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RESEARCH PAPER

Dermal Delivery of ETH-615, a Zwitterionic Drug

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ABSTRACT

ETH-615 is an amphoteric drug that forms a water-insoluble zwitterion at intermediate pH values. Increasing the aqueous solubility of ETH-615 through cyclodextrin complexation did not enhance transdermal delivery of the drug from saturated aqueous solutions. However, increasing the lipophilicity of the drug through masking of the anionic group with a pro-moiety increased the dermal and transdermal delivery of the drug. Furthermore, masking the anionic group enhanced the chemical stability of the drug, resulting in significant improvement of the shelf life of the drug in both aqueous and nonaqueous solutions.

Key Words: *Degradation; Hairless mouse skin; Lipophilic; Permeability; Prodrugs.*

INTRODUCTION

ETH-615 is an experimental anti-inflammatory drug (1). It is an amphoteric drug that forms a zwitterion (i.e., the molecule carries one cation and one anion) at pH between approximately 5 and 10. Although zwitterionic drugs are highly polar, their aqueous solubility is usually very limited at intermediate pH values. Previously, we showed that it is possible to enhance the aqueous solubility of ETH-615 through cyclodextrin complexation (2). However, due to the highly polar zwitterionic structure of ETH-615, the stability constants of its cyclodextrin complexes were several orders of magnitude smaller than those commonly observed for uncharged lipophilic compounds. In the present study, we attempted to enhance the solubility and dermal delivery of ETH-615 through prodrug formation.

Dermal drug delivery can be divided into the following steps:

1. Drug dissolution in the topical drug vehicle.
2. Diffusion of the dissolved drug molecules to the surface of the skin.
3. Partition of the drug molecules from the vehicle into stratum corneum (i.e., the outermost layer of epidermis).

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4. Permeation of the drug molecules through the epidermis into the dermis.

In general, the main permeation barrier is the lipophilic stratum corneum. However, the drug must be soluble to some extent in the vehicle to be able to partition from the vehicle into stratum corneum. Dermal delivery of zwitterionic drugs is particularly challenging. Although zwitterionic drugs are highly polar, their solubility in both aqueous and nonaqueous solutions is usually very limited. This can hamper successful dermal delivery of topically applied zwitterionic drugs. Cyclodextrin solubilization of ETH-615 did not have any significant effect on the transdermal delivery of the drug. In the present study, three different prodrugs of ETH-615 were synthesized, and their physicochemical properties and transdermal delivery were evaluated.

EXPERIMENTAL

Materials

1,2-O-Isopropylidene-rac-glycerol and 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDAC) were purchased from Sigma-Aldrich (St. Louis, MO). 4-Dimethylaminopyridine (DMAP) and phenol were purchased from Merck (Darmstadt, Germany). Leo Pharmaceutical Products (Ballerup, Denmark) kindly donated ETH-615 (**1**). [^{1}H] Nuclear magnetic resonance (NMR) ($CDCl_3$) values were as follows: δ 4.56 (s, 2H), 4.61 (s, 2H), 5.34 (s, 2H), 6.63 (d, J = 9.1 Hz, 2H), 6.86–7.04 (m, 5H), 7.22–7.35 (m, 3H), 7.55 (t, J = 7.2 Hz, 1H), 7.74 (t, J = 7.3 Hz, 2H), 7.83 (d, J = 8.1 Hz, 1H), 8.06 (d, J = 8.4 Hz, 2H), 8.14 (d, J = 8.5 Hz, 1H), 8.21 (d, J = 8.6 Hz, 1H). All other compounds and solvents used throughout this study were commercially available products of special reagent or analytical grade.

Synthesis

For synthesis of (±)-1,2-*O*-isopropylidene-3-(ETH-615)-glycerol (**2**), ETH-615 (0.4 g per 0.8 mmol) and EDAC (0.4 g 2 mmol) were dissolved in dichloromethane (20 ml) and stirred for 15 min. 1,2-Isopropylidene glycerol (0.13 g per 1 mmol) and DMAP (20–50 mg) were added to the solution, and the solution was heated under reflux for 5 hr. The mixture was then concentrated by evaporation under reduced pressure, diluted with ether (30 ml), and washed three times with water (30 ml). The organic phase was dried ($MgSO_4$) and concentrated under reduced pressure; the oily residue was purified by silica gel 60 (0.063–0.200 mm) column chromatography, eluted with a mixture of toluene and methanol (98/2). The title compound was obtained as a yellowish oil (0.4 g, 82% yield).

