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INTERACTION OF ANTISEPTIC COMPOUNDS WITH INTERCELLULAR LIPIDS OF STRATUM CORNEUM

H. Aki

Department of Pharmaceutics, Faculty of Pharmaceutical Sciences, Fukuoka University, 8-19-1 Nanakuma, Jonan-ku, Fukuoka 814-0180, Japan

Abstract

The interaction between three kinds of antiseptic compounds and components of intercellular lipids in the stratum corneum was characterized in terms of thermodynamics at pH 7.5 and 25°C, and the different mechanisms used to penetrate the stratum corneum were clarified. Anionic surfactants such as benzalkonium chloride and benzethonium chloride mainly bound to cholesterol (CH) and cholesterol sulfate with high affinity (10^5– 10^6 M^{-1}) to extract endogenous CH from the stratum corneum and penetrated through the intercellular route. Chlorhexidine gluconate also bound to CH and accumulated in the stratum corneum without removing endogenous CH. An amphoteric surfactant of dodecyldiaminoethylglycine hydrochloride seemed to be incorporated into the lipid bilayer and bound to ceramide with its polar end close to the lipid polar heads by hydrophobic interaction.

Keywords: benzalkonium chloride, benzetonium chloride, chlorhexidine gluconate, dodecyldiaminoethylglycine hydrochloride, microcalorimetry, stratum corneum, thermodynamics

Introduction

Stratum corneum, the outermost layer of skin, is the primary barrier to percutaneous penetration. The stratum corneum is a multilayered wall-like structure in which keratin-rich corneocytes are embedded in an intercellular lipid-rich matrix [1]. There are two possible pathways of drug permeation through the intact stratum corneum: intercellular and transcellular routes. The diffusional pathway through the stratum corneum is the intercellular route, and mainly involves three fractions, ceramide, fatty acids, and cholesterol (CH) and its derivatives. Ceramide is N-acyl-linked sphingolipids and has been suggested to have a central role in the barrier function of the stratum corneum [2]. Cholesterol, another major component of the stratum corneum, is required for homeostasis of the permeability barrier and ordering of the bilayer array [3].

Previously, the dorsal skin of rats and hairless mice was treated with a solution of ^{14}C-benzyldimethyldodecylammonium chloride (^{14}C-BKC). By whole body and microautoradiography, heavy deposits of ^{14}C-BKC at the surface of the skin and penetration into the epidermis and dermis at 24 h after the application, were observed [4]. BKC is a cationic surfactant that is widely used as a topical antiseptic agent. Even be-

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low the usual concentration of 0.1%, skin irritation and associated inflammatory responses are often induced by its application. The interaction between benzalkonium chloride (BKC) and the components of stratum corneum, in a model of intercellular lipids in human stratum corneum and homogenized rat stratum corneum, has been studied by titration microcalorimetry at pH 7.5 and 37°C [5]. BKC was strongly bound to CH and its derivatives, which were most likely extracted from the stratum corneum with increasing concentrations of BKC. It was suggested that BKC penetrated the stratum corneum by disrupting the structure of the lipid bilayer through extraction of CH. In this study, the binding of other antiseptic compounds, a cationic surfactant of benzethonium chloride (BZC), an amphoteric surfactant of dodecyldiaminoethylglycine hydrochloride (AEG), and chlorhexididine gluconate (CHG), to the components of intercellular lipids in the stratum corneum was investigated in order to clarify the mechanisms of interaction. These compounds are all quaternary ammonium compounds and show a disinfecting and cleaning action by causing a decrease in the surface tension and protein denaturation of the bacterial cell membrane.

Experimental

Materials

BKC, BZC and CHG were purchased from Sigma Chemical (St. Louis, MO, USA) and AEZ from Nissan Chemicals Co. (Tokyo, Japan). Ceramide, cholesterol monohydrate (CH), cholesterol 3-sulfate (CHsulf), cholesterol palmitate (CHpal), and palmitic acid were obtained from Sigma Chemical and used without further purification. All other chemicals were analytical grade.

Isothermal titration microcalorimetry

Microcalorimetric experiments were performed with a Thermal Activity Monitor 2277 system (Järfälla, Sweden) at 298.15 K. The reaction cell was initially filled with a 3.0 mL of a suspension or solution of one of the components of the stratum corneum in Tris buffer (pH 7.5). Heat flow was measured by injecting each antiseptic compound in 15–20 portions of 15 μL into the cell. The dilution heat of each compound was measured separately using Tris buffer as a titrand and subtracted from the reaction heat. A calorimetric titration curve was obtained by plotting the values of the heat of binding *vs.* the total concentration of the compounds added, from which the best fit values of binding affinity (K) and enthalpy change (ΔH) were calculated simultaneously by computer [6].

