약제학회지(1996), 제26권 제 1호
J. Kor. Pharm. Sci., Vol. 26, No. 1, 29-32(1996)

Percutaneous Absorption of Recombinant h-EGF through Normal, Stripped and First-Degree Burn Skin

Ae-Ri Cho*, Jung-Uk Lee, Byung-Lak Ahn, Joo-young Chung,**

Yeo-Wook Koh, Chang-Koo Shim†**

College of Pharmacy, Seoul National University,
**College of Pharmacy, Duksung Women's University, Seoul, Korea.*
***R & D Center, Dae Woong Pharmaceutical Co., Ltd.*

(Received January 25, 1996)

상피세포 성장인자의 경피흡수: 정상피부, 각질제거피부 및 화상피부에 있어서

조애리* · 이정욱 · 안병락 · 정주영 · 고여욱** · 심창구†**

*서울대학교 약학대학 약제학교실, *덕성여자대학교, 교양학부, **대웅제약 중앙연구소*

(1996년 1월 25일 접수)

In vivo and *in vitro* skin permeation of recombinant ^{125}I-EGF through normal, stripped and the first degree burn skin were studied. The *in vitro* skin permeation rate through the first degree burn skin (296 cpm/cm^2/hr) and the stripped skin (1131 cpm/cm^2/hr) were 3.5 times and 13 times higher, respectively, as compared with the one through normal skin. *In vivo* absorption study with the first degree burn skin, the peak concentration of EGF in the skin was achieved at 1-3 hr and decreased afterward up to 8 hr with an elimination constant of 1.31×10^{-3} g/ml/hr. To investigate the higher elimination rate of EGF in burn skin, binding and metabolism studies were conducted. No significant metabolism of EGF in burn skin (100°C, 5-second burning) was observed. With the presence of unlabelled-EGF, ^{125}I-EGF permeation through the burn skin showed higher permeation rate than the one without unlabelled-EGF. The result may indicate that EGF-receptor binding play a role in determining the skin permeation rate.

Introduction

Since its first isolation from submaxillary gland of a mouse, epidermal growth factor (EGF) has been recognized as having a very important role in wound healing process.[1] EGF-treatment significantly reduced the average healing time in 12 patients who had partial-thickness skin wounds or second-degree burn skin wounds.[2] However, the pharmacokinetics of EGF after topical application has not been understood clearly. So, we first studied the *in vitro* and *in vivo* skin permeation of EGF through the first degree burn skin and compared with those of normal skin and stripped skin. And we investigated the effect of EGF-receptor binding and metabolism of h-EGF during initial post-burn period on the skin permeation profile of EGF.

Experimental

Preparation of first degree burn skin

Male hairless mice (5~6 wks, weighing 30±5 g) were anesthetized with intraperitoneal injection of urethane (1.2 g/kg). The first degree burn skin was made by pressing a stainless-steel heating pad which was soaked in 100°C boiling water for 2 min on the abdominal side

†To whom correspondence should be addressed.

for 5 seconds. The degree of burn was well controlled and very reproducible in terms of burn area ($2\times4\,cm^2$) and the intensity of burning. The reproducibility of burn skin was confirmed by performing *in vitro* diffusion study with burn skin.

Preparation of stripped skin

After anesthetizing the mouse, the stratum corneum layer was stripped off 20 times using acetate adhesive tape (Scotch Magic Tape, Korea 3M Ltd., Korea).

***In vitro* diffusion study**

Each skin specimen was mounted on the side by side diffusion cell. Twenty ml of EGF (Daewoong Pharm. Co., Korea) aqueous solution (EGF: 0.5 μg/m*l*, ^{125}I-EGF: 0.3~0.4 μCi) containing 40% PEG 400 (Junsei Chemical Co., Japan) was applied on the stratum corneum side, and the skin surface was wrapped with polyvinyl acetate film. Ten milliliter of distilled water was put into the receptor cell. Gentamicin sulfate (Yuhan Pharm. Co., Korea) of 50 μg/m*l* was included in receptor solution to prevent bacterial contamination during diffusion study. The total surface area of skin is 2.16 cm^2. At predetermined time interval, 100 μ*l* sample from receptor solution was taken and total radioactivity was counted by gamma counter(Cobra Auto-Gamma, Packard, Canberra Co., U.S.A.)