The [^{1}H] NMR ($CDCl_3$) values were as follows: δ 1.41 (s, 3H), 1.49 (s, 3H), 3.88 (dd, J = 8.5 and 5.8 Hz, 1H), 4.15 (dd, J = 8.5 and 6.2 Hz, 1H), 4.37–4.49 (m, 3H), 4.53 (s, 2H), 4.58 (s, 2H), 5.31 (s, 2H), 6.64 (d, J = 9.1 Hz, 2H), 6.89–7.04 (m, 5H), 7.16–7.34 (m, 3H), 7.52 (t, J = 7.5 Hz, 1H), 7.66–7.72 (m, 2H), 7.80 (d, J = 7.5 Hz, 1H), 8.02–8.16 (m, 4H).

For (±)-1-(ETH-615)-glycerol (**3**), a solution of (±)-1,2-*o*-isopropylidene-3-(ETH-615)-glycerol (**2**) (0.2 g per 3 mmol) in a mixture of water, acetic acid, and acetone (90:9:1) was heated to 50°C–60°C and stirred at room temperature for 4 hr. The reaction mixture was then concentrated under reduced pressure. The oily residue was purified by silica gel 60 (0.063–0.200 mm) column chromatography and eluted with a mixture of toluene and methanol (9:1). This yielded the desired product as a clear oil (0.1 g, 63% yield).

The [^{1}H] NMR ($CDCl_3$) values were as follows: δ 2.65 [s(broad), 2H], 3.63–3.79 (m, 2H), 4.04–4.08 (m, 1H), 4.38–4.41 (m, 2H), 4.53 (s, 2H), 4.58 (s, 2H), 5.31 (s, 2H), 6.61 (d, J = 9.1 Hz, 2H), 6.85–7.04 (m, 5H), 7.16–7.35 (m, 3H), 7.52 (t, J = 7.5 Hz, 1H), 7.64–7.74 (m, 2H), 7.80 (d, J = 7.5 Hz, 1H), 7.96 (d, J = 8.4 Hz, 2H), 8.06 (d, J = 8.4 Hz, 1H), 8.16 (d, J = 8.6 Hz, 1H).

For ETH-615-phenol (**4**), ETH-615 (0.4 g per 0.8 mmol) and EDAC (0.4 g per 2 mmol) in dichloromethane (20 ml) was stirred for 15 min. Phenol (0.09 g per 1 mmol) and DMAP (20–50 mg) were added to the solution, and the solution was refluxed for 5 hr. The mixture was concentrated under reduced pressure, diluted with ether (30 ml), and washed three times with water (30 ml). The organic phase was dried ($MgSO_4$) and concentrated under reduced pressure. The oily residue was then purified by silica gel 60 (0.063–0.200 mm) column chromatography, eluted with toluene, and crystallized from methanol. The title compound was obtained as a white solid (0.4 g, 92% yield).

The [^{1}H] NMR ($CDCl_3$) values were as follows: δ 4.53 (s, 2H), 4.58 (s, 2H), 5.31 (s, 2H), 6.64 (d, J = 9.1 Hz, 2H), 6.89–7.04 (m, 5H), 7.16–7.34 (m, 11H), 7.55–7.90 (m, 4H), 8.01–8.35 (m, 3H).

Quantitative Determinations

The quantitative determinations of ETH-615 and its derivatives were done on a high-performance liquid chromatographic (HPLC) component system from Merck-

Hitachi (Merck, Darmstadt, Germany). The system consisted of a LaChrom pump L-7000 solvent delivery system operated at 1.0 ml/min, a Merck-Hitachi AS4000 autosampler with a temperature-controlled sample rack (150 mm, 4.6 mm id, 5-μm bead), a cyano-column (Beckman Instruments, Fullerton, CA), and a LaChrom UV Detector L7400 operated at 230 nm. The mobile phases consisted of MeOH and 0.03 M phosphoric acid (75:25), and the retention times were as follows: compound **1**, 4.3 min; compound **2**, 6.0 min; compound **3**, 2.6 min; and compound **4**, 6.2 min.

