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Optimization of Topical Erythromycin Formulations by Ion Pairing

Key Words
Acne vulgaris
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Ion pair
Partition coefficient

Abstract
Erythromycin (ERY) is used in the topical treatment of acne vulgaris. In order to decrease the amount of microorganisms markedly, the antibiotic must penetrate into the sebaceous follicles. Firstly, the aim of this study was to improve the lipophilicity of ERY by ion pairing. Secondly, a formulation with optimized penetration of the ion pair was developed. Thirdly, the optimized formulation was compared with formulations containing ethanol and with the commercial product Zineryt®. The determination of lipophilicity was based on partition coefficients (PC) and on the penetration of ERY into a modified multilayer membrane system (MMS). It was shwon that the penetration of ERY into a lipophilic acceptor system was three times higher when ion pairing between ERY and octadecansulfonate was used in comparison with the penetration of the ERY base alone. The dosage of the antibiotic used can be markedly reduced by optimizing a vehicle for the ion pair.

Introduction

In addition to increased sebum secretion, the unstable state of the sexual hormones during puberty and follicular hyperkeratosis, infection of sebaceous follicles by microorganisms, mostly *Propionibacterium acnes,* is an important part of the pathomechanism of acne vulgaris [1–4]. In recent years, concerns about possible side effects of systemic antibiotics have led to the use of topical antibiotics for the treatment of acne vulgaris. ERY is one among the various topical antibiotics that have been studied and shown to be effective [5–8]. The main problem with topical ERY is its insufficient penetration into the different

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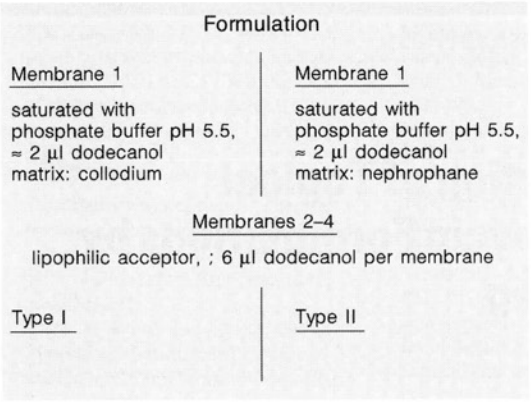

Fig. 1. Modified multilayer membrane system types I and II.

skin layers and also into the lipids of the sebaceous follicles. This is a consequence of the hydrophilic properties of ERY. Therefore, the aims of this study were:

- to increase the lipophilicity of ERY using ion pairing with suitable counter-ions;
- to find a suitable vehicle for the ion pair using a modified multilayer membrane system (MMS), and
- to compare the results obtained with formulations containing ethanol as well as the commercial product Zineryt®.

Materials and Methods

Materials

The substances were obtained from the following sources: erythromycin (Synopharm GmbH, Germany), collodium, macrogolstearate (Caesar & Loretz GmbH, Germany), glycerol, sodium sulfonates, propylene glycol (Fluka Chemie AG, Germany), octanol, dodecanol (E. Merck, Germany), buffer substances (Chemische Werke Berlin, Germany), nephrophane membranes (Filmfabrik Wolfen, Germany).

Methods

Analytical. ERY was determined with a method described by Dabrowska [9]; 0.2 ml of a 0.1 *N* NaOH solution and 3 ml chloroform were added to the respective membrane and shaken for 30 min. The chloroform phase was added to a mixture of 3 ml phosphate buffer (pH = 5.3) and 3 ml bromcresol purple (BCP) solution (0.1727 g BCP + 4 ml 0.1 *N* NaOH + 196 ml water) and again shaken for 30 min. After this, the chloroform phase was completely separated from the BCP solution and moderately shaken with 2 ml of a 0.1 *N* NaOH solution. The UV absorbance of the NaOH phase was measured at 590 nm using an UV-120-02 spectrophotometer (Shimadzu, Germany).

Synthesis of the Ion Pairs. 50 mg ERY and the equivalent amount of the sodium salt of the counter-ion were dissolved in 50 ml phosphate buffer (pH = 5.5). The solutions were mixed and 20 ml dichloromethane were added. The mixture was shaken for 20 min and the phases separated. The dichloromethane phase was washed with phosphate buffer (pH = 5.5), dried with Na_2SO_4, filtrated and evaporated. The ion pairs were characterized by different analytical methods [10].

Determination of the Partition Coefficients (PC). A defined amount of ERY (0.3–0.5 mmol/ml) in the phosphate buffer (saturated with n-octanol) was mixed with a solution of the counter-ion in phosphate buffer. A defined volume of n-octanol (saturated with phosphate buffer) was added and the mixture was shaken for 3 h at 32 °C. The amount of ERY in the buffer was determined as described above. The PC values were calculated according to Martin et al. [11]. In all cases, the sodium salts of the counter-ions were used.

