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Effect of Several Electrolyzed Waters on the Skin Permeation of Lidocaine, Benzoic Acid, and Isosorbide Mononitrate

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The effects of several electrolyzed waters were evaluated on the permeation of model base, acid and non-ionized compounds, lidocaine (LC), benzoic acid (BA), and isosorbide mononitrate (ISMN), respectively, through excised hairless rat skin. Strong alkaline-electrolyzed reducing water (ERW) enhanced and suppressed the skin permeation of LC and BA, respectively, and it also increased the skin permeation of ISMN, a non-ionized compound. On the contrary, strong acidic electrolyzed oxidizing water (EOW) enhanced BA permeation, whereas suppressing LC permeation. Only a marginal effect was observed on the skin permeation of ISMN by EOW. These marked enhancing effects of ERW on the skin permeation of LC and ISMN were explained by pH partition hypothesis as well as a decrease in skin impedance. The present results strongly support that electrolyzed waters, ERW and EOW, can be used as a new vehicle in topical pharmaceuticals or cosmetics to modify the skin permeation of drugs without severe skin damage.

Keywords electrolyzed water; alkaline reducing water; acidic oxidizing water; skin permeation; penetration enhancement

INTRODUCTION

Electrolyzed water is obtained by the electrolysis of water with and without a little salt. The typical electrolyzed waters presently available are summarized in Table 1 (The scientific board of functional water foundation, 2001). These electrolyzed waters show different physicochemical properties and functions depending upon the kind of salt (ingredient) and its quantity to be added, the structure of the electrolysis chamber, electrolysis conditions, and so on. The obtained electrolyzed waters are divided into alkaline electrolyzed reducing water (ERW) (strong alkaline-ERW, alkaline ion water) mainly used for washing materials or improving several pathological conditions in humans, and acidic electrolyzed oxidizing water (EOW) (strong acidic EOW, slightly acidic electrolyzed water, acidic ion water), which mainly has sterilization and astringent effects.

Two kinds of EOW have already obtained pharmaceutical affairs law approval in Japan; the proposed efficacy is washing disinfection for fingers or endoscopes in gastrointestinal inspection. On the contrary, some ERW have been found to have an emulsification effect (Konomatsu, Sugibayashi, Sugibayashi, & Ishii, 2003) of oil and and anti-oxidization effect (Lee, Kim, Ryoo, Lee, & Park, 2006; Nara et al., 2001). Moreover, Shirahata et al. (1997) reported the elimination ability of active oxygen by active atomic hydrogen hypothesis. However, Hiraoka et al. (2004) reported that the antioxidant activities were derived from molecular hydrogen and/or reductive vanadium ion. Thus, it is necessary to further consider these actions in detail.

There are three types of electrolysis chamber systems to make electrolyzed water: non-diaphragm-type one-chamber system or a two- or three-chamber system with a diaphragm (Table 1). For example, a middle cell chamber exists between anodal and cathodal cell chambers for three-cell-type electrolysis, where each chamber is separated by a diaphragm. Ultrapure water, pure water, or tap water can be applied to anodal and cathodal cells, and the middle cell is filled with pure water or sodium chloride solution to start electrolysis. Several ions, such as Na^+, H^+, Cl^-, and OH^-, are electrophoresed to the cathodal or anodal cell from the middle cell, depending on the current. This three-cell-type electrolysis system is more suitable for reducing ion concentration in the produced electrolyzed water than in the two-cell-type electrolysis system.

The main electrode reactions during electrolysis with a diaphragm using sodium chloride solution are shown in Equations 1–5. In the anode, oxygen (O_2) and hydrogen (H^+) ions are generated from water (H_2O) (Equation 1), and chlorine (Cl_2) arises from chlorine ion (Cl^-) (Equation 2). The produced chlorine reacts with water to become hypochlorous acid (HClO) and hydrochloric acid (HCl) (Equation 3); therefore, the anode solution becomes acidified. In addition, the dissolved oxygen (DO) level increases. In contrast, hydrogen (H_2) and hydroxide ions (OH^-) are produced in the cathode (Equation 4). Moreover,

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TABLE 1
Typical Electrolyzed Water

	Electrolyte Cell							
	Diaphragm	Electrode	Ingredient	pH	ORP (mV)	Available Chlorine Concentration (ppm)	Application	Rp.[a]
Strongly alkaline electrolyzed reducing water	+	Cathode	NaCl	11–11.5	–900	<1	Cleaning, emulsification, antioxidation	2, 3
Alkaline ion water	+	Cathode	Calcium lactate	9–10	—	—	Pathological condition improvement	
Hypochlorous electrolyzed water	—	—	NaCl	8–9	—	80–100	Sterilization	
Strongly acidic electrolyzed oxidizing water	+	Anode	NaCl	2.2–2.7	1,100	20–60	Sterilization, deodorizing	4
Acidic ion water	+	Anode	Calcium lactate	4–6	—	—	Astringent	
Slightly acidic electrolyzed oxidizing water	—	—	2–6% HCl	5–6.5	800	10–30	Sterilization	
	—	—	NaCl	5–6	—	50–80	Sterilization	

