 495 nm, emission wavelength: 515 nm. The quantitative measurement of pyridoxamine was performed by the method of Bisp et al., column: KANTO KAGAKU Mightyss RP-18, mobile phase A: 3.5 mM 1-octanesulfonic acid and 1.2 mM triethylamine in 0.033 M phosphoric acid, mobile phase B: acetonitrile, flow rate: 1.2 ml/min, elution conditions: isocratic (A:B=50:50), post-column reagent: 770 mM K2HPO4 buffer containing 5.26 mM sodium bisulfite, flow rate of the post-column reagent: 0.3 ml/min, excitation wavelength: 328 nm, emission wavelength: 393 nm.

**MTT Assay** After the conjunctiva and cornea had been placed in the Ussing chamber, as described above, either PLA or STDHF was added to the mucosal side when the bioelectric parameters had stabilized. Then, 120 min after the addition of PLA or STDHF, the mucosal side was replaced by superfusing with 50 ml fresh BRS. In case of conjunctiva, MTT was then immediately added to the fluid on the serosal side to give a final concentration of 0.3 mg/ml, enabling the stratified conjunctival cells with superficial location of tight junction barrier to make contact with MTT reagent. On the other hand, MTT was added to both mucosal and serosal fluids in case of the cornea which consist of epithelial, stromal, and endothelial layers. Three hours after the addition of MTT, the fluid on the serosal side (conjunctiva) or mucosal and serosal sides (cornea) was replaced with fresh BRS. After the conjunctiva and cornea had been punched out, the purple formazan product was solubilized in 0.04 N HCl-isopropanol solution. The absorbance at 570 nm was then measured using a UV–Vis spectrophotometer (UV-1600, SHIMADZU, Kyoto, Japan).

**Data Analysis** Unidirectional fluxes (J) for hydrophilic compounds were estimated from the steady-state slope of the cumulative amount appearing in the receiver fluid over time. The apparent permeability coefficient (Papp) was calculated by normalizing the flux against the effective permeation area (0.44 cm2) and initial solute concentration.

All data are presented as means±S.E. Statistical significance among group means was determined by Student’s t-test. A p<0.05 was considered as significant.

**RESULTS**

**Effects of the Concentration and Molecular Weight of PLA on the Permeation of FD-4 and TEER in Conjunctiva** The permeation profiles of FD-4 through the conjunctival epithelium in the presence of different concentrations and molecular weights of PLA are shown in Fig. 1. In this study, PLA was applied 120 min after the start of the permeation experiment and, hence, the permeability over 0—120 min in each experiment serves as its own control. Application of PLA increased the permeability of FD-4 compared...
Fig. 1. Cumulative Amount of FD-4 Passing through the Rabbit Conjunctiva in the Presence of Different Concentrations and Molecular Weights of PLA

- PLA (10), b) PLA (50), c) PLA (100).

Fig. 2. Time-Courses of the Conjunctival TEER during the Permeation Study in the Presence of Different Concentrations and Molecular Weights of PLA

- PLA (10), b) PLA (50), c) PLA (100). Arrow, apical application of PLA. Each data point represents the mean±S.E. (n=4).

Fig. 3. Relationship between FD-4 Papp and 1/TEER

FD-4 Papp and 1/TEER in the rabbit conjunctiva at 0 min (○), 80 min (■), 100 min (▲) and 120 min (●) after addition of 0.1 mg/ml PLA (50).

with that before application. The enhancing ratios calculated from the Papp before and after application of PLA at 0.01, 0.1, and 1 mg/ml were 1.1-, 3.2-, and 13-fold for PLA (10), 1.3-, 9.7-, and 21-fold for PLA (50), 2.4-, 9.8-, and 24-fold for PLA (100), respectively. In contrast, the TEER of the conjunctiva at 120 min after application of PLA (10) at 0.01, 0.1, and 1 mg/ml were reduced to 98%, 38%, and 5% of the baseline, 102%, 12%, and 3% of the baseline for PLA (50), 100%, 19%, and 1% of the baseline for PLA (100), respectively (Fig. 2). Thus, enhancing effect seems to be dependent of concentration and molecular weight of PLA. As shown in Fig. 3, a good correlation ($r^2=0.884$) was observed between Papp and 1/TEER in the study at 0.1 mg/ml PLA (50), suggesting that PLA enhances the paracellular permeability in the conjunctiva.

