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Comparative pharmaceutical evaluation of brand and generic clobetasone butyrate ointments

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ABSTRACT

In the present study, we performed comprehensive pharmaceutical evaluation among an original clobetasone butyrate (CLB) ointment product and three generic products. Although spherocrystal images were observed under a polarizing microscope for only Kindavate®, the original product, distribution of active and inactive ingredients was chemically equivalent between the original and generic medicine by the attenuated total reflection infrared spectroscopy. These results suggest that the spherocrystals observed in Kindavate® are composed of hydrocarbon. On GC/MS, it was revealed that linear alkanes having 25–27 carbon atoms are densely present in Sun White®, the base used in Kindavate®. On the other hand, linear alkanes having 22–31 carbon atoms were broadly distributed in most other white petrolatums. In the CLB ointment products, the distribution equivalent of linear alkane to Sun White® was observed only in Kindavate®. Thus, the GC/MS method is extremely useful for identification of white petrolatum used in the ointment.

A similar amount of CLB among the pharmaceutical products was detected in the skin tissue by skin accumulation test, although there were the differences in rheological properties and the quality of white petrolatum.

The present results will be very useful for pharmacists in selecting medicine products that match the needs of the patient. Such pharmaceutical information will help spread objective knowledge about products in the future, and will contribute to the appropriate selection of medication.

1. Introduction

In Japan, political measures that recommend the use of generic medicines from the point of view of medical cost reduction have been introduced in recent years. However, the spread of generic products has been delayed because of dissatisfaction and anxiety among pharmacists, and a lack of patient awareness of generics (Yanagihara et al., 2009; Sakurai et al., 2011), as well as differences in knowledge of pharmaceutical properties of generic medicines between pharmacists and doctors (Shibata et al., 2011). Thus, to select the appropriate generic medicines, pharmacists should actively disclose information about the pharmaceutical characteristics, which not only include medicine efficacy, but also the uniformity of active ingredient and additives, product use and pharmacokinetic properties, to doctors and patients.

According to the "Bioequivalence test guidelines for the generic medicine application of topical skin preparations in Japan" issued by the Ministry of Health, Labor and Welfare of Japan, bioequivalence testing of generic topical agents has been set based on the characteristics of the medicine products (http://www.nihs.go.jp/drug/be-guide/GL061124_hifu.pdf). Thus, there are no clear standards for pharmaceutical properties such as viscosity and quality of additives used in the product. As a result, if there are generic medicines having completely different properties from the original medicine, adverse effects such as unexpected allergies not seen in the original medicines may occur.

Microscopic imaging systems that employ X-ray fluorescence, infrared, near infrared, terahertz, raman and various other spectroscopic techniques have recently become very useful analytical tools in the fields of pharmaceutical design and quality control. The information obtained by these technologies has been used to evaluate generic products and medicines purchased on the

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internet (Veronin and Youan, 2004; Westenberger et al., 2005). Many studies have been conducted to investigate the distribution and blend uniformity of the active pharmaceutical ingredients and excipients in powders (Abhay et al., 2004; Bellamy et al., 2008; Li et al., 2008; Ma and Anderson, 2008; Shi et al., 2008). We have successfully analyzed semi-solid products using attenuated total reflection infrared (ATR-IR) spectroscopy, and reported that the distribution of active and inactive ingredients differs between innovator and generic products of alclometasone dipropionate ointment, a liquid droplet dispersion-type ointment (Yamamoto et al., 2012a).

On the other hand, significant differences in rheological properties have been observed between white petrolatum grades (Pandy and Ewing, 2008), suggesting the importance of understanding the properties of the base used in ointment products.

We investigated the spreadability of clobetasone butylate (CLB) ointments products using a spread meter, and reported that the yield value of Kindavate®, an original product, was significantly higher than that of three generic ointments (Yamamoto et al., 2012b). CLB ointment products, which are used primarily as anti-inflammatory preparations for conditions such as atopic dermatitis, can be used even in children in Japan as a steroidal ointment. In addition, some generic versions are currently available on the market. Therefore, objectively determining the pharmaceutical properties of ointment products is more effective from the point of view of proper medicine use.

In the present study, we observed the microscopic properties of CLB ointment products by using microscopic infrared spectroscopy and polarization microscopy. Next, we studied the rheological characteristics of CLB ointment products by viscometer, and performed identification of white petrolatum used as a base by the gas chromatography/mass spectrometer (GC/MS) method. In addition to these studies, we performed skin permeation studies of CLB by using the skin of hairless mice, in order to study the degree of efficacy among products. Then, we comprehensively evaluated the results obtained from these studies, and tried to pharmaceutically clarify the differences among CLB ointment products.