Determination of log K_{ow}

The partition coefficient was measured by the shake flask method (3). The aqueous phase consisted of 0.10 M, pH 7.3 phosphate buffer, which had been saturated by *n*-octanol, and the oil phase consisted of *n*-octanol saturated with the phosphate buffer. The initial drug concentration in the oil phase was 3×10^{-3} M. The drug concentration in each phase was determined by HPLC, and the log K_{ow} value represents the log value of the oil/water (i.e., C_O/C_W) partition coefficient.

Kinetic Studies

The degradation studies of ETH-615 (**1**) and its derivatives (compounds **3** and **4**) in aqueous 0.01 M acetate buffer solutions were carried out by adding a stock solution (50 μl) of the compound to be tested in methanol to an aqueous buffer solution (1.5 ml), previously equilibrated at 60°C in a temperature-controlled sample rack. The initial concentration was 8×10^{-5} M. The pH of the final reaction mixture was determined with a pH meter (PW 9420, Philips, UK) standardized at 60°C. All reactions were run under pseudo-first-order conditions. Aliquots (100 μl) were injected into the column at various time intervals, and the pseudo-first-order rate constants k_{obs} were determined from the disappearance of the compound by linear regression of a natural logarithm of the peak height versus time. The reported values are the means of three separate experiments plus or minus the standard deviation (SD).

The photochemical degradation of ETH-615 and its derivatives (compounds **3** and **4**) was evaluated in anhydrous methanol solutions by continuously monitoring the absorption at 200 nm (Perkin-Elmer Lambda 3A UV/Vis spectrophotometer). The initial concentration of the compounds was 1.5×10^{-3} M. The observed pseudo-first-order rate constant k_{obs} was obtained by linear regression of the absorption units. HPLC analysis during the degradation studies verified that the decrease in absorbance was proportional to the decreased concentration of the original compound. The reported values are the means of three separate experiments plus or minus the standard deviation.

The degradation of ETH-615 and its derivatives (compounds **2**, **3**, and **4**) was evaluated in 97% human plasma at 37°C. A methanolic stock solution (100 μl) of the compound to be tested was added to 3 ml of the plasma that had previously been equilibrated at 37°C. The initial concentration of the compounds was 2×10^{-4} M. Samples were taken (100 μl) at various time intervals and the reaction quenched by addition of 100 μl of 0.5 M aqueous perchloric acid solution. This was followed by dilution with 800 μl of the mobile phase and centrifugation at 4000 rpm for 20 min. An aliquot (100 μl) of the clear supernatant was injected directly into the HPLC system. The pseudo-first-order rate constants k_{obs} were determined from the disappearance of the compound by linear regression of a natural logarithm of the peak height versus time.

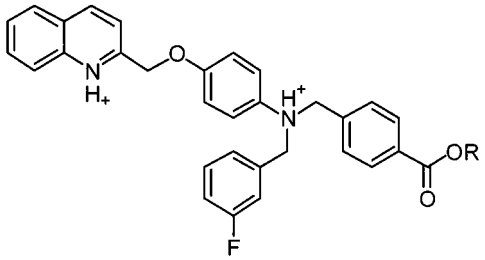
The degradation of ETH-615 and its derivatives (compounds **2**, **3**, and **4**) was evaluated in 97% hairless mouse skin homogenate, with 1 g of the skin homogenized in 20 ml of 0.5 M phosphate buffer at pH 7.6. The homogenate was kept at 37°C. A methanolic stock solution (100 μl) of the compound to be tested was added to 3 ml of the homogenate. The initial concentration of the compounds was 2×10^{-4} M. Samples were taken (100 μl) at various time intervals, and the reaction was quenched by addition of 900 μl of acetonitrile and centrifugation at 4000 rpm for 20 min. An aliquot (100 μl) of the clear supernatant was injected directly into the HPLC system.

