The influence of transdermal enhancers on percutaneous absorption of rhEGF gel

Jiaojiao Pu [a], Ju Wang [b]

Institute of Biomedicine, National Engineering Research Center of Genetic Medicine, Jinan University, Guangzhou 510632, China

[a]993653380@qq.com, [b]wangju168899@qq.com.

Abstract

To study the influence of transdermal enhancers on percutaneous absorption of rhEGF in gel and to get optimum concentration【Methods】The test for rhEGF Gel permeation through Kunming mice ventral skin in vivo was performed in a special improved Franz diffusion cell, the amounts of rhEGF of this permeation were determined with BCA. Influence of different concentration of azone and propylene glycol on the permeation of rhEGF through mice skin in vitro was observed. CCK-8 method was used to determine cytotoxicity in 3T3 cells after transdermal absorber addition. Western blotting method was used to determine rhEGF activity after transdermal absorber addition.【Results】Finally, dermal enhancers which contains 0.5% azone and 5%PG could make the needed time which the percent of cumulative permeation of rhEGF through mice skin in vitro large. 【Conclusion】0.5%azone in combination with 5% propylene glycol is the most suitable permeation enhancer of rhEGF Gel, the addition of transdermal absorber had no effect on rhEGF activity

Keywords

RhEGF; azone; propylene glycol; percutaneous absorption.

1. Introduction

There are two main areas of human skin: the epidermis (mainly consisting of four layers: the basal layer, the spinous layer, the granular layer and the outermost stratum corneum) and the dermis layer[1]. Skin barrier properties are mainly regulated by the stratum corneum that is mainly composed of multiple layers of keratin-rich corneocytes surrounded by lipid lamellae in a bilayer form[2].This form is the major barrier to the delivery of drugs through the skin[3]. Human skin is the most suitable model for assessment of percutaneous penetration of active compounds. However, its availability is limited. Therefore, mice skin is used for performing penetration tests, there are several strategies to overcome this skin barrier to successfully deliver active molecules[4]. They include the use of chemical permeation enhancers, electroporation, microneedles,ultrasound,photomechanical waves,etc[5].the use of chemical enhancers reversibly reduces skin barrier function by disrupting intercellular stratum corneum lipids. In this study, combination of azone and propylene glycol was used to evaluate the effect of different chemical promoters on rhEGF penetration.

2. Experimental detail

2.1 Reagents

PBS, DMEM medium, fetal bovine serum was purchased from HyClone, Cell Counting Kit-8 was purchased from DOJINDO.

2.2 Preparation of transdermal absorbent gels with different concentrations

Ka poem 940 0.6%, recombinant epidermal growth factor 1mg/ml, 0.5% azone +3% propylene glycol, 0.5% azone +5% propylene glycol or 0.5% azone+8% propylene glycol was added to rhEGF Gel respectively.

2.3 Ttransdermal absorption experiment

(1) Kunming mice were anesthetized, the hair was cut, the skin on the back and abdomen was stripped, the subcutaneous fat was removed, and the mice were soaked with normal saline, pressed with foil paper, under - 20 ℃ refrigerator, use in two days.

(2) The spare mice skin is removed from the refrigerator, restores it to room temperature, rinses it several times with normal saline. Taken the mouse skin with an appropriate area (1cmx1cm) between the diffusion pool and the receiving pool. The volume of the diffusion pool was 2ml, the volume of the receiving pool was 8ml, the cuticle of the skin was oriented toward the diffusion pool, PBS was used as the receiving solution, and the inner surface of the skin was immersed in the receiving solution. Seal the top with a sealing film to prevent the test solution from escaping.

(3) 32 ℃ constant temperature water bath, 230 r/min constant speed magnetic stirring, respectively at different time points from accept liquid sampling 1.5 ml, added immediately after each sampling volume and temperature of the same receiving liquid.

2.4 BCA method was used to measure the protein concentration.

(1) preparation of BCA working fluid: an appropriate amount of BCA working fluid is prepared according to the number of samples. The calculation method is: BCA working fluid volume=600 μL* 8+600μL * number of samples; BCA reagent A: BCA reagent B=50:1, an appropriate amount of BCA working fluid is prepared, fully mixed and ready for use.

(2) BCA working fluid was added to the 96-well plate, 200 μL per well, with 3 duplicate Wells for each sample and standard. In the preparation of standard music, the following concentrations were used:0.5mg/mL, 0.25mg/mL, 0.125mg/mL, 0.0625mg/mL, 0.015625mg /mL, 0 mg/mL.