Figures 4a and b show cumulative amount of FD-4 and the corresponding TEER across the rabbit cornea and conjunctiva at 120 min after application of PLA (10) at 0.01, 0.1, and 1 mg/ml were reduced to 98%, 38%, and 5% of the baseline, 102%, 12%, and 3% of the baseline for PLA (50), respectively. In contrast, the TEER of the conjunctiva was increased following the addition of PLA (50) by 9.78-, 6.81-, and 7.91-fold, respectively (Table 1). The Papp values of FD-4 at the baseline (7.39 ± 0.35 cm/s) 12.3 ± 0.35 cm/s) were increased in the presence of PLA (50) by 9.78-, 6.81-, and 7.91-fold, respectively (Table 1).

Effects of PLA (50) on the Permeation of Hydrophilic Compounds through Various Ocular Surface Tissues

Tables 1 and 2 show the Papp values of FD-4 and pyridoxamine in excised cornea, conjunctiva, and conjunctiva–sclera composite in the presence or absence of 0.1 mg/ml PLA (50), respectively. The conjunctival, corneal, and conjunctiva–sclera composite in the presence or absence of 0.1 mg/ml PLA (50) and control (0.05 mg/ml PLA). Arrow, apical application of PLA. Each data point represents the mean±S.E. (n=4).

Table 1. Papp Values of FD-4 across the Cornea, Conjunctiva, and Conjunctiva–Sclera Composite in the Presence and Absence (Control) of 0.1 mg/ml PLA (50)

<table>
<thead>
<tr>
<th></th>
<th>Cornea</th>
<th>Conjunctiva</th>
<th>Conjunctiva/sclera</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.25±0.05</td>
<td>7.39±1.23</td>
<td>2.48±0.56</td>
</tr>
<tr>
<td>PLA</td>
<td>1.69±0.35</td>
<td>72.2±14.4</td>
<td>19.6±3.12</td>
</tr>
<tr>
<td></td>
<td>[6.81]</td>
<td>[9.78]</td>
<td>[7.91]</td>
</tr>
</tbody>
</table>

Table 2. Papp Values of Pyridoxamine across the Cornea, Conjunctiva, and Conjunctiva–Sclera Composite in the Presence and Absence (Control) of 0.1 mg/ml PLA (50)

<table>
<thead>
<tr>
<th></th>
<th>Cornea</th>
<th>Conjunctiva</th>
<th>Conjunctiva/sclera</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>1.54±0.77</td>
<td>64.3±2.56</td>
<td>29.5±3.24</td>
</tr>
<tr>
<td>PLA</td>
<td>12.3±1.22</td>
<td>300±51.8</td>
<td>245±24.9</td>
</tr>
<tr>
<td></td>
<td>[7.98]</td>
<td>[4.67]</td>
<td>[8.31]</td>
</tr>
</tbody>
</table>

The effect of replacement of the mucosally applied PLA (50) was investigated using the conjunctiva. Figure 5 shows the time-courses of the conjunctival TEER after replacement of PLA (50).
tion (mazan production was significantly reduced by treatment in both conjunctiva and cornea (Figs. 6a, b). However, for-

PLA (50) (5 mg/ml) did not affect the formazan production treatment) (Fig. 6a). Application of a higher concentration of significantly different from that of the control (without any 120 min after application of 0.1 mg/ml PLA (50) was not sig-

in Fig. 6. In case of PLA, the production of formazan at tion of PLA and STDHF (a positive control) was compared the baseline at 120 min after replacement (240 min after initi-

action. The conjunctival TEER decreased to 17% of the base-

line value by addition of PLA (50) was recovered to 68% of the baseline at 120 min after replacement (240 min after initia-

tion of the experiment), suggesting the potentially reversible action of PLA on the conjunctival tight junction altera-

tions. The viability of the conjunctiva and cornea after applica-

tion of PLA was examined by MTT assay. Production of formazan after applica-

tion of PLA and STDHF (a positive control) was compared in Fig. 6. In case of PLA, the production of formazan at 120 min after application of 0.1 mg/ml PLA (50) was not signif-

dicantly different from that of the control (without any treatment) (Fig. 6a). Application of a higher concentration of PLA (50) (5 mg/ml) did not affect the formazan production in both conjunctiva and cornea (Figs. 6a, b). However, for-

mazan production was significantly reduced by treatment with 0.1 mg/ml STDHF, and a further reduction in production (ca. 0 μg/ml) occurred at a higher concentration of STDHF (5 mg/ml). On the other hand, significant reduction in formazan production was also observed in cornea, but much less than that in the conjunctiva (Fig. 6b).

