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Research Paper

Comparative Study of the Skin Penetration of Protein Transduction Domains and a Conjugated Peptide

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Purpose. We examined the ability of a protein transduction domain (PTD), YARA, to penetrate in the skin and carry a conjugated peptide, P20. The results with YARA were compared to those of a well-known PTD (TAT) and a control, nontransducing peptide (YKAc). The combined action of PTDs and lipid penetration enhancers was also tested.
Methods. YARA, TAT, YKAc, P20, YARA-P20, and TAT-P20 were synthesized by Fmoc chemistry. Porcine ear skin mounted in a Franz diffusion cell was used to assess the topical and transdermal delivery of fluorescently tagged peptides in the presence or absence of lipid penetration enhancers (monoolein or oleic acid). The peptide concentrations in the skin (topical delivery) and receptor phase (transdermal delivery) were assessed by spectrofluorimetry. Fluorescence microscopy was used to visualize the peptides in different skin layers.
Results. YARA and TAT, but not YKAc, penetrated abundantly in the skin and permeated modestly across this tissue. Monoolein and oleic acid did not enhance the topical and transdermal delivery of TAT or YARA but increased the topical delivery of YKAc. Importantly, YARA and TAT carried a conjugated peptide, P20, into the skin, but the transdermal delivery was very small. Fluorescence microscopy confirmed that free and conjugated PTDs reached viable layers of the skin.
Conclusions. YARA and TAT penetrate in the porcine ear skin *in vitro* and carry a conjugated model peptide, P20, with them. Thus, the use of PTDs can be a useful strategy to increase topical delivery of peptides for treatment of cutaneous diseases.

KEY WORDS: P20; peptides; skin; topical delivery; TAT; YARA.

INTRODUCTION

Topical and transdermal delivery of drugs is attractive in that it provides patient commodity and avoidance of first-pass hepatic metabolism (1). However, it is well-known that the delivery of macromolecules such as peptides to and across the skin is a difficult task due to the barrier function of the skin, provided by the highly organized structure of the stratum corneum (2). Several compounds and techniques have been studied to increase drug penetration in the skin, including penetration enhancers such as oleic acid (1,3,4), drug delivery systems such as transferosomes (5,6), and physical techniques such as electroporation and iontophoresis (7,8). Although these compounds and techniques have been shown to individually increase topical delivery of macromolecules, their combination has often been demonstrated to result in additive or even synergistic effects (9). For example, combination of penetration enhancers has been shown to increase skin permeability to macromolecules by up to 100-fold (4). An additive effect on transdermal flux of LHRH (29 times) was observed by pretreating the skin with chemical enhancers followed by iontophoresis (10). In addition, the iontophoresis of phospholipid liposomes was demonstrated to increase the percutaneous delivery of cyclosporin A (11). Despite this progress, topical and transdermal delivery of peptides and proteins in therapeutics remains difficult. This is due to problems associated with skin toxicity of chemical enhancers at high concentrations, inconvenience of using electrical apparatuses at home, and high production costs of sophisticated drug delivery systems (3).

Recently, a novel approach for delivery of several substances across biological membranes and tissue barriers has emerged. Protein transduction domains (PTDs), also known as cell-penetrating peptides, are a class of small peptides capable of penetrating the plasma membrane of mammalian cells (12). Among the best-known PTDs are the HIV transcription factor TAT, the Antp peptide derived from the *Drosophila melanogaster* homeodomain protein, the herpes simplex virus protein VP22, and arginine oligomers (13–15). PTDs are characterized by a high content of positively charged arginine and lysine amino acid residues, suggesting that the positive charge and the guanidinium group of argi-

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nine residues are essential to the transport (16). These peptides have been reported to transport conjugated peptides, oligonucleotides, and even small particles such as liposomes across mammalian cells (15,17–19).

The mechanism involved in the membrane translocation of PTDs is controversial and may vary among different compounds (20). Several fluorescence-based studies have suggested that PTDs cross the plasma membrane by an energy-independent process, going directly to the cytoplasm and nucleus of the cells (21,22). However, recent reports have attributed these observations to artifacts resulting from cell fixation (14,23). Studies using living cells have demonstrated that membrane translocation occurs by endocytosis mediated by interaction of PTDs with negatively charged glycosaminoglycans on the surface of the cells (14,20). Yet, other studies have demonstrated that both nonendocytic and endocytic pathways may be involved in cellular internalization of PTDs and that the contribution of each mechanism depends on the PTD studied (16,24).