(3) The sample under test was taken from the refrigerator, vortex after blending, 10 μ l samples were joined to 200 μL working liquid, 37 ℃ avoid light incubation for 30 min.

(4) After incubation, the absorbance value of 562nm wavelength was detected, and the standard curve of the standard substance was drawn. The absorbance value of the sample to be tested was substituted into the equation to calculate the protein concentration.

2.5 Result analysis:

(1) After all samples were taken, the protein concentration (mg/ml) was measured by BCA method.

(2) to obtain the permeability at each time point, the cumulative permeability Q was first used for time regression, the slope of the equation is the transdermal rate P.

2.6 Cell culture

The 3T3 cells were purchased from the American Type Culture Collection (ATCC; Manassas, VA) and cultured in DMEM (Invitrogen, Carlsbad, CA) supplemented with 10% fetal bovine serum (FBS; Invitrogen) at 37 °C in an incubator with 5% CO2.

2.7 Western blotting

3T3 cells were grown to 70–80% confluence and were seeded at a concentration of 1.4 × 106 cells/well in a 6-well plate, rhEGF or rhEGF with0.5%azone and 5%PG was added to the medium for half an hour.

Equal amounts of protein were separated by polyacrylamide gel electrophoresis and transferred to polyvinylidene fluoride (PDVF) membranes. membranes were blocked in Tris-buffered saline (TBS) containing 5% bovine serum albumin (BSA) and 0.5% Tween 20 for 2 h ,and then probed overnight at 4 °C with β-actin(Mouse anti-human; 1:2000), p-EGFR2 (rabbit anti-mouse; 1:1000), EGFR (rabbit anti-human; 1:2000).The immunoreactive proteins were visualized on X-ray films by developing with an enhanced chemiluminescence system (GE Healthcare, Buckinghamshire, UK). The relative protein concentrations were quantified by densitometry using Quantity One 1-D Analysis Software (Bio-Rad Laboratories, Hercules, CA).

2.8 Cytotoxicity detection of EGF gel contains transdermal absorber

(1) observe 3T3 cells under the microscope, and remove and digest when the cells are 80% full.

(2) cells were inoculated in 96-well plate at a density of 4000 cells/well, 150 μL complete medium for each well, PBS was added around the 96-well plate, the cells were placed in the ultra-clean table for 5min, then stabilized and transferred to the cell culture box for 24h.

(3) The cells were treated with dilution of 0-50 μg/mL for 48h at different times.

(4) after 48h, serum-free medium was prepared, and CCK8 working solution =10:1.With a volley suck out medium, 100 μl per hole CCK 8 working liquid , 37 ℃ after incubation for 30 min, enzyme OD450 standard instrument detection.

2.9 Statistical analysis

All data were presented as mean ± SD and statistically significant was determined by one-way ANOVA. $P < 0.05$ was considered statistically significant.

3. Results and discussion

3.1 Standard curve determination

The measured curve value of OD562 is shown in table 1, and the standard curve is drawn as shown in Fig1(A). The standard curve has a good linear relationship.

Table.1OD value of standard curve

(mg/ml)	1	0.5	0.25	0.125	0.0625	0.03125	0
value1	0.4982	0.304	0.2047	0.1577	0.1286	0.1141	0.1031
value2	0.4817	0.3015	0.2131	0.1557	0.1275	0.1137	0.1023
value3	0.4803	0.3035	0.2059	0.1607	0.1288	0.1124	0.1046
average value	0.48673	0.303	0.2079	0.15803	0.1283	0.1134	0.103333

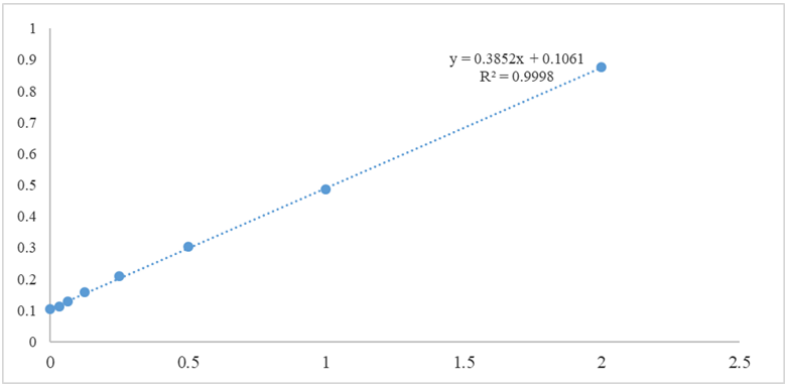


Fig.1 standard curve

3.2 Comparison of cumulative permeability of different transdermal absorbers

The maximum permeability of Con group was 0.258mg; When 0.5% azone and propylene glycol 2% were added to rhEGF gel, the maximum permeability achieved to 0.358mg at 6h; When 0.5% azone and propylene glycol 5% were added to rhEGF gel, the maximum permeability achieved to 0.669mg at 4.5h; When 0.5% azone and propylene glycol 8% were added to rhEGF gel, the maximum permeability achieved to 0.521mg at 6h.