***In vivo* absorption study**

Fifty ml ^{125}I-EGF, dissolved in 40% PEG, was rubbed for 50 sec into a 8 cm^2 area. The EGF was left on the skin for periods of 1, 3, 6, and 8 hrs. The residue was carefully washed off three times by gentle cleaning with cotton balls soaked with water, 70 % ethanol, and water, successively. And the abdomen site was excised and the total radioactivity of the recovered skin site was counted by gamma counter.

Results and Discussion

Fig. 1 shows the *in vivo* skin permeation profiles of EGF across various skin model. Based on the initial 4 hrs skin permeation profile, 0.7% and 10% of applied dose were permeated through the first degree burn skin and the stripped skin, respectively. Based on steady-state skin permeation profiles (0~24 hr), permeation rates were determined. The *in vitro* skin permeation rate through the first degree burn skin(296.5 cpm/cm^2/hr) and the stripped skin (1131.3 cpm/cm^2/hr) were 3.5 times and 13 times higher,

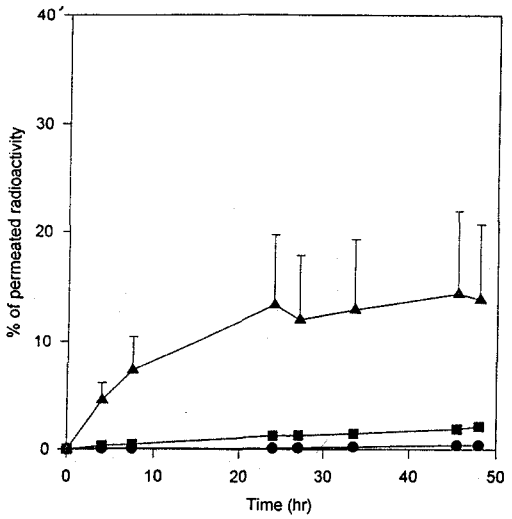

Figure 1 — *In vitro* skin permeation profiles of ^{125}I-EGF across various skin model.
-●- Normal skin(n=3), -■- Burned skin(n=3), -▲- Stripped skin(n=3)

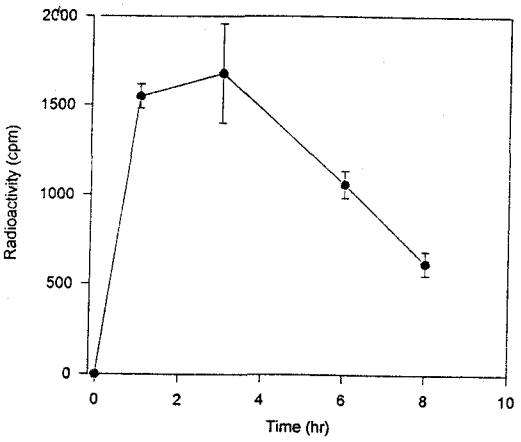

Figure 2 — Radioactivity in burn skin sample after topical application of ^{125}I-EGF solution for 1, 3, 6 and 8 hours.

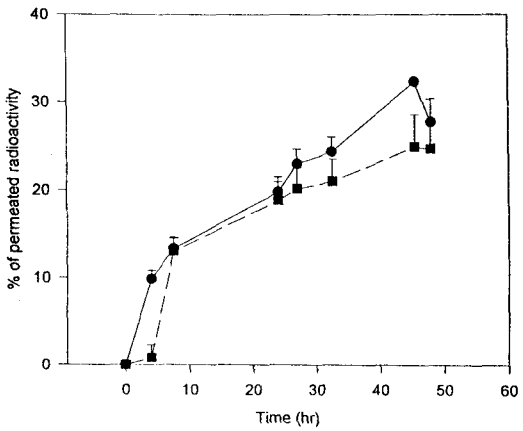

Figure 3 — *In vitro* skin permeation profiles of ^{125}I-EGF across stripped skin model.
●: EGF 10 μg+^{125}I-EGF 0.4 μCi(n=3), ■: ^{125}I-EGF 0.4 μCi(n=3).

respectively, as compared with the one through normal skin(84.9 cpm/cm^2/hr) (data not shown).