The use of PTDs to achieve skin penetration of conjugated molecules is an interesting and noninvasive strategy for topical and/or transdermal delivery. The ability of PTDs to penetrate in the skin of living animals was first demonstrated by Rothbard *et al.* (25) using a heptarginine oligomer. This PTD increased the skin penetration of conjugated cyclosporin, resulting in functional inhibition of cutaneous inflammation. Other PTDs have also been demonstrated to penetrate in the skin of living animals and to carry conjugated compounds (26,27).

Recently, molecular modification of TAT has led to the discovery of a new, optimized PTD, YARAAARQARA (YARA) (28). This PTD has been shown to penetrate intact strips of porcine coronary artery and human saphenous vein smooth muscle, carrying a phosphopeptide analogue of the heat shock protein (HSP) 20, namely P20 (18,29,30). The first aim of the current study was to evaluate the ability of YARA to penetrate in the skin *in vitro*. The penetration of YARA was compared to that of the well-known PTD TAT and of the nontransducing peptide YKALRISRKLAK (YKAc); all peptides have similar molecular weight. Our second aim was to examine the influence of chemical penetration enhancers (monoolein and oleic acid) on the topical and transdermal delivery of YARA, TAT, and YKAc. Our third aim was to verify the ability of YARA and TAT to increase the skin penetration and percutaneous delivery of a conjugated model peptide, P20. This peptide is hydrophilic and has a high molecular weight (2005 Da). Many peptides with similar characteristics have therapeutic potential for treatment of skin diseases (6,31), and their skin penetration has been shown to be extremely poor (32).

MATERIALS AND METHODS

Materials

Reagents for peptide synthesis, including amino acids, were purchased from Advanced ChemTech (Louisville, KY, USA), Anaspec (San Jose, CA, USA), Applied Biosystems (Foster City, CA, USA), and Novobiochem (San Diego, CA, USA). Fluorescein-5-isothiocyanate (FITC "Isomer 1") was purchased from Molecular Probes (Eugene, OR, USA). Monoolein was obtained from Quest (Naarden, The Netherlands) and oleic acid from Sigma (St. Louis, MO, USA). All solvents and chemicals were of analytical grade. Freshly excised porcine ears were obtained from a local abattoir (Southwest Meat Processing, Queen Creek, AZ, USA).

Peptide Synthesis

Fluorescein isothiocyanate (FITC)-labeled peptides, including YARA (YARAAARQARA, MW: 1668), TAT (YGRKKRRQRRR, MW: 2020), YKAc (YKALRISRKLAK, MW: 1907), P20 (WLRRASAPLPGLK, MW: 2005), YARA-P20 (YARAAARQARAWLRRASAPLPGLK, MW: 3111), and TAT-P20 (YGRKKRRQRRRWLRRASAPLPGLK, MW: 3466) were synthesized using an Automated Peptide Synthesizer (Apex 396, Advanced ChemTech, Louisville, KY, USA) and solid phase technique. FITC was linked to a β-alanine residue added to the N-terminus of the peptide. The peptides were purified by fast protein liquid chromatography (FPLC; Akta Explorer, Amersham Pharmacia Biotech, Piscataway, NJ, USA) using a reversed-phase column and identified by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF-MS; Applied Biosystems, Foster City, CA, USA) or electrospray ionization mass spectrometry (ESI-MS; Waters Corporation, Milford, MA, USA).

Formulations

Except in the experiments involving chemical penetration enhancers, FITC-labeled peptides were dissolved in phosphate-buffered saline (PBS; 10 mM, pH 7.2); the peptide concentration was 100 μM. In the experiment involving penetration enhancers, PBS could not be used as a solvent because of the lipophilic nature of monoolein and oleic acid. Propylene glycol was used as a solvent, as it solubilizes both lipids and peptides. Formulations of FITC-TAT, FITC-YARA, and FITC-YKAc (100 μM) in propylene glycol containing 10% (w/w) monoolein, 5% (w/w) oleic acid, or none of these penetration enhancers were prepared. The formulations were prepared by mixing monoolein or oleic acid with propylene glycol and adding the peptides to the system immediately thereafter.

In Vitro Skin Penetration

To evaluate the topical and transdermal delivery of the peptides, we applied the formulations of FITC-labeled TAT, YARA, YKAc, P20, YARA-P20, or TAT-P20 on the surface of freshly excised porcine ear skin mounted in a Franz diffusion cell.

Porcine ear skin was used as model skin for *in vitro* skin penetration studies because of its similarity with human skin, especially regarding histologic and biochemical properties and permeability to drugs (33). Freshly excised porcine ears were obtained from a local abattoir. The skin from the outer surface of the ear was carefully dissected; making sure that the subcutaneous fat was maximally removed (34). Maximum care was taken to maintain the integrity of the skin, which was assured by histology. The cleaned porcine ear skin was immediately mounted in a Franz diffusion cell (diffusion area of 1 cm^2; Laboratory Glass Apparatus, Inc, Berkeley, CA, USA), with the stratum corneum facing the donor compartment (where the formulation was applied) and the dermis

facing the receptor compartment, which was filled with PBS (100 mM, pH 7.2, 3 ml). The receptor phase was maintained at 37°C and under constant stirring. To achieve higher reproducibility, the skin samples were equilibrated to the diffusion cell conditions for 30 min before application of any formulation.