[a]Classification of the electrolyzed water used in this study.

hydrogen peroxide (H_2O_2) and hydroxide ions (OH^-) are generated from oxygen and water (Equation 5), and H_2O_2 breaks down to hydroxide ions (Equation 6). As a result, the solution is alkalized in the cathode. The DO level decreases, whereas dissolved hydrogen (DH) increases (Equations 4 and 5). Thus, ERW and EOW are produced in the cathode and anode, respectively, by electrolysis of NaCl solution as follows:

Electrode reaction at anode

$$2H_2O \rightarrow O_2 + 4H^+ + 4e^- \quad (1)$$

$$2Cl^- \rightarrow Cl_2 + 2e^- \quad (2)$$

$$Cl_2 + H_2O \leftrightarrow HOCl + H^+ + Cl^- \quad (3)$$

Electrode reaction at cathode

$$2H_2O + 2e^- \rightarrow H_2 + 2OH^- \quad (4)$$

$$O_2 + 2H_2O + 2e^- \rightarrow H_2O_2 + 2HO^- \quad (5)$$

$$H_2O_2 + 2e^- \rightarrow 2OH^- \quad (6)$$

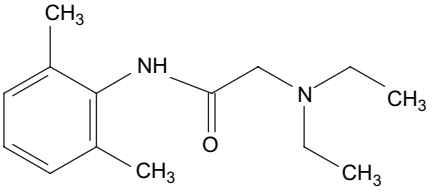
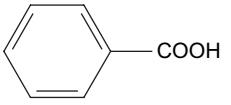
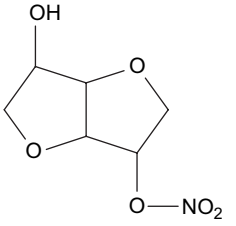
These electrolyzed waters are seldom studied for medical and pharmaceutical efficacy although they can be used as unique ingredients for external pharmaceutical formulations or cosmetics. Then, the purpose of this study was to investigate the effects of several electrolyzed waters on the skin permeation of model compounds. In this study, lidocaine (LC), benzoic acid (BA), and isosorbide mononitrate (ISMN) were selected as model base, acid, and non-ionized compounds, respectively, and the effects of electrolyzed waters were evaluated on the drug permeability through excised hairless rat skin. The structural formula, molecular weight (MW) and pK_a of the model drugs are summarized in Table 2. Moreover, the contribution of ionized and non-ionized fractions of the drugs was estimated for steady-state skin permeation by determining the permeability coefficient of each fraction. LC was used in this study. In addition, the influence of electrolyzed waters was evaluated on the skin barrier ability by measuring the amount of protein leakage and skin impedance during the application of electrolyzed water to the stratum corneum side and/or the dermis side of hairless rat skin. To evaluate the dermal irritation of these electrolyzed waters, MTT test and phospholipid leakage examination were carried out using excised hairless rat skin.

EXPERIMENTAL

Materials

LC and ISMN were purchased from Tokyo Kasei Industry Co., Ltd. (Tokyo, Japan). BA and NaCl were purchased from

TABLE 2
Chemical Structures and Physicochemical Properties of Model Compounds

Compound	Structure	MW	pK_a
Lidocaine (LC)		234.34	8.30
Benzoic acid (BA)		122.12	4.18
5-Isosorbide mononitrate (ISMN)		191.14	—

Wako Pure Chemical Industries, Ltd. (Osaka, Japan). Other reagents and solvents were of reagent or high-performance liquid chromatograph (HPLC) grade and used without further purification.

ERW and EOW were produced by a Redox Water Type 1S' Device (Water Design Co., Ltd., Tokyo, Japan) in our laboratory. The device is a three-chamber type with a diaphragm (see *Introduction*). In addition, purified water was obtained from a distilled water producer (STILL ACE SA-2000A, EYELA, Tokyo, Japan). NaCl was used as an electrolysis auxiliary agent. Furthermore, ERIC S-100, a strong alkaline ERW, was supplied by A. I. System Products (Kasugai, Aichi, Japan). Distilled water for injection (JP grade) was purchased from Otsuka Pharmaceutical Co., Ltd. (Tokyo, Japan).