**DISCUSSION**

In the present study, we investigated the effect of PLA on the permeability of hydrophilic compounds in excised rabbit cornea, conjunctiva, and conjunctiva/sclera composite using the *in vitro* Ussing chamber technique. To assess the safety of PLA, the effects of PLA on integrity and viability were also examined by TEER monitoring and MTT assay.

The baseline conjunctival FD-4 Papp of 7.39±1.23 (×10⁻⁸ cm/s) observed in this study was consistent with the previous observation by Horibe et al.¹⁸ The corneal Papp of FD-4 at baseline (0.25 ×10⁻⁸ cm/s) was one order lower than that in the conjunctiva. Such a difference in baseline Papp is reasonable because similar differences in Papp between cornea and conjunctiva were observed in earlier studies using timolol and polyethylene glycol.¹⁹,²⁰ On the other hand, vita-

min B₆ derivatives including pyridoxamine are known to be transported *via* a carrier-mediated system in Caco-2 cells,³² although the possible expression of such a system has not been investigated in the conjunctiva. Considering the *K₅₀* (11.99±1.41 μm) and *V₅₀* (67.63±3.87 pmol·mg protein⁻¹·3 min⁻¹) in Caco-2 cells, the involvement of such a system would be negligible at the concentration used (2.5 mg/ml (10 mM)).

Enhancement of FD-4 permeation and the corresponding reduction in the conjunctival TEER were observed by applying PLA. These effects were dependent on the molecular size and concentration of PLA (Figs. 1, 2). These findings were similar to previous observations obtained in the excised rabbit nasal epithelium.²¹ PLA (50) also enhanced the permeation of both pyridoxamine and FD-4 *via* the cornea, conjunctiva, and conjunctiva/sclera composite by factors of 4.7- to 9.8-fold compared with the respective control (Tables 1, 2). These finding suggest that topically applied PLA can in-

crease the ocular absorption of instilled drugs *via* both corneal and noncorneal pathways. The observed enhancing potency of PLA was comparable with that obtained by other enhancer candidates, such as EDTA and bile salts, examined in previous studies involving the cornea and conjunctiva. However, the minimal tissue damage produced by PLA is a significant advantage compared with the other types of en-

hancers discussed below. Furthermore, the enhancing effect of PLA (50) in the conjunctiva alone was comparable with that in the conjunctiva/sclera composite, suggesting that the alteration in the conjunctival integrity induced by PLA in-

volved the composite because a stromal tissue, such as the sclera, is not a significant barrier to the permeation of pyri-

doxamine and FD-4.

The enhancing effect of PLA is likely to be associated with an increase in paracellular permeation in the conjunc-

tiva because an increased permeation of both hydrophilic FD-4 and pyridoxamine and a corresponding reduction in TEER were observed. In fact, a good correlation between FD-4 Papp and 1/TEER in the presence and absence of PLA was obtained (Fig. 3). Thus, PLA appears to increase the paracellular permeability as reported in monolayers such as Caco-2 and MDCK cells and excised rabbit nasal epithelium.¹⁴,²¹ In addition, PLA induced same degree of factor enhancement for both pyridoxamine and FD-4 regardless of the difference in their molecular size (Tables 1, 2). Since our simulation uniformly increased the Papp of hydrophilic com-

pounds with a broadly distributed molecular weight when the area fraction of the permeation pathway was increased (un-

published data), PLA may increase area fraction of the permeation pathway for pyridoxamine and FD-4. Because the paracellular permeability is restricted by the function of the tight junctions which are formed from multiple proteins in-

cluding Claudins, occludin, zonula occludens, *etc.*²⁴,²⁵ PLA may induce dissociation of tight junction assemblies to in-
crease the paracellular permeation of hydrophilic molecules. Ohtake et al. reported that application of PLA to nasal mucosa changed the location of tight junction-related proteins, such as occludin and ZO-1, from the plasma membrane to the cytoplasm.\(^\text{26}\) Another polycation, protamine, also showed that the expression and distribution of claudin-1 and occludin were altered in MDCK monolayers.\(^\text{27}\) Thus, tight junction-associated proteins in ocular surface tissues, such as the cornea and conjunctiva, are likely to be disassembled in the presence of polycations like PLA. However, it is not clear whether polycations produce a similar change in tight junction-associated proteins in stratified epithelia, such as the cornea and conjunctiva. Such protein changes need to be confirmed in further studies.