PBS solutions or propylene glycol formulations of the peptides (70 μl each) were applied to the skin surface (donor compartment). The concentration of FITC-YARA, FITC-TAT, and FITC-YKAc in the skin (an indicator of topical delivery) and receptor phase (an indicator of transdermal delivery) was determined at 4 h post-application. The concentrations of FITC-P20, FITC-YARA-P20, and FITC-TAT-P20 in the skin and receptor phase were determined at 0.5, 1, 2, 4, and 8 h post-application.

At the end of the experiment, skin surfaces were thoroughly washed with distilled water to remove excess formulation and carefully wiped with a tissue paper. To separate the stratum corneum (SC) from the remaining epidermis (E) and dermis (D), the skin was subjected to tape stripping. The skin was stripped with 15 pieces of adhesive tape (3M, St. Paul, MN, USA), and the tapes containing the SC were immersed in 3 ml of a water:methanol (1:1 v/v) solution, vortexed for 2 min, and bath sonicated for 30 min. The remaining [E+D] was cut in small pieces, vortexed for 2 min in 2 ml of a water: methanol (1:1 v/v) solution, and homogenized using a tissue grinder for 1 min and bath sonication for 30 min. The resulting mixture was centrifuged for 1 min. The peptide present in the receptor phase was concentrated (10×) as follows. Samples (2 ml) of the receptor phase were lyophilized for 24 h, and the residue was dissolved in 200 μl of a hidroalcoholic (20% of ethanol) solution.

All solutions were subjected to fluorimetry analysis using a Gemini SpectraMax platereader (Molecular Devices, Sunnyvale, CA, USA) with excitation at 495 nm and emission at 518 nm. The method was linear within the concentration range studied (0.05–2.0 μM). To evaluate the recovery of the peptides from the skin in the extraction procedure, tissues sections (1 cm^2) were spiked with 20 μl of 0.2 and 0.5 mM solutions of the peptides. The skin sections were homogenized using a tissue grinder, vortex-mixer, and bath sonicator, as described above. The recovery of the peptides was 83–90%, depending on the peptide. We accounted for such a recovery percentage in the quantification of peptides.

Histology

At 4 h postapplication of FITC-labeled YARA, TAT, YKAc, P20, YARA-P20, and TAT-P20, the diffusion area of skin samples were frozen using isopentane at –30°C, embedded in Tissue-Tek OCT compound (Pelco International, Redding, CA, USA), and sectioned using a cryostat microtome (Leica, Wetzlar, Germany). The skin sections (8 μm) were mounted on glass slides. The slides were visualized without any additional staining or treatment through a 20× objective using a Zeiss microscope (Carl Zeiss, Thornwood, NY, USA) equipped with a filter for FITC and AxioVision software.

Statistical Analysis

The results are reported as means ± SD. Data were statistically analyzed by nonparametric Kruskal-Wallis test followed by Dunn post-test (6). The level of significance was set at $p < 0.05$.

RESULTS

Topical and Transdermal Delivery of PTDs

Our first aim was to evaluate the ability of PTDs to penetrate in the skin and permeate across this tissue, so that they could be used as carrier for topical and/or transdermal delivery of peptides (results shown in Fig. 1). In this experiment, PBS was used as vehicle. We determined the penetration of FITC-YARA, FITC-TAT, and FITC-YKAc in the SC and [E+D] as well as their transdermal delivery at 4 h postapplication. The penetration of the control, nontransducing peptide FITC-YKAc in both SC and [E+D] was very small, and no peptide was found in the receptor phase (indicating no transdermal delivery). On the other hand, the penetration of FITC-YARA and FITC-TAT in the SC and [E+D] was 8–10 times higher than that of the control peptide. The transdermal delivery of FITC-YARA and FITC-TAT was small at 4 h postapplication, and there was no significant difference in the amount of FITC-YARA detected in the receptor phase compared to FITC-TAT. Only 0.053 ± 0.009 nmol of FITC-YARA and 0.058 ± 0.008 nmol of FITC-TAT were found in the receptor phase, which means that the amount of YARA and TAT that penetrated into the skin (SC+[E+D]) was re-