Measurement of Physicochemical Properties of Electrolyzed Waters

Physicochemical properties of several electrolyzed waters, pH, oxidation–reduction potential (ORP), DO, electric conductivity (κ), osmolality (Π), and surface tension (γ) were measured. Concentrations of Na^+ and Cl^- were also measured in the electrolyzed waters. The concentration of H^+ or OH^- was estimated from pH of EOW or ERW, respectively, as ions that may affect the osmolality of electrolyzed waters. In these physicochemical property measurements, an ion and pH meter (IM-55G, DKK-TOA Corporation, Tokyo, Japan), a portable DO meter P series (DO-14P, DKK-TOA Corporation), an electric conductivity meter G series (CM-30G, DKK-TOA Corporation), an osmotic pressure Osmo Stat (OM-6040, Arkray, Inc., Kyoto, Japan), and a fully automatic surface tension meter (CBVP-Z, Kyowa Interface Science Co., Ltd., Niiza, Saitama, Japan) were used. Na^+ and Cl^- were measured by an atomic absorption spectrophotometer (AA-6500, Shimadzu Corporation, Kyoto, Japan) and ion chromatography analysis (HIC-6A, non-suppressor type, Shimadzu Corporation), respectively. The ion chromatographic system consisted of a pump (LC-9A), an electrical conductivity detector (CDD-6A), a chromatopac (C-R5A), a column oven (CTO-6AS), and an IC column (Shim-pack IC-AC, 4.6 mm i.d. × 100 mm, all from Shimadzu Corporation). The mobile phase was 1.0 mM *p*-hydroxybenzoic acid + 1.1 mM *N*,*N*-dimethylaminoethanol solution for Cl^- analysis. The flow rate was 1.0 mL/min and the column temperature was maintained at 40°C.

Animals

Male hairless rats (WBN/ILA-Ht) weighing 241 ± 12 g were obtained from the Life Science Research Center, Josai University (Sakado, Saitama, Japan). All animal experiments were performed under the guidelines of the Life Science Research Center, Josai University.

In Vitro Skin-Permeation Experiments

Abdominal skin was excised from hairless rats under pentobarbital anesthesia (50 mg/kg i.p.) after the hair was shaved. This fresh skin sample was immediately used after excision. The

skin was then set on the two-chamber diffusion cell (Okumura, Sugibayashi, Ogawa, & Morimoto, 1989) with an effective diffusion area of 0.95 cm^2. Several electrolyzed waters (2.5 mL) containing LC, BA, or ISMN at a concentration of 0.1%, and physiological saline (2.5 mL), were applied on the stratum corneum and dermis sides, respectively. Both the donor and the receiver solutions were stirred by a magnetic stirrer (Multi stirrer M-1, Iuchi, Osaka, Japan) at 500 rpm using a star head bar. Moreover, both cells were maintained at 32°C by warm water circulation to the water jacket of diffusion cells. The solution on the dermis side was periodically sampled to measure the skin permeation of drugs and the same volume of fresh saline was added to keep the receiver volume constant. All permeation experiments were conducted for 6 h. Various buffer solutions with different pH values (3.5, 6.3, 7.3, 8.3, 9.3, 10.3, and 12.2) were prepared using GTA wide-area buffer solution with the same molar of 3,3-dimethylglutaric acid, Tris (hydroxymethyl) aminomethane and 2-amino-2-methyl-1,3-propanediol. Buffer solutions (pH 11.4 and 12.1) were prepared using Na_2CO_3 or Na_2HPO_4–NaOH. 1M HCl or 1M NaOH was used to adjust the pH.

The permeability coefficient of ionized and unionized LC was estimated by evaluating the contribution of each fraction to the steady-state total flux. In this study, the permeability coefficients, P_i and P_u, of ionized and unionized LC were calculated, respectively, by curve-fitting the P versus pH profile using the nonlinear least-squares method (algorithm; quasi-Newton method) in the Solver function of Microsoft Excel software to Equation 7.

$$P = P_u\alpha + P_i(1-\alpha) = \frac{P_u}{1+10^{pK_a-pH}} P_i\left(1-\frac{1}{1+10^{pK_a-pH}}\right) \quad (7)$$

HPLC analysis

LC, BA, and ISMN concentrations in each sample solution were measured using HPLC. The HPLC system consisted of a pump (LC-10AD), UV detector (SPD-10A), integrator (C-R 3A), system controller (SCL-10AVP), autoinjector (SIL-10AXL), and column oven (CTO-10A) (all from Shimadzu Corporation). An ODS reverse-phase column (Wakopak Wakosil-II 5C18 HG, 4.6 mm i.d. × 250 mm, Wako Pure Chemical Industries, Ltd.) was used. The mobile phase was acetonitrile : 0.1% phosphoric acid solution containing 5 mM sodium 1-heptane sulfonate = 35 : 65 for LC, acetonitrile : 0.05 M glycine buffer solution (pH 2.5) = 50 : 50 for BA and acetonitrile : water=10:90 for ISMN. Their flow rates were adjusted to 1.0 mL/min. Detection was at UV 230, 230, and 220 nm for LC, BA, and ISMN, respectively. The column temperature was maintained at 40°C. Internal standard method was applied for the determination of LC and BA using ethyl *p*-hydroxybenzoate and butyl *p*-hydroxybenzoate, respectively, as an internal standard substance. Absolute calibration method was used for ISMN.

In Vitro Skin Impedance Measurement

Right and left abdominal skins were excised from hairless rats under sodium pentobarbital anesthesia after shaving the hair as in the permeation experiments. The excised skin was sandwiched between the two-chamber diffusion cell sets as in the permeation experiments. Electrolyzed water (2.5 mL) was applied on both sides of the cell, and stirred by a star head bar, while maintaining the whole set at 32°C. Skin impedance was measured periodically using an impedance meter (Sinusoidal, 10 Hz, Advance R&D Co., Ltd., Tokyo, Japan) with silver-silver chloride electrodes immersed in stratum corneum and dermis side cells.