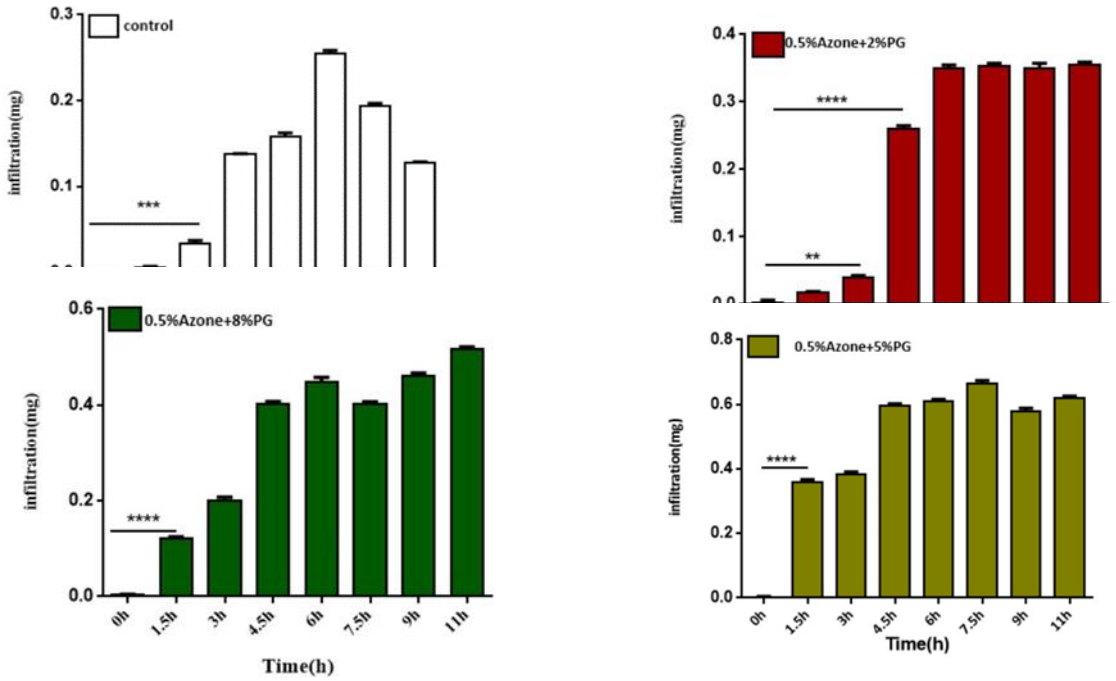


Fig.2 cumulative permeability of different transdermal absorbers
(a)cumulative permeability of the Con group;
(b)cumulative penetration of rhEGF gel with
0.5%azone+2%PG;(c)0.5%azone+5%PG;(d)0.5% azone+8%PG;

3.3 Transdermal rates of gels with different transdermal absorbers

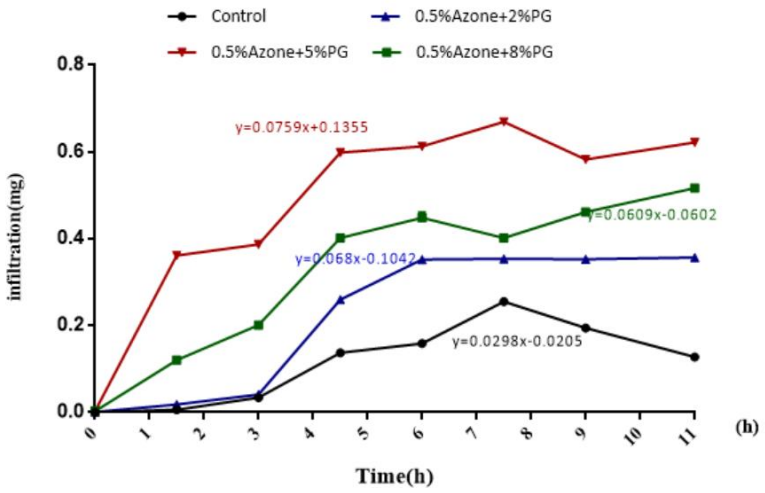


Fig.3.Transdermal rates of gels with different transdermal absorbers

Linear regression was performed with the cumulative permeability as the ordinate and time as the abscissa, the slope of the equation is the infiltration rate.