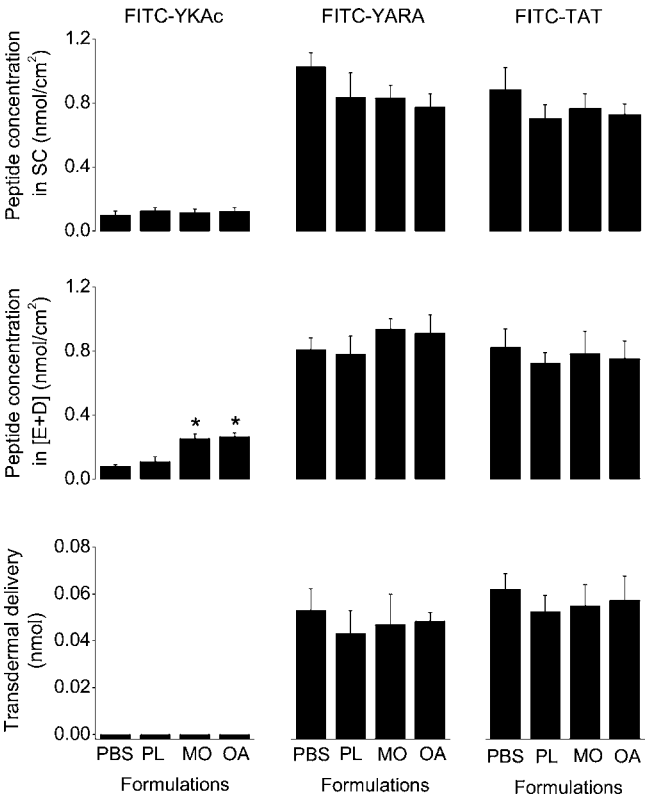

Fig. 1. *In vitro* peptide penetration in the SC, [E+D], and their transdermal delivery after 4 h using PBS or formulations containing the penetration enhancers monoolein (MO, 10% w/w) or oleic acid (OA, 5% w/w). The number of replicates is 4–8 per experimental group. *$p < 0.05$ compared to propylene glycol solution. PL, propylene glycol; SC, stratum corneum; [E+D], epidermis without stratum corneum plus dermis. Mean ± SD, n = 4–8.

spectively 34 and 30 times higher than the amount that permeated across the skin.

Influence of Penetration Enhancers on Topical and Transdermal Delivery of PTDs

We next evaluated the influence of monoolein and oleic acid on the topical and transdermal delivery of FITC-labeled YARA, TAT, and YKAc (results shown in Fig. 1). In this experiment, the permeation enhancers and peptides were dissolved in propylene glycol. Compared to PBS, propylene glycol did not influence the skin penetration of the YKAc, YARA, and TAT at 4 h postapplication. Notably, addition of monoolein or oleic acid to the formulations significantly ($p < 0.05$) increased (~2.5 times) the penetration of the nontransducing peptide, FITC-YKAc, in [E+D]. The same penetration enhancers, however, failed to further increase the already high topical or the transdermal delivery of TAT and YARA.

Transport of the Conjugated Peptide P20 Into and Across the Skin by PTDs

Having demonstrated that FITC-YARA penetrates in the skin in a similar extent to FITC-TAT, we evaluated its ability to increase the penetration of a conjugated peptide. We attached the peptide P20 to FITC-YARA and FITC-TAT and evaluated their topical and transdermal delivery as a function of time. The PTDs studied were able to carry conjugated P20 into SC and [E+D] (Fig. 2). When P20 was conjugated to YARA and TAT, its penetration in both SC and [E+D] was significantly higher ($p < 0.05$) than that of nonconjugated P20 at all time points studied (Figs. 2A–2F). The concentration of YARA-P20 and TAT-P20 in [E+D] was progressively increased ($p < 0.05$) from 0.5 to 4 h postapplication (Figs. 2E and 2F), but no further increase was found between 4 and 8 h. The concentration of the PTD-P20 conjugates in the viable layers of skin ([E+D]) was 5 to 7 times higher than that of nonconjugated P20 at 4 and 8 h postapplication. The maximal rate of penetration of YARA-P20 and TAT-P20 in the whole skin (SC + [E+D]) was achieved at 1 h postapplication (Figs. 2K–2L). The transdermal delivery of FITC-YARA-P20 and FITC-TAT-P20 was very small (0.031 ± 0.011 nmol and 0.027 ± 0.009 nmol for FITC-YARA-P20 and FITC-TAT-P20, respectively); the peptides were detected in the receptor phase only at 8 h postapplication. FITC-P20 did not permeate across the skin at all.