Skin Permeability Studies

Female hairless mice (3CH/Tif hr/hr) (Bommice, Denmark) were sacrificed by cervical dislocation, and their full-thickness skins were removed. The outer surface of the skin was rinsed carefully with 35% (v/v) aqueous methanol solution, followed immediately by rinsing with distilled water. The skin was placed in Franz diffusion cells of type FDC 400 15 FF (Vangard International Inc., USA). The surface area of the skin in the diffusion cell was 1.77 cm^2. The receptor phase (12 ml) consisted of pH 7.4 phosphate buffer saline containing 0.3% (w/v) Brij-58 to ensure sufficient drug solubility in the receptor phase. The receptor phase was sonicated under vacuum prior to usage to remove dissolved air. The receptor phase was stirred with a magnetic bar and kept at 37°C by circulating water through an external jacket. The donor phase consisted of a saturated solution of the compound in pro-

Table 1

*Structure and Physicochemical Properties of ETH-615 (**1**) and Its Three Derivatives*



Compound	*R*	MW (g/mol)	mp[a] (°C)	Solubility in Propylene Glycol[b] (mg/ml ± sd)	Log K_{ow}[c]	Retention[d] (min)
1	H	491	154	1.11 ± 0.10	2.89	4.3
2		605	Oil	0.31 ± 0.03	3.69	6.0
3		565	Oil	3.30 ± 0.51	2.45	2.6
4		567	136	0.10 ± 0.02	3.00	6.2

[a] The melting point is not corrected.
[b] The solubility in propylene glycol at room temperature (22°C– 23°C) ± SD.
[c] The logarithm of the partition coefficient between *n*-octanol and water at room temperature.
[d] The retention time obtained in the HPLC system used for quantitative determination of the compounds.

pylene glycol obtained by heating a suspension of the compound in an ultrasonic water bath (40°C for 30–40 min). After equilibration at room temperature for 3 days and filtration through a 0.45-μm membrane filter, the donor phase (2.0 ml) was applied to the skin surface, and the donor chamber was covered with aluminum foil. Samples (100 μl) of receptor phase were removed from the cells at various intervals of up to 48 hr and replaced with a fresh buffer solution. The samples were kept frozen until analyzed by HPLC.

The solubility of the compound in propylene glycol was determined by quantitative determination of the

Table 2

Observed Pseudo-First-Order Rate Constants k_{obs} *for the Rate of Disappearance of the Compounds in Aqueous 0.01 M Acetate Buffers at 60°C and Protected from Light, in Anhydrous Methanol Solution at 200 nm in a UV Absorbance Detector at Ambient Temperature (~25°C), in Human Plasma at 37.0°C, and in Hairless Mouse Skin Homogenate at 37.0°C*

	k_{obs} (hr^{-1})				
	Aqueous Acetate Buffer				
Compound	pH 4.0	pH 5.0	Methanolic Solution	Human Plasma	Mouse Skin Homogenate
1	0.82 ± 0.03	0.15 ± 0.03	0.062 ± 0.033	0.033	0.063
2	n.d.[a]	n.d.[a]	0.040 ± 0.024	0.25	0.077
3	0.11 ± 0.00	0.10 ± 0.01	n.d.[a]	0.22	0.087
4	0.09 ± 0.00	0.10 ± 0.01	No degradation[b]	No degradation[b]	1.05

[a] Not determined.
[b] No degradation was observed over a period of 12 hr.

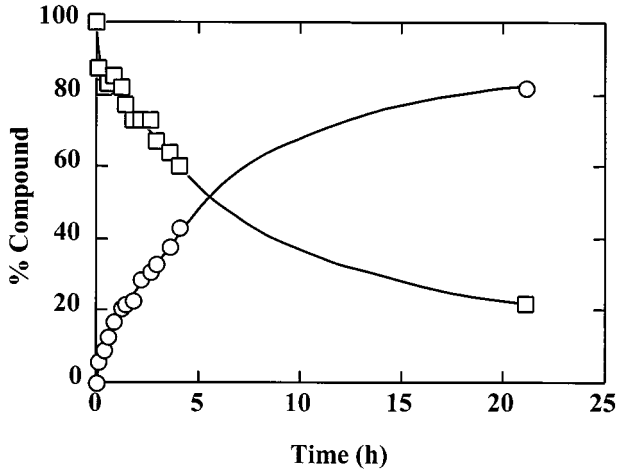


Figure 1. The disappearance of compound **2** and the appearance of ETH-615 (compound **1**) in hairless mouse skin homogenate at 37°C.

compound after appropriate dilution with the mobile phase in the saturated donor phase.