Penetration Studies: MMS. The model system used has been described previously [12]. A four-layer membrane system with dodecanol as lipid, but with a first membrane different composition was used. By saturating it with phosphate buffer at pH 5.5, the first membrane was acidic, simulating the acid layer of the skin. The dodecanol content of the first membranes was two thirds less than that of membranes 2–4. Membranes 2–4 had a lipid content of about 6 µl dodecanol per membrane [13]. Therefore, 2–4 represented the lipophilic acceptor, simulating a lipid target such as the sebaceous follicle. To study the penetration of formulations containing ethanol, membranes were used with collodium (MMS, type I) and nephrophane (MMS, type II) as a matrix (fig. 1). Identical ERY concentrations were used for the base and the ion pair: 2.0% ERY are equivalent to 2.8% ERY/octadecansulfonate (OS) and 4.67% ERY are equivalent to 6.54% ERY/OS. Penetration profiles (amount of penetrating ERY per time) and the area under the concentration-time curve (AUC) were used to evaluate penetration.

Table 1. PC of ERY and ERY in combination with different counter ions in the system n-octanol/phosphate buffer at different pH values (mean ± SD, n = 8)

Counter-ion	PC between n-octanol/phosphate buffer		
	pH 6.0	pH 6.8	pH 7.4
Azelainate	<0.01	4.7±0.8	5.3±0.3
Dehydrocholate	0.3±0.1	4.5±0.2	10.9±0.2
Desoxycholate	5.4±0.7	9.2±0.3	10.1±0.5
Dodecanoate	<0.01	4.2±0.8	5.9±0.2
Dodecylsulfate	20.6±1.7	21.7±1.1	20.1±0.7
Hexylsalicylate	6.2±0.7	10.5±0.3	10.5±0.4
Octadecansulfonate	28.5±2.1	29.1±3.3	27.1±2.5
Octanoate	4.9±0.1	7.5±1.1	15.1±0.2
Erythromycin	0.2±0.2	2.9±1.2	5.5±0.4

Results

Increasing the Lipophilicity of ERY by Ion Pairing

The lipophilicity of ERY with and without counter-ions was characterized measuring the PC in the system n-octanol/phosphate buffer. Counter-ions which are able to increase the lipophilicity of ERY were identified by the determination of PCs of ERY, and of ERY in combination with different counter-ions in the system n-octanol/phosphate buffer. As shown in table 1, dodecylsulfate (DS), OS and hexylsalicylate (HSS) are able to increase the lipophilicity of ERY markedly. No increase in the PC was observed when the amount of counter-ion was increased. To study the influence of different counter-ions on the penetration of 1% formulations of ERY into a lipophilic acceptor, MMS type I was used. The vehicle consisted of 15% macrogol stearate (MS), 10% propylene glycol (PG) and 75% glycerol (GL). Table 2 shows the amount of ERY penetrating into the lipophilic acceptor of membranes 2–4. When the ion pairs ERY/OS, ERY/HSS and ERY/DS were used, the amount of ERY penetrating into the lipophilic acceptor may be twice as large as that with the ERY base.

Table 2. Penetration of ERY and ERY with different counter-ions into the MMS (mean ± SD, n = 8, t = 60 min)

Counter-ion	ERY penetrating in membranes 2–4, %
Octansulfonate	9.3±2.5
Dodecansulfonate	14.7±1.7
Tetradecansulfonate	21.2±1.9
Octadecansulfonate	22.5±4.4
Hexylsalicylate	17.2±3.4
Dodecylsufate	25.4±5.8
Dehydrocholate	8.9±1.9
Erythromycin	11.1±2.8

Furthermore, it can be seen that the aliphatic sulfonates enhance the penetration of ERY into the lipophilic acceptor. The penetrating amount increased with increasing alkyl chain length.