In Vitro Protein Leaching Experiment from Skin

The excised hairless rat skin was set in the two-chamber diffusion cell. Electrolyzed water and physiological saline (2.5 mL each) were applied on the stratum corneum and dermis sides, respectively. Only the receiver side (stratum corneum side) was stirred by a star head bar, and the cells were maintained at 32°C. An aliquot of receiver solution (0.5 mL) was sampled, and the same volume of the fresh electrolyzed water was added 0, 3, and 9 h after beginning the experiment. The leaching protein in the sample solution was determined using a bicinchoninic acid (BCA) protein assay kit (Pierce Biotechnology, Inc., Rockford, IL, USA). Determination was carried out according to the "enhanced test tube protocol" (Brown, Jarvis, & Hyland, 1989; Smith et al., 1985).

MTT Assay

The stratum corneum was stripped from hairless rat skin with adhesive tape 20 times under anesthesia using sodium pentobarbital after shaving and wiping with saline. Purified water, ERW, S-100, EOW, 0.02 M NaOH, and 0.01 M HCl (200 μL each) with or without isotonization of NaCl impregnated into a sheet of plaster of 15 mm diameter for a patch test was applied to the abdominal skin. Physiological saline was used as a negative control. The plaster was wrapped with surgical tape to prevent removal from the abdominal skin and exposed for 24 h. The application site of the skin was excised under sodium pentobarbital anesthesia and excess fat was trimmed. The center of the excised skin was punched out to a diameter of 8 mm using a biopsy punch. The tissue sample was washed with saline, soaked with 2 mL of 0.333 mg/mL MTT solution, and incubated under 5% CO_2 at 37°C. The washed tissue was transferred to a test tube and 1 mL of 0.04 M HCl-isopropanol was added. The sample was left in a dark room at room temperature for 12 h and then formazan was extracted. The concentration of formazan was measured at 570 nm by a UV-visible spectrophotometer (UV-160A, Shimadzu Corporation).

In Vitro Phospholipid Leaching Experiments from Skin

The excised abdominal skin was set in a two-chamber diffusion cell. The electrolyzed water and physiological saline

(2.5 mL each) were applied on the stratum corneum and dermis sides, respectively. Only the receiver side (stratum corneum side) was stirred by a star head bar, and the cells were maintained at 32°C. Receiver solution (0.5 mL) was sampled and the same volume of fresh electrolyzed water was added 0, 3, and 9 h after beginning the experiment. The phospholipid in the extracted solution was determined by a phospholipid C-Test Wako kit (Wako Pure Chemical Industries, Ltd.).

Data Analysis

Tukey–Kramer test following non-repeated measures ANOVA was used for statistical analysis. Correlation was authorized by Pearson's correlation coefficient test. A level of probability of 0.01 or 0.05 was taken as the level of significance.

RESULTS

Table 3 shows the physicochemical properties of the electrolyzed waters used in this experiment. The purity of the purified water (Rp. 0) was compared with that of water for injection JP (Rp. 1); no difference was found. The pH of ERW (Rps. 2 and 3) and EOW (Rp. 4) were determined to be about 12.06 and 2.49, respectively. ORP of ERW (Rps. 2 and 3) and EOW (Rp. 4) were positive and negative values, respectively. Na^+ concentration was high for Rps. 2 and 3, whereas low for Rp. 4. In contrast, Cl^- concentration was high for Rp. 4 and low for Rps. 2 and 3. A good correlation was confirmed between electric conductivity (κ) or osmolality (Π) and Na^+ concentration for ERW (Rps. 2 and 3). Although the Π value for all electrolyzed waters mostly correlated with the sum of Na^+ and Cl^- concentration, H^+ and OH^- may also affect the Π value. H^+ and OH^- concentrations were calculated from the pH of each electrolyzed water, Rps. 2, 3, and 4; however, the sum of Na^+, Cl^-, H^+, and OH^- concentrations was not enough to explain the exact Π value of each electrolyzed water. Although the surface tension (γ) of Rps. 2 and 4 showed almost the same value as purified water (Rp. 0), a very low value was observed for Rp. 3. Unfortunately, the reason for this is unknown.

Figure 1A shows the time course of the cumulative amount of LC, a basic model compound that permeated through the excised hairless rat skin from several electrolyzed waters. The cumulative amount of LC over 6 h for ERW, Rps. 2 and 3 was 5.2- and 2.5-fold, respectively, compared with that for purified water (Rp. 0) used as a control. On the contrary, the cumulative amount of LC for Rp. 4 was 0.071-fold compared with that for Rp. 0.

Figure 1B shows the time course of the cumulative amount of BA, an acidic model compound that permeated through the excised rat skin from electrolyzed waters. The cumulative amount of BA over 6 h was 1.8-fold for EOW, Rp. 4, and 0.13- and 0.13-fold for Rps. 2 and 3 compared with that for Rp. 0.