Visualization of the Skin Penetration of Peptides Using Fluorescence Microscopy

Pictures of the control skin are shown in Fig. 3. As expected (35), the skin treated with PBS presented a very weak autofluorescence (especially the SC). Fluorescent images of the skin sections treated with FITC-labeled YARA, TAT, and YKAc are shown in Fig. 4. Treatment of the skin with FITC-YARA and FITC-TAT resulted in a strong fluorescent staining of SC and viable epidermis. Some fluorescence could also be observed in the dermis, demonstrating that these PTDs were able to cross the SC and reach the viable layers of the skin. On the other hand, FITC-YKAc was predominantly localized in the SC, and only a very weak fluorescence was observed in the epidermis. When the skin was treated with FITC-labeled YARA or TAT conjugated with P20, we also observed the presence of strong fluorescence in the SC and viable epidermis (Fig. 5). When the skin was treated with FITC-P20, fluorescence was found only in the SC.

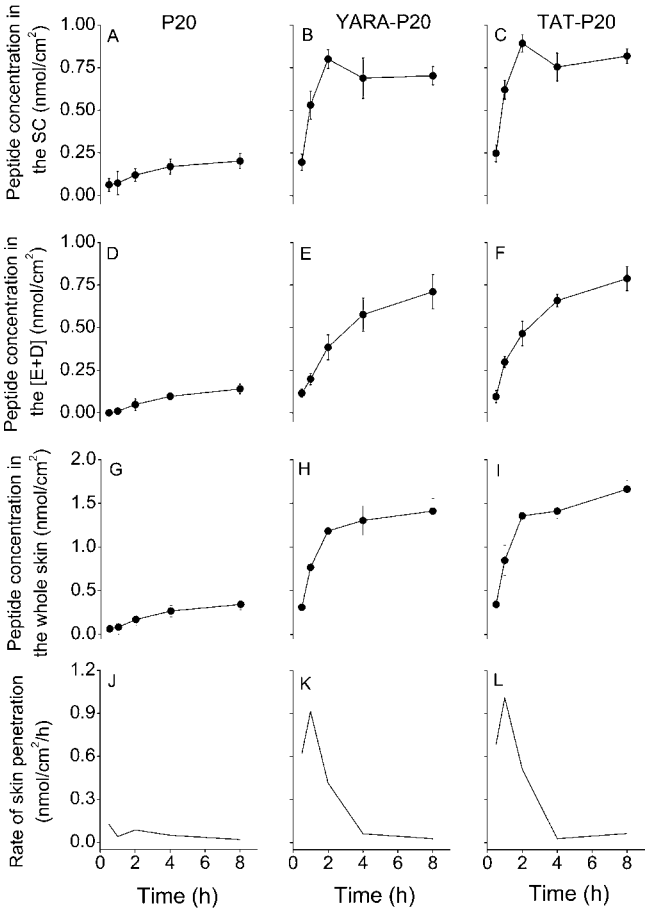

Fig. 2. Time-course of *in vitro* peptide penetration in the SC (A–-C), [E+D] (D, F) and whole skin (G–I) after 0.5, 1, 2, 4, or 8 h. The figure also shows the rate of skin penetration, calculated using the penetration of the peptides in the whole skin (J–L). The number of replicates is 6–8 per experimental group. SC, stratum corneum; [E+D], epidermis without stratum corneum plus dermis. When P20 was conjugated to YARA and TAT, its penetration in both SC and [E+D] was significantly ($p < 0.05$) higher than that of nonconjugated P20 at all time points studied. Mean ± SD, n = 6–8.

DISCUSSION

In the current study, we demonstrated the ability of the PTD YARA to penetrate in the skin of porcine ears *in vitro*. Despite the fact that YARA was demonstrated to transduce into cells more effectively than TAT *in vitro* and *in vivo* (28), we found no significant difference in the ability of these two peptides to penetrate in the skin. On the other hand, the skin penetration of a nontransducing peptide of similar molecular weight, YKAc, was negligible in both SC and [E+D], which is expected because this peptide is hydrophilic and has a high molecular weight (1907 Da).

The influence of chemical enhancers on the skin penetration of peptides was evaluated using propylene glycol formulations containing monoolein and oleic acid. The use of propylene glycol as a solvent had no influence on the topical and transdermal delivery of the peptides studied. Formulations