Search for a Suitable Vehicle for the Ion Pair

Next, a vehicle for the ion pair was optimized as concerns the requirements for an ERY formulation for the external treatment of acne vulgaris. ERY is not stable in aqueous

Table 3. Comparison of the penetration of the ERY base and ERY/OS from different formulations into the lipophilic acceptor of MMS type I (mean ± SD, n = 8, t = 60 min)

Formulation	ERY penetrating in membranes 2–4, %	
	2.8% ERY/OS	2.0% ERY
15% MS 10% PG 75% GL	20.6 ± 1.4	7.3 ± 0.8
15% MS 10% PG 3% ZnO 72% GL	16.4 ± 2.1	10.9 ± 1.6
15% MS 10% PG 10% ZnO 65% GL	17.1 ± 0.9	7.9 ± 1.2
10% PG 10% HG 80% GL	17.2 ± 3.9	12.8 ± 3.8
15% CS 10% PG 10% HG 65% GL	26.6 ± 3.6	15.3 ± 1.0

PG = Propylene glycol; GL = glycerol; MS = macrogol stearate; HG = hexylene glycol; CS = cetylstearyl alcohol.

solution [14, 15]. Some products for external use are stable for only a short time or because they contain a high percentage of alcohol, which is not at all suitable for the treatment of acne vulgaris [16]. Therefore, an MS-based vehicle was developed. First, the ratio of PG and GL was varied. The penetration of the ion pair ERY/OS and the ERY base into the lipophilic acceptor of the MMS are compared in figure 2.

To improve the galenic acceptance of the topical formulation, 3 or 10% zinc oxide (ZnO) was added. As shown in table 3, there was no significant change in the penetration of the ion pair. Formulations 4 and 5 shown in table 3 have very good penetration properties. Nevertheless, these formulations are not acceptable for the treatment of acne vulgaris for galenic reasons.

Comparison with Ethanol-Containing Solutions

Collodium, used as a matrix in MMS, is soluble in ethanol. Therefore, studies with a modified MMS (type II) were carried out. Firstly, the penetration of ERY from Zineryt (4.67% ERY) was compared with the penetration of ERY from a 6.54% solution of the ion pair ERY/OS in the solvent of Zineryt. Because of the insufficient stability of ERY, Zineryt consists of two parts. One part consists of ERY together with zinc acetate, and the other part is the solvent of Zineryt containing ethanol and 45% diisopropyl sebacate. The penetration of ERY from Zineryt and of ERY/OS from the solvent of Zineryt is shown in figure 3. The penetration of ERY is enhanced when the ion pair ERY/OS is used. As shown in table 4, the same amount of ERY penetrates from Zineryt (4.67% ERY) as from the 2.8% formulation of ERY/OS in the solvent of Zineryt.

Secondly, the penetration of ERY (optimized vehicles) into MMS type I and type II was also studied and compared with the penetration from the formulations shown in figure 3. Using 15% MS, 10% PG, 75% GL or 15% MS, 10% PG, 3% ZnO, 72% GL as vehicles with 2.0% ERY, the same amount of ERY penetrated into the lipophilic acceptor as from Zineryt (see table 4). When the 2.8% formulation of the ion pair ERY/OS was applied, the penetration of ERY was double.

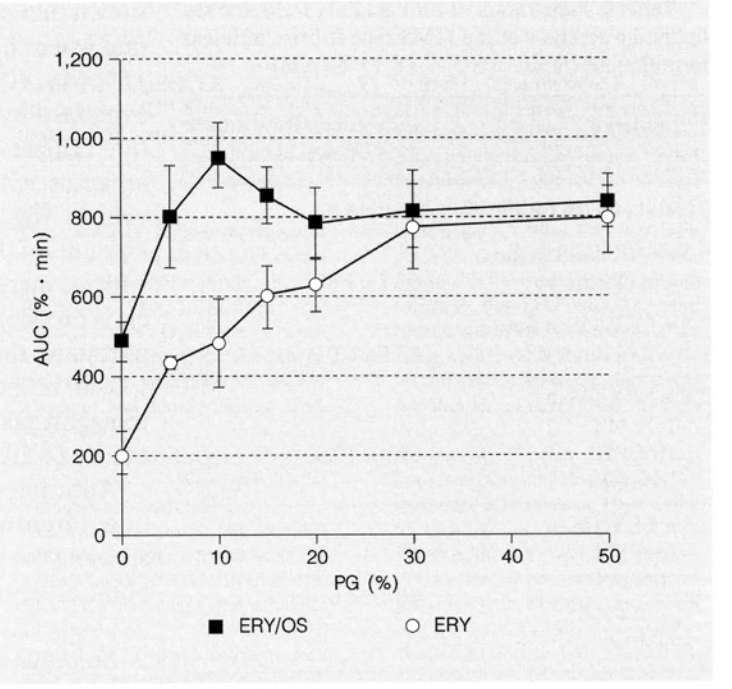

Fig. 2. Penetration of ERY and ERY/OS into membranes 2–4 of MMS type I depending on the content of PG, vehicle: 15% MS, x% PG, (85–x)% GL (mean ± SD, n = 6).