Theoretically, the contribution of the unionized fraction was larger in skin permeation than that of the ionized fraction. In this study, ISMN, a unionized water-soluble drug, was also used in

TABLE 3
Physicochemical Properties of Electrolyzed Water

Rp.		pH	ORP (mV)	DO (mg/L)	κ (mS/m)	Na^+ (mEq/L)	Cl^- (mEq/L)	Π (mOsm)	H^+ or OH^- (mEq/L)	γ (mN/m)
0	Purified water	5.71	193.2	6.91	0.28	1.7×10^{-3}	3.2×10^{-2}	0	1.9×10^{-3}	70.8
1	Injection solvent	5.70	177.1	6.65	0.17	—	—	0	2.0×10^{-3}	70.6
2	Alkaline ERW	12.15	–1068	5.50	245	10	5.1×10^{-1}	20	8.3×10^{-1}	69.3
3	S-100	11.96	–216.6	7.56	722	86	8.2	113	5.3×10^{-1}	29.2
4	Acidic EOW	2.49	955.9	12.16	216	1.6×10^{-1}	5.3	10	3.2	65.6

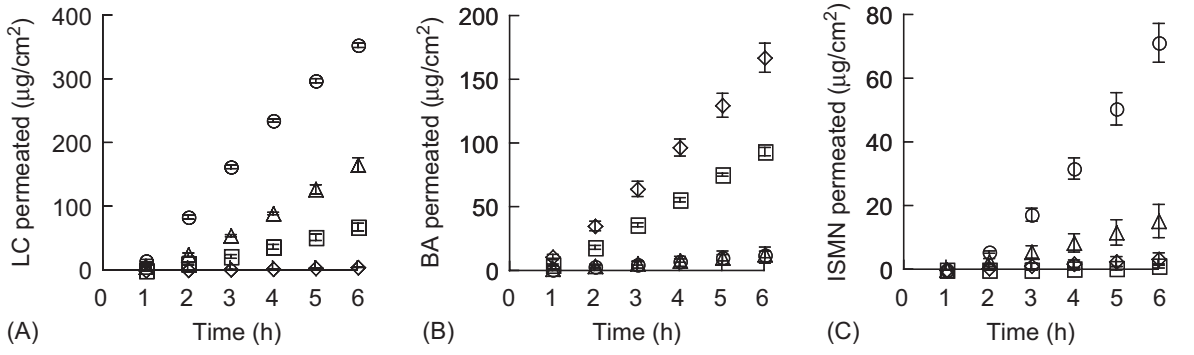


FIGURE 1. Effect of electrolyzed waters on the permeation of lidocaine (LC) (A), benzoic acid (BA) (B), and isosorbide mononitrate (ISMN) (C) through excised hairless rat skin. Symbols: □: Rp. 0, ○: Rp. 2, Δ: Rp. 3, ◇: Rp. 4. Each point represents the mean ± *SE* of 3–4 experiments.

addition to weak electrolyzed drugs, LC and BA. Figure 1C shows the time course of the cumulative amount of ISMN that permeated through excised hairless rat skin from several electrolyzed waters. Interestingly, the cumulative amount of ISMN over 6 h was 57- and 12-fold from ERW, Rps. 2 and 3, respectively, and 2.7-fold from EOW Rp. 4, compared with Rp. 0. The permeability coefficient of each compound is summarized in Table 4. Table 5 shows pH changes of the donor solution under each permeation experiment. No big pH changes in the donor solution were observed before and after the permeation experiment.

The contribution of ionized and unionized fractions of LC was investigated for the total steady-state flux of the drug through skin. In other words, the cumulative amount of LC permeation was determined through excised hairless rat skin from several buffer solutions (final pH: 6.6, 7.5, 8.3, 9.2, 10.1, 11.1, 11.7, and 12.1) to estimate the permeation coefficient, P_i and P_u, for ionized and unionized fractions of LC. The obtained result is shown in Figure 2A. The cumulative amount of LC permeation increased with an increase in donor pH. Table 6 summarizes each permeability coefficient in the steady state.

The observed permeability coefficients in the steady state were plotted as a function of pH, and the contribution of P_i and P_u was estimated by Equation 7. P_i and P_u were calculated to be 2.77×10^{-7} and 4.70×10^{-6} cm/s, respectively. Thus, P_i was 1/17 of P_u. In the case of indomethacin from our previous study (Hayashi, Sugibayashi, & Morimoto, 1992), P_i and P_u were 1.50×10^{-7} and 2.79×10^{-5} cm/s, and the contribution of P_i was 1/190 of P_u. The contribution of P_i for LC was larger than that for indomethacin. The total permeability coefficient of LC from Rp. 2 was significantly larger than that from buffer solutions having the same pH as Rp. 2, as shown in Figure 3A.

Figure 2B shows the time course of the cumulative amount of ISMN permeated through excised hairless rat skin from several buffer solutions (final pH: 3.6, 7.0, 9.7, 11.2, and 12.0). The cumulative amount of ISMN permeated was increased dependent on buffer pH. The permeability coefficient of ISMN from Rp. 2 was remarkably larger than that from buffer solutions, and the enhancing effect for ISMN (ratio of permeability coefficient against that for purified water) was much higher than that for LC (Figure 3B).