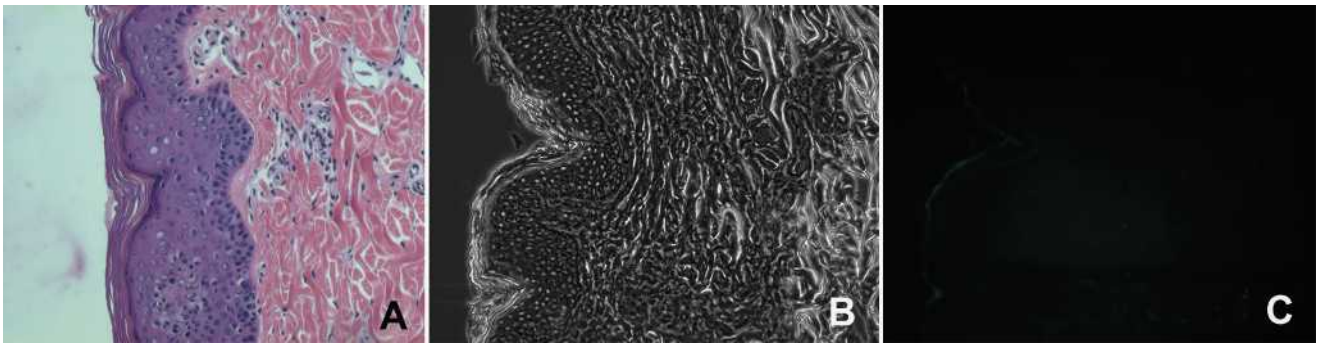

Fig. 3. Skin treated with PBS for 4 h. (A) Hematoxylin staining, (B) unstained skin section observed under halogen light, (C) unstained skin section observed under fluorescent light to show autofluorescence. Sections were visualized through a 20× objective.

containing monoolein or oleic acid did significantly enhance the penetration of the control, nontransducing peptide YKAc in the skin. This observation is consistent with the fact that monoolein and oleic acid act by several mechanisms to increase the permeability of the SC to drugs, including peptides (36,37). These mechanisms include modification of lipid domains and extraction of lipids from the SC (10,36–38). On the other hand, neither monoolein nor oleic acid influenced the topical and transdermal delivery of YARA or TAT. The results suggest that the chemical penetration enhancers studied can be useful to increase the skin penetration of peptides, but only when these have no transduction ability and do not penetrate in the skin at a high extent by themselves.

The skin penetration of YARA-P20 and TAT-P20 was very fast, and the conjugates were able to penetrate in the SC and [E+D] to a higher extent compared to P20 alone. Fluorescence microscopy analysis confirmed that YARA-P20 and TAT-P20 crossed the SC barrier efficiently, and revealed that these relatively large molecules were homogeneously distributed in viable epidermis. It has been shown that conjugates of PTDs-peptides can penetrate very fast in the mice skin, achieving high concentrations as fast as 1 h postapplication (39,40). Robbins *et al.* (39) observed only slight differences in the skin penetration of heptarginine-hemaglutinin epitope from 0.5 to 1 h postapplication. In the current study, we observed that the maximum rate of skin penetration of the con-