Fig.2 shows *in vivo* skin absorption study with the first degree burn animal model. The peak concentration of EGF in the skin was achieved at 1~3 hr and decreased afterward up to 8 hrs with apparent elimination constant (K_{el}) of 1.31×10^{-3} g/m*l*/hr. As apparent absorption and elimination constant reflect the sum of intrinsic absorption and elimination processes, the decline profile after 3 hrs may indicate that as time proceeds, the elimination constants increase due to modulation of EGF-receptor or increase of protease release after burn shock cell damage proceeds.[3] So, we investigated the effect of EGF-receptor binding on the skin permeation profile by incorporating cold EGF into donor solution during *in vitro* skin permeation study. With the presence of cold-EGF, available binding sites for ^{125}I-EGF will decrease. So unbound free ^{125}I-EGF which has less difficulty to permeate through the skin showed higher permeation rate as shown in Fig.3. Degradation pathway has been known that EGF first binds EGF-receptor and internalization and lysosomal degradation of EGF proceed.[4] Table 1 shows

Table 1 — *Metabolism of EGF in First-Degree Burn Skin after Topical Application in vivo(n=2~3)*

Sample		Application time(hr)				
		1/2	1	3	6	8
Ppt(cpm)		877±51	777±152	346±84	320	760
Sup & Ab	Ppt (cpm)	386±57	491±61	730±199	388	280
	Sup (cpm)	0	57±30	35±17	149	0
Total(cpm)		1262±109	1326±129	1112±286	857	440
Intact EGF (%)		100	96±2	97±2	83	100

Sup: supernatant; Ppt: precipitate; Ab: antibody

that the total radioactivity of ^{125}I-EGF recovered in pellet part decreased as time proceeds. If wound healing after burning is the timed process, as time proceeds, the degradation of EGF may increase and the elimination of EGF will increase result in less amount of EGF retained in the skin. This experimental result might be a possible explanation for the rapid elimination profile after 3 hrs *in vivo* absorption study as shown in Fig.2.

Conclusion

In normal skin, no absorption of EGF was observed. In stripped skin, significant amount of EGF was absorbed through the skin and reached the systemic circulation. *In vivo* absorption study with the first degree burn skin, the peak concentration of h-EGF in the skin was achieved at 1~3 hr and decreased afterward up to 8 hrs with the elimination constant (Kel:1.31×10^{-3} g/m*l*/hr). EGF-receptor binding and metabolism after burn-shock cell damage may play an important role in skin absorption of EGF.

Acknowledgment

This work was partially supported by the grant from the Ministry of Public Health and Welfare, KOREA.

References

1) G. Carpenter and S. Cohen, Epidermal growth factor, *Ann. Rev. Biochem.* **48** : 192-216 (1979).
2) G.L. Brown, L.B. Nanney J. Griffen, A.B. Cramer, J.M. Yancey, L.J. Curtinger, L. Holtzin, G.S. Schultz, M.J. Jurkiewicz and J. B. Lynch, Enhancement of wound healing by topical treatment with epidermal growth factor, *New Engl. J. Med.*, **321** : 76- 79 (1989).
3) B.A. Wenczak, J.B. Lynch and L.B. Nanney, Epidermal growth factor receptor distribution in burn wounds, *J. Clin. Invest.*, **90** : 2392-2401 (1992).
4) L.E. King, R.E. Gates, C.M. Stoscheck, L.B. Nanney, The EGF/TGF a receptor in skin, *J. Invest. Dermatol.*, **94** : 164s-170s (1990).