RESULTS AND DISCUSSION

Three different derivatives of ETH-615 were synthesized and characterized (Table 1). At room temperature, the solubility of the compounds in water (at neutral pH) was in all cases less than 10 μg/ml. The solubility of ETH-615 (i.e., compound **1**) had been determined previously to be about 70 μg/ml at pH 1.3, about 1 μg/ml at pH 3 to 7, and about 1 mg/ml at pH 8 (2). Compounds **2** and **3** were oils, but compound **4** was a crystalline solid. ETH-615 has a rather limited solubility in propylene glycol, and interestingly, eliminating the zwitterionic character of the drug by replacing the anion with a lipophilic moiety did not improve the solubility (Table 1). In general, masking of the anion moiety of ETH-615 did not improve its solubility in aqueous or nonaqueous solvents.

Compounds **2**, **3**, and **4** did not degrade to form ETH-615 in aqueous acetate buffer solutions or anhydrous methanol solution (Table 2). However, under enzymatic conditions, the compounds degraded to form quantitative amounts of ETH-615 (Fig. 1). Thus, these compounds can be regarded as true prodrugs of ETH-615. The promoieties protected the drug, resulting in significantly slower drug hydrolytic degradation in aqueous buffer solutions and also slower photochemical decomposition in methanol. However, enzymatic degradation of the prodrugs (i.e., compounds **2**, **3**, and **4**) was significantly faster than that of the parent drug (i.e., compound **1**). In fact, when the prodrugs were applied to hairless mouse skin, only the parent drug could be detected on the receptor side. The prodrugs were not able to penetrate the skin in their intact form. However, formation of a lipophilic prodrug could increase the dermal delivery of ETH-615. The permeability coefficient, as measured by the appearance of ETH-615 in the receptor phase, was about 10 times greater for compound **2** than for ETH-615 itself, or $44 \pm 11 \times 10^{-5}$ cm hr^{-1} compared with $4.4 \pm 1.4 \times 10^{-5}$ cm hr^{-1}, respectively (Table 3). The permeability coefficient for the drug in an aqueous 10% (w/v) 2-hydroxypropyl-β-cyclodextrin solution, which had been saturated with ETH-615, was only $0.6 \pm 0.1 \times 10^{-5}$ cm hr^{-1}. Compound **3** is much more hydrophilic than ETH-615 and resulted in decreased dermal delivery of the drug. Compound **4** was somewhat

Table 3

Flux and Permeability Coefficient (± SD) of ETH-615 Through Hairless Mouse Skin Calculated from the Appearance of ETH-615 in the Receptor Phase at 37°C

Compound	Flux ± SD (μg hr^{-1} cm^{-2})	Permeability Coefficient ± SD (cm hr^{-1})
1	$4.6 \pm 1.6 \times 10^{-2}$	$4.4 \pm 1.4 \times 10^{-5}$
2	$14 \pm 4 \times 10^{-2}$	$44 \pm 11 \times 10^{-5}$
3	(2×10^{-2})[a]	(0.5×10^{-5})[a]
4	No permeation	No permeation

The donor phase consisted of a saturated solution of ETH-615 or its derivatives in propylene glycol. Under the present conditions, the ETH-615 derivatives did not penetrate through the hairless mouse skin.

[a] Approximate value.

more lipophilic than ETH-615 and, thus, should have resulted in dermal delivery comparable to that of compound **2**. However, compound **4** was unable to deliver ETH-615 through the skin, probably due to very low solubility in the donor phase (i.e., in propylene glycol).

In conclusion, masking of the anionic moiety of the zwitterionic drug ETH-615 with a pro-moiety did not enhance the aqueous solubility of the drug and had only a minor effect on the solubility of the drug in propylene glycol. However, masking the anion and increasing the lipophilicity of the drug resulted in a 10-fold increase in the dermal delivery of the drug. Furthermore, masking the anion increases the chemical stability of the drug under nonenzymatic conditions.

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