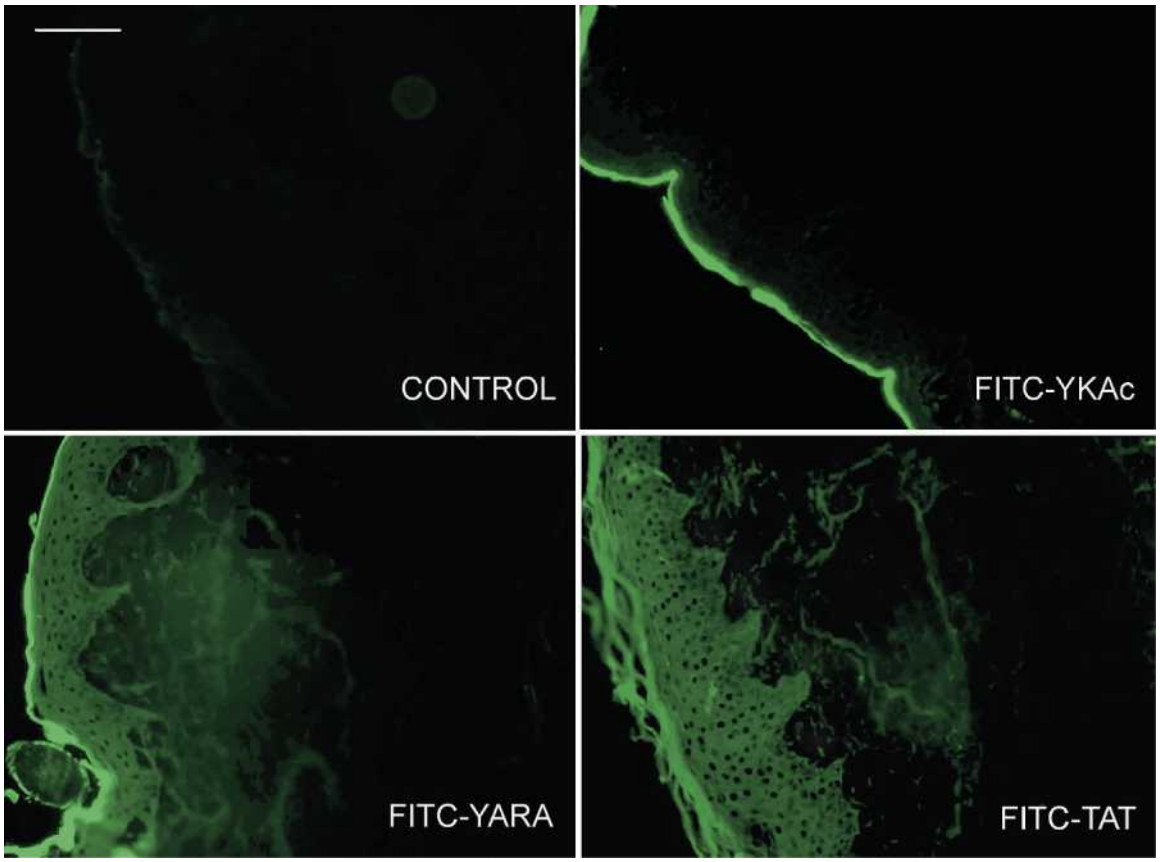

Fig. 4. Fluorescence microscopy of porcine ear skin sections 4 h after application of PBS (control), FITC-YKAc (nontransducing peptide), FITC-YARA, and FITC-TAT. Sections were visualized using FITC filter through a 20× objective. Scale bar, 100 μm. The experiment was performed in 3 replicates.

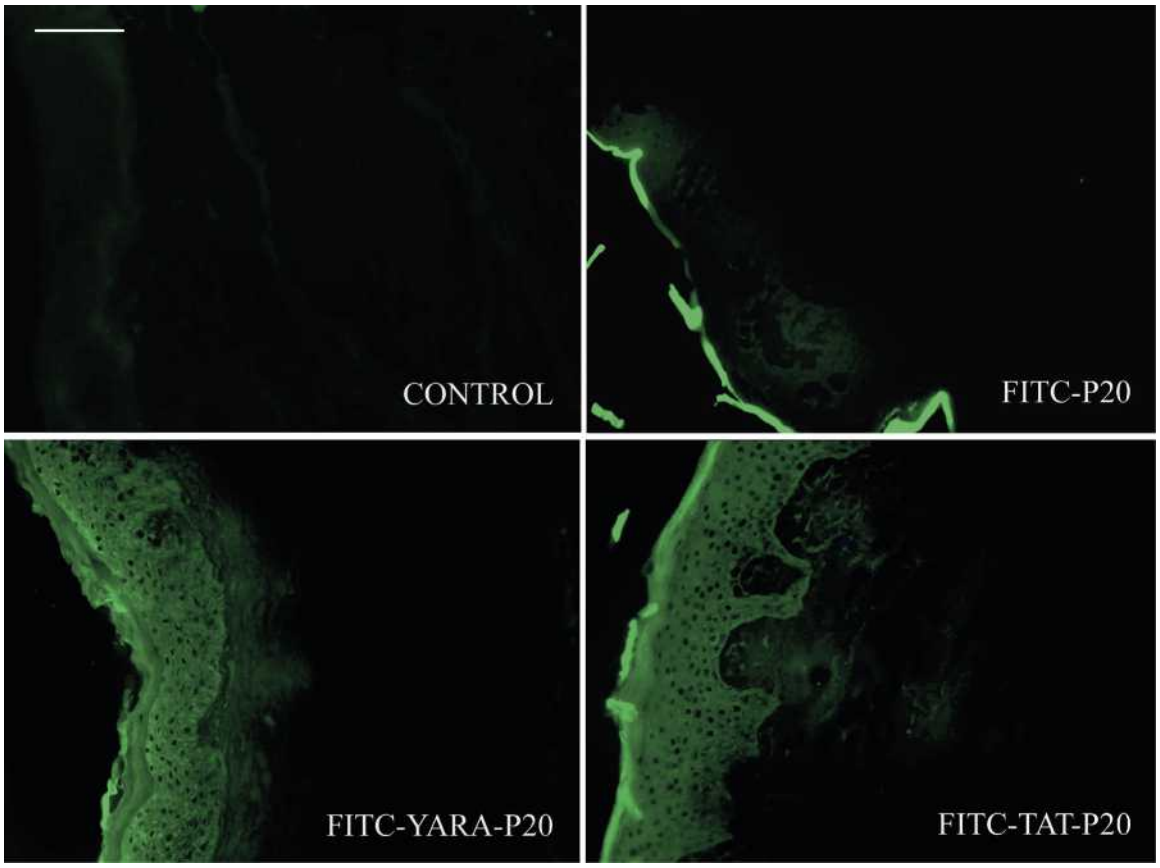

Fig. 5. Fluorescence microscopy of porcine ear skin sections 4 h after application of PBS (control), FITC-P20, FITC-YARA-P20, and FITC-TAT-P20. Sections were visualized using FITC filter through a 20× objective. Scale bar, 100 μm. The experiment was performed in 3 replicates.

jugates occurred at 1 h postapplication, but their concentration in the skin progressively increased until 4 h postapplication ($p < 0.05$). It is important to consider that the skin penetration of protein transduction domains conjugated to peptides might vary depending on the PTD and the experimental model skin used, as the mechanism of penetration might vary among different compounds, and the properties and characteristics of the skin might differ among animals (20,33).