Effect of electrolyzed water on the skin barrier was evaluated by measuring skin impedance after applying electrolyzed water to the stratum corneum and dermis sides of hairless rat skin. The obtained results are shown in Figure 4. Skin impedance was measured over 6 h after the application of electrolyzed water. As skin impedance was not stable until 2 h after application, probably because of skin hydration, the percent impedance against that 2 h after application of electrolyzed waters was then plotted. A gradual decrease in skin impedance was observed by Rps. 2, 4, and 0.01 M HCl. A slight decrease in impedance was observed by Rp. 3 and physiological saline. On the contrary, a significant decrease in impedance was obtained in 0.02 M NaOH. Physiological saline was used as an alternative, because skin impedance could not be measured by purified water (Rp. 0).

Figure 5 shows protein leaching from the skin surface 3 and 9 h after treatment with electrolyzed waters. This experiment investigated the influence of electrolyzed waters on the skin barrier using electrolyzed water applied to the stratum corneum side of hairless rat skin. Rp. 4 and 0.01 M HCl, with the same pH as Rp. 4, showed the same profile for protein leaching as purified water. Higher protein leaching was observed for Rps. 2 and 3 than for purified water. The amount of protein leakage,

TABLE 4
Permeability Coefficient (cm/s) of Model Compounds

Rp.		LC	BA	ISMN
0	Purified water	4.34×10^{-6} (1.0)	5.48×10^{-6} (1.0)	1.22×10^{-7} (1.0)
2	Alkaline ERW	2.11×10^{-5} (4.9)	7.18×10^{-7} (0.13)	5.49×10^{-6} (45)
3	S-100	1.24×10^{-5} (2.9)	6.56×10^{-7} (0.12)	9.51×10^{-7} (7.8)
4	Acidic EOW	3.26×10^{-7} (0.075)	9.79×10^{-6} (1.8)	2.05×10^{-7} (1.7)

Numbers in parentheses show the ratio of the permeability coefficient to Rp. 0.

TABLE 5
pH Changes of the Donor Solution Before and After Experiment

		LC		BA		ISMN	
Rp.		Initial	Final	Initial	Final	Initial	Final
0	Purified water	9.42	8.50	3.19	3.27	6.22	6.29
2	Alkaline ERW	12.03	11.70	11.08	10.11	12.35	11.87
3	S-100	11.89	11.70	11.55	11.34	12.25	11.98
4	Acidic EOW	4.10	4.48	2.41	2.43	2.46	2.50

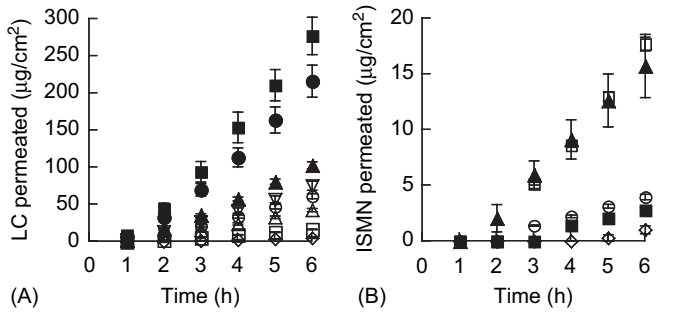


FIGURE 2. Effect of donor pH on the permeation of lidocaine (LC) (A) and isosorbide mononitrate (ISMN) (B) through excised hairless rat skin. Symbols: (A) ■: pH 12.1, ●: pH 11.7, ▲: pH 11.1, ▽: pH 10.3, ○: pH 9.3, Δ: pH 8.3, □: pH 7.3, ◇: pH 6.3, (B) □: pH 12.1, ▲: pH 11.4, ○: pH 10.0, ■: pH 7.0, ◇: pH 3.5. Each point represents the mean ± *SE* of 3–4 experiments.

TABLE 6
Permeability Coefficient of Lidocaine Through Excised Hairless Rat Skin

Final pH	*P* (cm/s)
6.6	3.48×10^{-7}
7.5	1.02×10^{-6}
8.3	2.61×10^{-6}
9.2	3.81×10^{-6}
10.1	4.64×10^{-6}
11.1	6.31×10^{-6}
11.7	1.43×10^{-5}
12.1	1.71×10^{-5}

however, was significantly low compared with 0.02 M NaOH with the same pH and 1% Triton-X-100, a positive control. Buffer solution pH 12.0 was used to prepare 1% Triton-X-100 instead of purified water, because protein leakage was not remarkable when using purified water.

No skin irritation was observed by electrolyzed waters from MTT assay and phospholipid leakage examination using excised hairless rat skin (data not shown).