Solubility determination of CH in aqueous solutions of antiseptic compounds

The equilibrium CH solubility was determined by adding an excess of CH to the Tris buffer (pH 7.5) solutions of 0.001–1.0 *w/v*% BKC, BZC, AEZ or CHG and agitating continuously for 24 h at 37°C. The samples were passed through a 0.45 μm filter (Toyo Co. Ltd., Japan) to analyze CH by the HPLC method described by Duncan *et al.* [7].

Extraction of CH from stratum corneum by antiseptic compounds

Sheets of stratum corneum were prepared by treating the dorsal skin of hairless rats with 0.5% crude trypsin (Type II, Sigma) in pH 7.5 phosphate-buffered saline for 1.5 h at 37°C [8]. At the end of the incubation period, the epidermis was separated from the dermis. After incubation at room temperature for 6 h, the stratum corneum was peeled away from the epidermis. The isolated stratum corneum was rinsed in distilled water; dried by vacuum desiccation (10^{-4} torr) for 24 h at room temperature; and powdered finely before use. About 3 mg of the stratum corneum was suspended in 2 mL of an aqueous solution containing each antiseptic compound. Methanol and aqueous solutions were used as controls. The samples were shaken violently for 15 min and centrifuged at 1.000 g for 10 min. Then the supernatant was treated as described above. For each experiment, the samples and controls were taken from the same skin. Each set of experiments was performed in seven rats.

Results and discussion

Interaction between antiseptic compounds and a model of intercellular lipids in human stratum corneum

Figure 1 shows the calorimetric titration curves for the binding of BZC (a), AEZ (b) and CHG (c) to the lipid mixtures as a model of the intercellular lipids in human stratum corneum and to the lipid mixtures without one of each component. The lipid composition of the system was ceramide (46.2% in mass/mass), CH (30.2%), CHpal (11.2%), CHsulf (2.2%) and palmitic acid (10.2%) [9]. Palmitic acid was essential in forming suspensions in the lipid mixtures. The heat of binding for all compounds de-

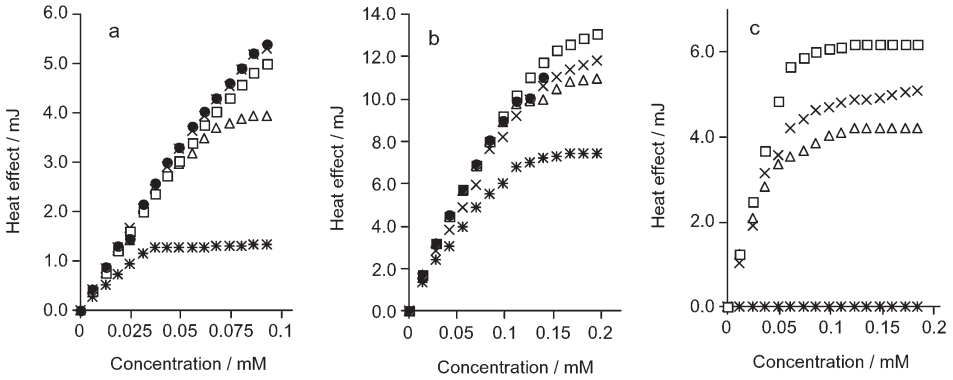

Fig. 1 Heat effect for the interaction of BZC (a), AEZ (b) and CHG (c) with model systems of intercellular lipids in human stratum corneum in a pH 7.5 Tris buffer solution at 25°C. ● – a model system of intercellular lipids; ■ – the model without ceramide; Δ – the model without CHsulf; * – the model without CH; × – the model without CHpal

creased significantly only in the absence of CH. In the case of CHG, the heat of binding decreased in the absence of CH and increased in the absence of ceramide. It was indicated that CH played an important role in the interaction between the antiseptic compounds and stratum corneum lipids.

Binding of antiseptic compounds to the components of intercellular lipids in stratum corneum

The heat of binding for BZC, AEG and CHG with ceramide, CH and CHsulf was examined at pH 7.5 and 25°C (Fig. 2). The initial concentrations of BZC, AEZ and CHG were 1.23 mM (0.055%), 2.79 mM (0.10%) and 2.45 mM (0.20%), respectively, and the reacting concentrations were lower than the critical micelle concentration (c.m.c). The concentrations of ceramide, CH, and Chsulf were 0.1–0.3 mM, 0.1~0.3 mM, and 0.05 mM, respectively. The binding affinity (K) and thermodynamic parameters (ΔG, ΔH, ΔS) of the compounds are summarized in Table 1 together with those of BKC, benzyldimethyldodecylammonium chloride (BKC12), benzyldimethylhexadecylammonium chloride (BKC14) and benzyldimethyltetradecyl-ammonium chloride (BKC16).