Although the metabolic activity in the skin is smaller than the activity in other tissues (such as mucosa), the stability of peptides in the skin is an important issue (41). Several authors have demonstrated that FITC-labeled macromolecules present good stability in biological tissues, including skin. The integrity of FITC-poly-lysine in the receptor phase of a diffusion cell was demonstrated by HPLC and mass spectrometry, even after the exposure of the compound to electrical current or ultrasound (42,43). FITC-labeled dextrans of different molecular weight had their structure integrity maintained after transdermal delivery, as demonstrated by size-exclusion chromatography (44). Last but not least, the integrity of FITC-oligonucleotides in the skin was demonstrated by Western blot (45). The stability of the PTDs used in this study has also been demonstrated before after incubation at 37°C for several hours in contact with biologic tissues. The pharmacological activity of the conjugate YARA-P20 was preserved after its incubation for 2 days at 37°C with vein segments (29).

Thus, topical administration of conjugates of PTD-peptides may have therapeutic potential for local skin disorders. Topical delivery of peptides has been increasingly studied due to the importance of these compounds for the treatment of skin diseases and for the improvement of skin properties (in the case of cosmetics). Topical administration of several peptides would be attractive, including TGF-β, leptin (both for wound healing), INF-α (antiviral), cyclosporin (for treatment of autoimmune diseases), bacitracin (for skin infections), and palmytoyl-glycyl-hystidyl-lysine tripeptide (for stimulation of collagen synthesis), among many others (6,11,25,31,46–48). In addition, several peptides have been applied to the skin and studied as antigens for the development of topical vaccines (49). In this context, the use of PTDs could be useful for successfully increasing peptides delivery to the skin, a significant achievement that could bring therapeutic benefits associated with avoidance of systemic side effects and patient commodity.

Event though the skin penetration of different PTDs has been reported in the literature (25–27,39), the exact mechanism of action remains unknown. The intercellular lipid domain of the stratum corneum differs from cell membranes not only on lipid composition, but also on water content and lipid/protein ratio (50). In addition, the outermost layer of the skin is composed of non-viable cells, and endocytosis is not expected. Hence, the mechanism for PTDs to penetrate in the skin is likely different from that for them to cross cell membranes. Rothbard *et al.* (25) suggested that the SC is a meta-

bolically active environment (although it is not constituted of viable cells), which can contribute to the transport of PTDs. Moreover, it is well known that several PTDs are able to interact with lipids (51), which may be important for their transport across the SC. Indeed, poly-L-arginine was demonstrated to increase the permeability of tight junctions of the nasal epithelium (52) and the transport of a dextran. This effect was triggered by interaction of poly-arginine with negatively charged lipids of the cell (53). The presence of tight junctions in the skin has already been demonstrated (54), and the disassembly of these structures by the PTDs studied might be important for their penetration into the viable layers of the skin. Moreover, PTDs might penetrate different layers of the skin, and the resulting gradient might be the force driving the penetration of PTDs in the skin (25).

Although the topical delivery of YARA-P20 and TAT-P20 was high, we found that their transdermal delivery was small and occurred slowly, at least *in vitro*. *In vivo*, however, the transdermal delivery of these compounds might be more substantial and faster, and further studies are necessary to evaluate whether topically administered PTD-P20 may produce effects in deeper tissues. This may be of special interest due to the recently demonstrated ability of P20 to cause vasodilation (29,30). Such an effect may be, for example, used for the topical treatment of sexual dysfunction in males and/or females.

CONCLUSIONS

We conclude that the PTDs YARA, TAT, and their conjugates with the peptide P20 rapidly penetrate in porcine skin *in vitro* at a high extent. These results suggest that PTDs can be used as carrier molecules to deliver peptides of therapeutic interest to the skin. We also conclude that the skin penetration of YARA and TAT is not further improved by formulations containing the chemical penetration enhancer monoolein or oleic acid, even though the same penetration enhancers improve the topical delivery of a large, but non-transducing, peptide. Finally, we conclude that the transdermal delivery of conjugated PTDs is small and slow, at least *in vitro*, despite their high penetration in the skin.

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