2. Experimental

2.1. Reagents

The CLB ointments Kindavate® Ointment (lot. 10057), an original medicine product by Glaxo Smithkline K. K. (Tokyo, Japan), Kinglon® Ointment (lot. TIC02), Mildvate® Ointment (lot. Y9301) and Paldes® Ointment (lot. 80117) 0.05%, generic versions by Tatsumi Kagaku Co., Ltd. (Ishikawa, Japan), Nichi-Iko Pharmaceutical Co., Ltd. (Toyama, Japan) and Iwaki Seiyaku Co., Ltd. (Tokyo, Japan), respectively, were analyzed.

Sun White®, Propeto®, White Petrolatum (JP, Kenei), White Petrolatum (JP, Kosakai) and White Petrolatum (JP, Yoshida), white petrolatums by Nikko Rika Corporation (Tokyo, Japan), Maruishi Pharmaceutical Co., Ltd. (Osaka, Japan), Kenei Pharmaceutical Co., Ltd. (Osaka, Japan) and Yoshida Pharmaceutical Co., Ltd. (Tokyo, Japan), were analyzed. The CLB used was a USP reference standard, and all other reagents used were reagent-grade chemicals by Wako Pure Chemical Industries, Ltd. (Osaka, Japan).

2.2. Polarization microscopy

The microscopic features of ointments were characterized by applying a small amount of the sample to a microscope slide, covering with a cover slip, and observing under an E-600-Pol polarizing microscope (Nikon Corporation, Tokyo, Japan) in reflection mode at 200× magnification.

2.3. Determining and imaging micro IR spectra

The pharmaceutical ingredients of CLB ointments are shown in Table 1. Spotlight400 (Perkin Elmer Japan, Yokohama, Japan), a line chemical imaging system equipped with an MCT linear array detector and a Ge ATR accessory, was used to collect IR spectra of the ointments. Each spectrum came from a 1.56 μm × 1.56 μm square pixel. The background was measured in air, and sample scans were recorded at 8 cm^{-1} spectral resolution with 2 scans across the range of 4000–750 cm^{-1}. Data were analyzed using Isys chemical imaging software (version 5.0; Malvern Instruments Ltd., Worcestershire, UK). The absorbance data for IR spectra were subtracted based on absorbance at 1780 cm^{-1} in order to remove the baseline effect. Offset data from the ointments were used to generate chemical images using a peak height method based on the characteristic peaks of the pure components.

2.4. Determination of rheological characteristics

Flow curves of shear rate against shear stress were obtained using a viscometer (TV-30; Toki Sangyo Co., Ltd., Tokyo, Japan). The temperature of the base plate was 25 ± 0.1 °C. The shear rate was varied from 3.83 to 9.58 s^{-1}. The hysteresis loop area was calculated as the area surrounded by the hysteresis loop using the trapezoidal method.

2.5. Estimation of white petrolatum used in CLB ointment product by GC/MS method

First, approximately 100 mg of ointment was dispersed in 3 mL of hexane. Then, 5 mL of acetonitrile was added, and the resulting mixture was shaken for 10 min. One milliliter of the acetonitrile bottom layer was collected and diluted with acetonitrile to a volume of 20 mL. The resulting liquid was analyzed using a HP 6890 gas chromatograph (Agilent Technologies, Inc., Santa Clara, CA) equipped with a JMS-AM II15 mass spectrometer (JEOL Ltd., Tokyo, Japan). GC/MS was conducted according to the following conditions:

For GC: column, HP-INNOWAX polar capillary column (30 m × 0.32 mm i.d. × 0.25 μm film thickness, cross-linked polyethylene glycol); column temperature, 70 °C (2 min) → (15 °C/min) → 150 °C (0 min) → (3 °C/min) → 200 °C (0 min) → (8 °C/min) → 280 °C (0 min) → (30 °C/min) → 300 °C (5 min); injection temperature, 250 °C; injection volume, 2.0 μL (splitless) and gas flow rate, 1.2 mL/min (He, constant flow).

For MS: electric ionization (70 eV, 300 μA); interface temperature, 280 °C; ion source temperature, 200 °C; and analysis mode; SIM *m/z* 85.

2.6. In vitro skin absorption of CLB

Skin accumulation studies were conducted with excised skin of hairless mice (Laboskin®, Hos/HR-1 Male, 7 weeks; Sankyo Labo Service Corporation, Inc., Tokyo, Japan) as an accumulation membrane. Skins were mounted on a Franz diffusion cell with the stratum corneum side facing upwards into the donor compartment. The donor compartment was filled with 300 mg of each ointment. The receiver solution was a 40% PEG400 solution. Stirring rate and temperature were maintained at 600 rpm and 32 °C, respectively. The receiver compartment had a volume of 7 mL, and the available diffusion area between compartments was 1.77 cm^2. At appropriate intervals, 500-μL aliquots of receiver fluid were withdrawn, and an equal volume of fresh buffer solution was supplied to the receiver compartment.

After 24 h, skin samples were removed from the cells, and ointment was carefully removed with a micro spatula, followed by