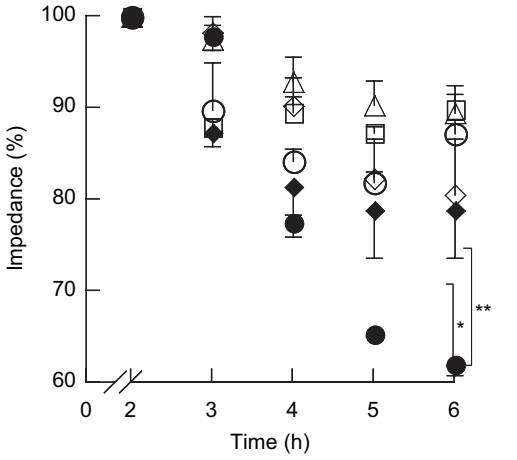


FIGURE 4. Effect of electrolyzed water on in vitro skin impedance. Symbols: □: physiological saline, ○: Rp. 2, Δ: Rp. 3, ◇: Rp. 4, ●: 0.02 M NaOH, ◆: 0.01 M HCl. Each point represents the mean ± *SE* of 3–4 experiments (*$p < .05$, **$p < .01$).

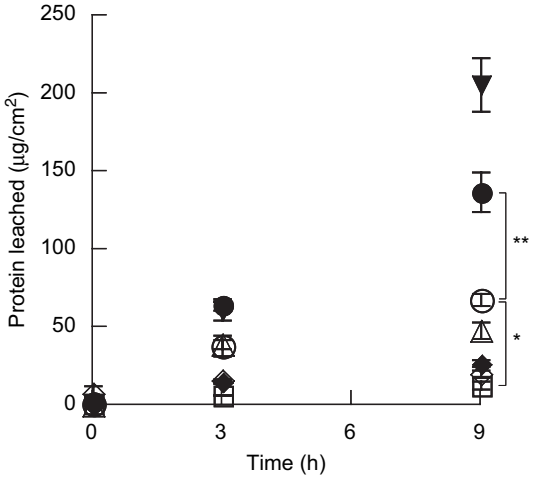


FIGURE 5. Influence of electrolyzed water on protein leaching from excised hairless rat skin. Symbols: □: Rp. 0, ○: Rp. 2, Δ: Rp. 3, ◇: Rp. 4, ●: 0.02 N NaOH, ◆: 0.01 N HCl, ▼: Triton-X-100 (pH 12.0). Each point represents the mean ± *SE* of 3–4 experiments (*$p < .05$, **$p < .01$).

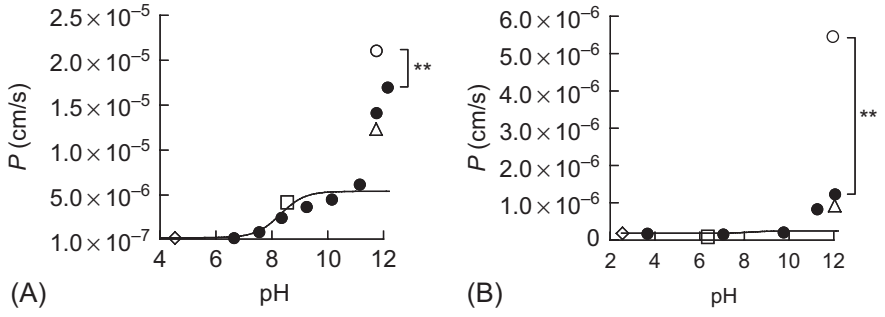


FIGURE 3. Relationship between the skin permeability coefficient of lidocaine (A) or isosorbide mononitrate (B) and donor pH. Symbols: □: Rp. 0, ○: Rp. 2, Δ: Rp. 3, ◇: Rp. 4, ●: buffer solution. Line was obtained by curve fitting (**$p < .01$).

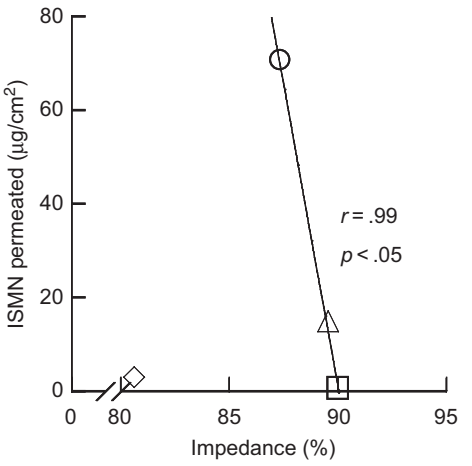


FIGURE 6. Relation between the amount of isosorbide mononitrate (ISMN) permeated and the skin impedance. Symbols: □: Rp. 0, ○: Rp. 2, Δ: Rp. 3, ◇: Rp. 4.

Figure 6 summarizes the relation between the logarithm of the cumulative amount of ISMN permeated over 6 h and the skin impedance at 6 h. Bigger decrease in impedance showed higher skin permeation of ISMN. A significant negative correlation was observed between the skin impedance and the cumulative amount of ISMN permeated when Rp. 4 was excepted.