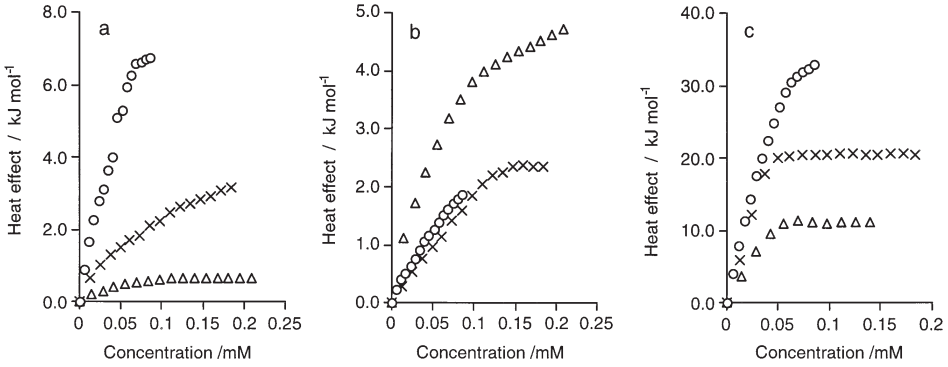

Fig. 2 Binding of BZC, AEZ and CHG to a – ceramide, b – cholesterol and c – cholesterol sulfate in pH 7.5 Tris buffer solution at 25°C o – BZC; Δ – AEZ, and × – CHG

The binding affinity of the compounds to ceramide increased in the order of CHG ($1.46 \cdot 10^4$ M^{-1})<BZC ($3.47 \cdot 10^4$ M^{-1})<AEZ ($9.25 \cdot 10^4$ M^{-1}). Those of BKC increased with the increasing alkyl chain-length of BKC. The binding of the compounds to ceramide was characterized by a small negative ΔH value and positive ΔS, reflecting hydrophobic interaction. Although binding to CH was also induced by hydrophobic interaction, K values conversely increased in the order of AEZ ($2.23 \cdot 10^4$ M^{-1})<BZC ($6.95 \cdot 10^4$ M^{-1})<CHG ($1.35 \cdot 10^5$ M^{-1}). The binding of BKC and BZC to CHsulf was characterized by large negative ΔH and ΔS values and the K val-

ues of BKC decreased as the alkyl chain-length of BKC increased, indicating that the binding was induced by ionic interaction.

Table 1 Binding affinities and thermodynamic parameters for antiseptic compounds binding to components of intercellular lipids in stratum corneum at pH 7.5 and 25°C

	$K/10^3$ M^{-1}	$-\Delta G$/kJ mol^{-1}	$-\Delta H$/kJ mol^{-1}	ΔS/J mol^{-1} K^{-1}
Ceramide				
BKC12	5.20±0.39	21.2	16.3	16.6
BKC14	54.1±9.0	27.0	15.1	39.9
BKC16	75.4±4.4	27.8	15.5	41.5
BZC	34.7±1.4	25.9	12.4	45.2
AEZ	92.5±6.5	28.3	1.54	89.9
CHG	14.6±1.4	23.8	4.43	64.8
Cholesterol				
BKC12	61.7±7.8	27.3	11.5	53.2
BKC14	192.8±19.1	30.2	16.7	45.1
BKC16	479.7±14.3	32.4	24.0	28.3
BZC	69.5±2.3	27.6	2.80	83.3
AEZ	22.3±1.9	24.8	4.98	66.5
CHG	135.4±12.0	29.3	2.74	89.1
Cholesterol sulfate				
BKC12	93.0±11.4	28.4	52.2	–80.1
BKC14	49.4±7.9	26.8	55.3	–95.7
BKC16	8.42±1.76	22.4	46.6	–81.2
BZC	38.3±5.7	26.2	45.9	–66.3
AEZ	37.7±4.4	26.1	12.4	46.0
CHG	33.9±2.2	25.8	22.5	11.2

Dissolution behavior of stratum corneum CH in antiseptic solutions

The solubility of CH in aqueous solutions of the antiseptic compounds is shown in Fig. 3. The solubility increased sharply from near the c.m.c. values of BKC, BZC and AEZ. CH, however, was quite insoluble in the CHG solution. In the suspension of homogenized stratum corneum, the concentration of total CH also increased with the concentrations of BKC, BZC and AEZ. Figure 4 shows the relative extent of endogenous CH extracted from the stratum corneum of hairless rats by the antiseptic compounds. Results were expressed as a percentage of the total amount of CH extracted from stratum corneum by methanol. The concentrations of antiseptic compounds employed in this experiment were typical of those used for the disinfection of hands. In stratum corneum treated with 0.1% BKC and BZC, more than 50% of the endogenous