As shown in Fig.3, the infiltration rate of Con group (rhEGF only) was 0.0298mg/h, when 0.5% azone and propylene glycol 2% were added to rhEGF gel, the penetration rate was 0.068mg,when 0.5% azone and propylene glycol 5% were added to rhEGF gel, the penetration rate was 0.0759mg/when 0.5% azone and propylene glycol 8% were added to rhEGF gel, the penetration rate was 0.0759mg/h.

3.4 Effects of rhEGF gel after 0.5%azone+5%PG addition on cytotoxicity of 3T3 cells.

The rhEGF gel was diluted 20 times into an aqueous solution, and then aqueous solution was diluted 1.5 times. Within 48 hours, rhEGF was applied to 3T3 cells as a concentration ranging from 1.94 g/mL to 33 g/mL.Fig.4 represents that There was no significant difference between the administration group and the control group, indicating that the addition of 0.5%Azone+5%P in the gel had no cytotoxicity under 50μg/ml.

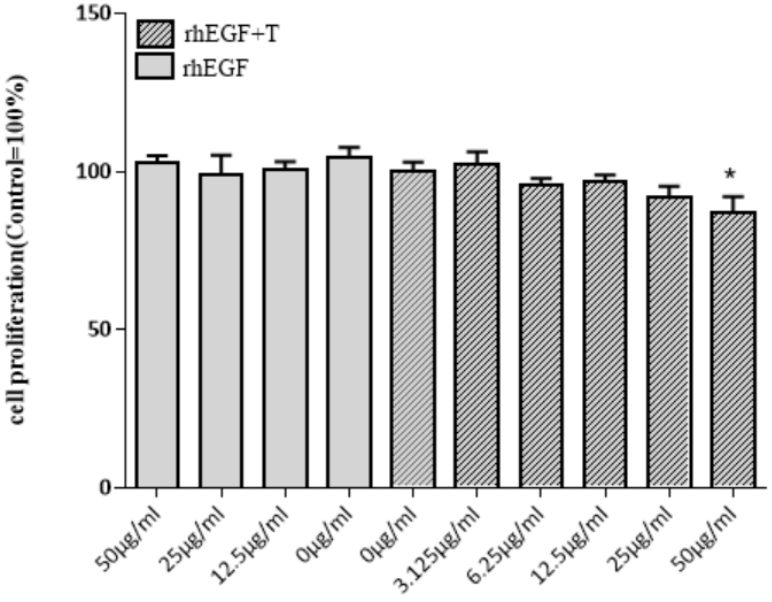
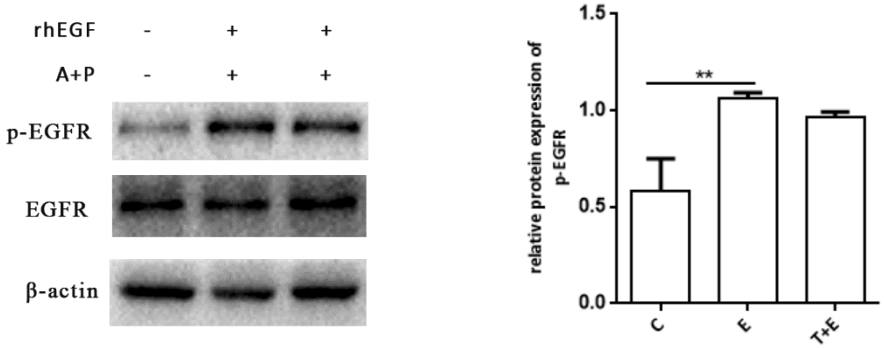


Fig.4 CCK8 cell toxic detection

3.5 Effect of transdermal absorbent on rhEGF activity.



A B

Fig.5 Effect of transdermal absorbent on rhEGF activity.

(a) Western blotting to detect EGFR;

(b) Relative protein expression analysis of p-EGFR.

rhEGF can cause phosphorylation of EGFR. In Fig.5 (A), E represents administration of rhEGF, and E+T represents administration of rhEGF+ transdermal absorber.

The results showed that 0.5%azone+5%PG added to rhEGF gel did not affect the recombinant protein activity.

4. Conclusion

In this study, Addition of 0.5% azone in combine with 5%PG largely enhanced transdermal absorption rate of rhEGF Gel. Addition of transdermal absorber does not affect the recombinant protein activity.

Acknowledgements

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