DISCUSSION

pH of Rps. 2 and 3 was about 12 because OH^- was generated according to Equations 4–6, whereas pH of Rp. 4 was 2.5, because H^+ is generated according to Equations 1 and 3. ORP is a potential generated from electron exchange in an oxidation-reduction reaction system. It is a measure to quantitatively evaluate ease of oxidizing (reducing) of substance or be oxidized (be reduced). ORP should be determined by the balance of DO and DH and the concentration of several coexistent ions (Okouchi, Suzuki, Sugano, Kagamimori, & Ikeda, 2002; Pourbaix, 1966). DO of Rp. 4 was higher than that of drinking water-quality standards, and the result supported Equation 1. DH is expected to be high in Rps. 2 and 3 by Equation 4, although DH was not measured in the present experiment. Little ORP change was obtained (from 928.4 to 963.4 mV) in Rp. 4 by N_2 bubbling, whereas a remarkable increase was obtained (from −1097.4 to 2.6 mV) in Rp. 2 by the treatment. Because the saturated concentration of DH (1.55 mg/L, 25°C, 1 atm) is generally far smaller than the saturated concentration of DO (8.11 mg/L, 25°C, 1 atm) in water, Rp. 2 would be more affected by N_2 bubbling to escape DH than Rp. 4.

Na^+ or Cl^- concentration was high in Rps. 2 and 3 or Rp. 4, respectively, because Na^+ or Cl^- move from the middle cell at electrolysis to the cathodal or anodal cell, respectively; however, Na^+ concentration of Rp. 2 was half that of 0.02 M NaOH having the same pH as Rp. 2 (20 mEq/L). In a similar manner, Cl^-concentration of Rp. 4 could be reduced to half compared with that of 0.01 M HCl with the same pH as Rp. 4 (10 mEq/L). Because other ions to Na^+ or Cl^- existed in Rp. 3, despite a low concentration (comfirmed by ion chromatography, data not shown), these ions are also related to the osmolality of Rp. 3.

The skin permeation of LC and BA from several electrolyzed waters almost followed the pH partition hypothesis. The skin permeability of LC from Rps. 2 and 3, which are alkalic, was higher, and that from Rp. 4, which was acidic, was lower than that from Rp. 0. On the contrary, the permeability of BA from Rp. 4 was higher and that from Rps. 2 and 3 was lower. According to the pH partition hypothesis, however, the permeability of ISMN, an unionized drug, must be constant, independent of the solvent pH. Then, the skin-permeation profile of LC and ISMN was evaluated from several buffer solutions having different pHs as to whether it accorded with the pH partition hypothesis. The permeation of LC from buffer solutions exactly followed the pH partition hypothesis. When the unionized fraction of LC increased, the permeation amount of LC also increased depending on the pH. The permeation amount of ISMN was also dependent on the pH. The skin injury may take place by a high pH more than 11. The permeability coefficient of LC and ISMN from Rp. 2 was remarkably larger than that from buffer solution with the same pH, as shown in Figure 3. ISMN permeability was increased by electrolyzed waters, suggesting a permeation-enhancing effect of electrolyzed water. Because the dermal appendage route, such as hair follicles, is considered important for the permeability of hydrophilic compounds (Ogiso et al., 2002), Rp. 2 may increase this permeation pathway. It was proved from the *Results* and *Discussion* that the increase in skin permeability, which cannot be explained by pH partition hypotheses, must be related to the permeation enhancement effect by electrolyzed waters.

A decrease in skin impedance was observed by 0.02 M NaOH and 0.01 M HCl. Changes in impedance are greatly related to the skin permeability of ionic compounds and hydrophilic compounds; therefore, 0.02 M NaOH and 0.01 M HCl affects hydrophilic pathways in the skin barrier. Because Rps. 2 and 4 must affect the hydrophilic pathway to decrease skin impedance, this must explain the increased permeability of ISMN from Rp. 2, but the reason for no increase in the permeability of ISMN from Rp. 4 is unknown. Because little skin permeability of ISMN from Rp. 4 was observed, the present correlation analysis was done except for Rp. 4. A good correlation was recognized between the skin impedance and the cumulative amount of ISMN permeated from electrolyzed waters.

Protein leaching is related to structural destruction of the stratum corneum, which occupies most of the skin surface, and alkaline, especially 0.02 M NaOH, which caused solubilization and destruction of the stratum corneum. Increased pK_a of a compound (i.e., increase in alkalinity) increased skin irritation (Nangia, Andersen, Berner, & Maibach, 1996). On the contrary, ERW and EOW showed low skin irritation compared with 0.02 M NaOH with the same pH and 1% Triton-X-100,

MTT assay-positive materials such as surface-active agents and lactic acid. These electrolyzed waters are highly safe, which was determined by protein leaching from the skin surface in hairless rats.

CONCLUSION

These results suggest a high skin-permeation enhancement effect by electrolyzed waters. The mechanism is related to an increase in drug permeability through appendage routes, such as hair follicles, in addition to the permeation increase explained by the pH partition hypothesis. Electrolyzed waters are generally safe, so they can be used as new vehicles in topical pharmaceuticals or cosmetics, although the entire mechanism has not been elucidated.

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