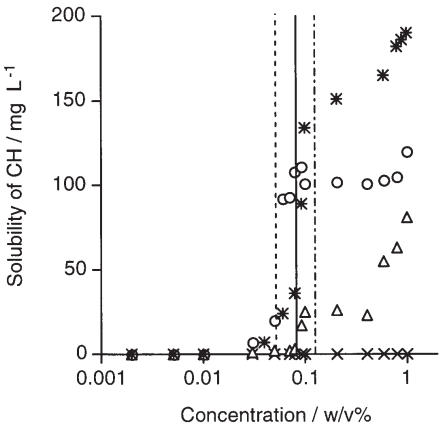

Fig. 3 Solubility of CH in antiseptic solutions. * – BKC12; o – BZC; Δ – AEZ, and × – CHG; — – c.m.c. of BKC12; - - - – c.m.c. of BZC, and -·-·- – c.m.c. of AEZ

CH was extracted, suggesting that the cationic surfactants increase the permeability of the skin. In fact, BKC showed enhanced activity for percutaneous absorption of enoxacin compared to an anionic surfactant of sodium laurylsulfate and a non-ionic surfactant of Polysorbate 80 [10].

These results indicated that only cationic surfactants such as BKC and BZC were effective as solubility enhancers for CH. It seemed that at concentrations above the c.m.c of BKC and BZC, the interaction between the cationic surfactant and endogenous CH resulted in CH being incorporated into the micelle of the cationic surfactant.

Mechanism of the interaction between antiseptic compounds and intercellular lipids in stratum corneum

In this study, the thermodynamics of the binding of antiseptic compounds to the intercellular lipids of stratum corneum were investigated. Cationic surfactants, BKC and BZC, mainly interacted with CH and CHsulf in patches of the ordered lipid structure, and probably penetrated the stratum corneum by extracting CH and disrupting the lipid bilayer at concentrations above c.m.c (Fig. 4). CHG bound to CH with higher affinity (10^6 M^{-1}) by hydrophobic interaction than any other component of the stratum corneum (Table 1). However, the mechanism of the interaction between CHG and the intercellular lipids was significantly different from that of BKC and BZC. CH was practically insoluble in the CHG solution (0.2 mg in 100 mL of CHG). Even when the concentration of CHG increased, the solubility of CH hardly changed (Fig. 3). Thus, it seemed that CHG bound to endogenous CH strongly was adsorbed into the stratum corneum without the removal of CH.

The amphoteric surfactant of AEZ most strongly bound to ceramide by hydrophobic interaction among the antiseptic compounds used in this study. AEZ pos-

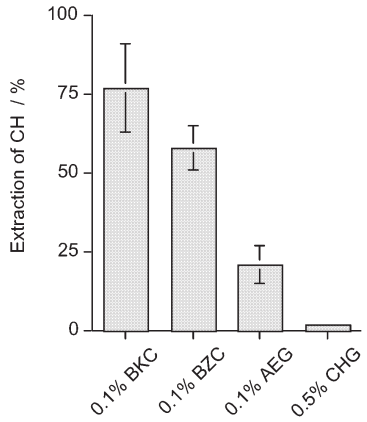

Fig. 4 Relative extent of endogenous CH extracted from stratum corneum of hairless rats by antiseptic compounds. Results were expressed as a percentage in comparison with the total CH extracted from stratum corneum by methanol. Each column represents the mean ±SD of seven experiments

sesses an aliphatic carbon chain (C12) plus a diaminoethylglycine group and is completely different in chemical structure from BKC, BZC and CHG. Thus, AEZ was probably incorporated into the lipid bilayer and bound to ceramide with its polar end close to the lipid polar heads. Further study is needed to elucidate whether AEZ disrupts and increases the fluidity of the lipid region.

Conclusions

Isothermal titration microcalorimetry was employed to investigate the interactions between three kinds of antiseptic compounds and the components of intercellular lipids in the stratum corneum. From the binding and thermodynamic characterization, different mechanisms were found: cationic surfactants such as BKC and BZC were bound to CH and CHsulf by hydrophobic and ionic interaction, respectively, and most likely extracted CH and its derivatives from the stratum corneum above the c.m.c.; an amphoteric surfactant AEZ was incorporated into the lipid bilayer and bound to ceramide with high affinity (10^6 M^{-1}) by hydrophobic interaction; CHG was bound to only CH and accumulated in the stratum corneum.

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