Application of PLA markedly reduced the conjunctival integrity as indicated by the reduction in TEER (Fig. 2). However, when the applied PLA was replaced with fresh BRS 120 min after PLA application, the observed reduction in TEER by ca. 15% had returned to 70% of the baseline value 120 min after the replacement (Fig. 5), suggesting that the observed change in the conjunctival integrity was transient. Ohtake et al.\(^\text{28}\) showed that PLA of different molecular weights and concentrations produced a transient and reversible enhancement of the nasal absorption of FD-4 \textit{in vivo}. Their findings indicate that the potential enzymatic degradation of PLA and mucociliary clearance are responsible for the transient action and the maintenance of epithelial function after treatment with PLA in terms of the reversible action in \textit{in vivo} situations. Although our \textit{in vitro} study in this paper differs to some extent from the previous \textit{in vivo} nasal studies, the permeation enhancement and the corresponding change in the conjunctival integrity seem to be transiently induced only in the presence of PLA. Such a transient effect may also apply to the \textit{in vivo} ocul ar absorption of drugs, considering the estimated restoration of tear fluid of 2—3 min and the rapid clearance of instilled eye drops (80% or more) during the first 15—30 s.\(^\text{29}\)

However, the result of MTT assay showing that the viability of the conjunctiva was not affected by PLA (50 mg/ml) treatment at 5 mg/ml for 120 min clearly demonstrates the potential advantage of PLA from a safety point of view (Fig. 6). In addition to the previous findings that PLA did not induce significant leakage of cytosolic components \textit{in vitro}\(^\text{30}\) and obvious morphological alterations \textit{in vivo} in the nasal epithelium,\(^\text{15}\) our findings from the MTT assay confirm the safety of PLA at least as far as acute cellular toxicity is concerned. In contrast, STDHF, a typical bile salt which was used as a positive control, significantly reduced the production of formazan (Fig. 6a). Although STDHF at 5 mg/ml also induced a significant reduction in formazan production in the cornea, the difference in production between cornea and conjunctiva (Figs. 6a, b) might be due to potential difference in sensitivity to the cytotoxicity as reported in cultured corneal and conjunctival epithelial cells.\(^\text{31}\) In our previous observation, STDHF at 5 mg/ml had the same potential as PLA at 5 mg/ml for nasal permeation of FD-4 (4.9-fold for STDHF, 4.8-fold for PLA),\(^\text{31}\) thus STDHF acts as a positive control in this study. Because STDHF appears to produce the nasal absorption enhancement of FD-4 in rats, but induces concomitant cellular and epithelial damage in the previous studies,\(^\text{16}\) the enhancing action of PLA is essentially different from that of bile salts.

Among the amino acid polymers, PLA may be more advantageous than PLL as an absorption enhancer in terms of safety and mechanical aspects. Ohtake et al.\(^\text{32}\) showed that intracarotid infusion of 5 mg PLA (MW 10 kDa) increased the brain distribution of albumin due to the enhanced permeability of the blood brain barrier.\(^\text{29}\) The effect was 15 times greater than that induced by 5 mg PLL (MW 10 kDa), suggesting the potential greater ability of PLA for enhancement of solute membrane permeability compared to PLL. On the other hand, PLL appears to induce both reduction of cell viability and cellular disruption as indicated by MTT assay in 293T cells\(^\text{30}\) and leakage of cytosolic components such as LDH in bronchial epithelium,\(^\text{15}\) respectively. In our preliminary MTT assay study, the production of formazan (1.0 ±0.2 μg/ml) 120 min after application of 5 mg/ml PLL (MW 34 kDa) in conjunctiva was significantly lower than that produced by 5 mg/ml PLA (50) (5.2 ±1.8 μg/ml) (p<0.05). Thus, PLA is likely to be more eligible as an absorption enhancer compared to PLL. However, further studies including frequent and long-term administration will be required to establish the safety of PLA as the enhancer.

In conclusion, PLA appears to enhance the permeation of FD-4 and pyridoxamine through ocular surface tissues, such as the cornea, conjunctiva, and conjunctiva/sclera composite without producing any significant epithelial damage in terms of TEER recovery and cellular viability. PLA may be a useful permeation enhancer for ocular drug delivery \textit{via} both the corneal and noncorneal pathways. Investigations to identify ideal absorption enhancers may expand the potential use of instillation as an alternative to invasive subconjunctival injection and implantation. Further studies are in progress to confirm the safety and mechanistic aspects of PLA.

**REFERENCES**