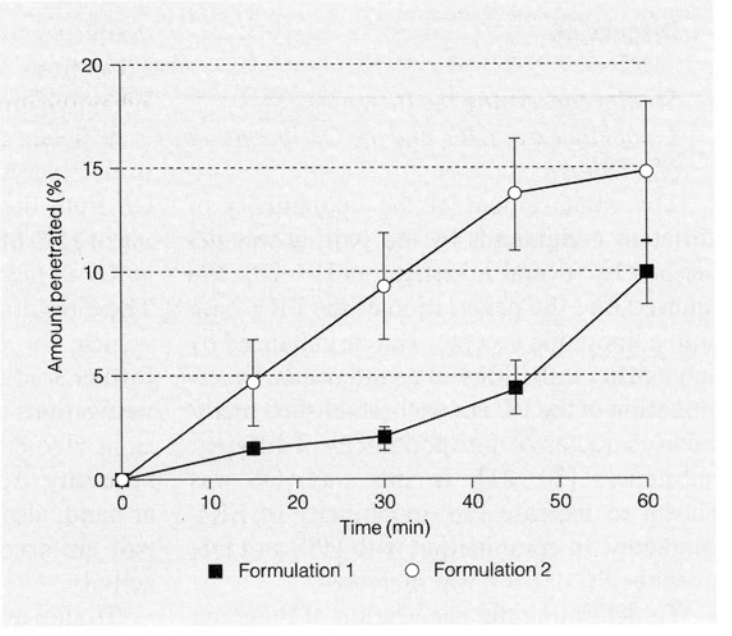

Fig. 3. Penetration of ERY from Zineryt (4.67% ERY) (formulation 1) and from ERY/OS, 6.54% in the solvent/Zineryt (formulation 2) into the lipophilic acceptor of MMS type II (mean ± SD, n = 6).

Table 4. Penetration of ERY and ERY/OS into the lipophilic acceptor of the MMS type II from different formulations (mean ± SD, n = 8, t = 60 min)

Formulation	ERY penetrating into membranes 2–4, µg
Zineryt (4.67% ERY)	36.9 ± 9.0
6.54% ERY/OS in the solvent of Zineryt	63.7 ± 9.6
2.8% ERY/OS in the solvent of Zineryt	32.7 ± 6.0
2.8% ERY/OS in 15% MS 10% PG 75% GL	64.8 ± 10.0
2.8% ERY/OS in 15% MS 10% PG 3% ZnO 72% GL	61.4 ± 4.0

Discussion

Studies concerning the Increase of the Lipophilicity of ERY and the Optimization of a Vehicle

The enhancement of the lipophilicity of different compounds by ion pairing was described by several investigators [17–20]. We showed that the penetration of the ERY base into a lipophilic acceptor can be enhanced by ion pairing with different counter-ions. Determination of the PC is a well-established method to characterize the lipophilicity of different substances [20, 21]. In this way, OS was shown to increase the lipophilicity of ERY markedly. In combination with HSS and DS, even the PC of ERY was increased.

To determine the penetration of these ion pairs, a modified MMS was used with an acid membrane layer on the top and lipophilic layers below. The penetration studies have shown that penetration of this ion pair is optimal when the vehicle is composed as follows: 15% MS, 10% PG and 75% GL. This can be explained by the low solubility of ERY/OS in the vehicle and the high solubility in the acceptor with lower amounts of PG in the vehicle (fig. 2). In contrast, the increasing amount of ERY in the lipophilic acceptor with an increasing amount of PG in the vehicle is probably caused by the convective transport into the acceptor system of ERY dissolved in PG [10] (fig. 2). This convective transport takes place due to the enhanced solubility of ERY in the vehicle effected by PG.

Addition of 3 or 10% ZnO to the formulation improved the acceptance without decreasing the penetration of ERY into the lipophilic acceptor system.

Studies concerning the Comparison with Formulations Containing Ethanol

First, it was shown that the penetration of 6.54% ERY/OS in the solvent of Zineryt is doubled compared with the penetration of ERY from Zineryt (4.67% ERY). A 2.8% ERY/OS formulation in the solvent of Zineryt is sufficient to reach the same penetration as from Zineryt. The penetration of 2.8% ERY/OS from the optimized formulation consisting of 15% MS, 10% PG and 75% GL is about twice as high as that of ERY from Zineryt. These results underline the importance of the vehicle for drug penetration. Nevertheless, further studies, most importantly in vitro investigations using excised human skin as well as in vivo experiments on acne patients, are necessary to confirm these results. On the other hand, also microbial studies using the ion pair are necessary to prove its antimicrobial activity.

To sum up, using a suitable ion pair and an optimized vehicle, the dosage of the antibiotic applied in the treatment of acne vulgaris can be reduced markedly.

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