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Assessment of the physical properties and stability of mixtures of tetracycline hydrochloride ointment and acyclovir cream

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ABSTRACT

In dermatology, ointments are often mixed as part of drug therapy, but mixing often leads to incompatibility. Three combinations of tetracycline ointment (TC-o) and acyclovir cream (ACV-cr) were prepared at a TC-o:ACV-cr ratio of 1:1 using a brand-name ACV-cr and two generic ACV-cr (samples TC-o + ACV-A, TC-o + ACV-B, and TC-o + ACV-C). Microscopic examination revealed separation in TC-o + ACV-C. Viscosity and elasticity measurement indicated that the storage modulus (G') and loss modulus (G'') of each of the TC-o + ACV-cr mixtures behaved similarly to those of an ACV-cr and the loss tangent ($\tan\delta$) behaved similarly to that of a TC ointment. In addition, differences in the storage modulus (G') and loss modulus (G'') of the TC-o + ACV-cr mixtures were noted. To assess stability, each TC-o + ACV-cr mixture was stored away from direct sunlight at 25 °C and an RH of 84% and at 4 °C (in a refrigerator). HPLC revealed that the ACV content in each TC-o + ACV-cr mixture remained at 95–105% for up to 14 days under both sets of storage conditions. A decline in TC content in each TC-o + ACV-cr mixture was not noted with storage at 4 °C but was noted over time with storage at 25 °C and an RH of 84%. In addition, significant differences in the percent decline in TC content in each TC-o + ACV-cr mixture occurred with storage at 25 °C and an RH of 84%. Thus, differences in physical properties and stability may occur when combining brand-name and generic drugs, and temperature and humidity may be the cause of the TC-o's incompatibility.

1. Introduction

In dermatology, ointments are often mixed as part of drug therapy to relieve symptoms and improve feel and compliance (Kizu et al., 2004). However, there are no basic data on the clinical effectiveness of and adverse reactions to such mixtures, and mixing is sometimes done based on experience rather than on evidence (Gao and Li Wan Po, 1994). There are reports of physical changes and changes in drug penetration accompanying a decline in content and changes in the characteristics of bases once ointments are combined (Fukami et al., 2006; Guin et al., 1993; Wohlrab, 1984). In actual practice, many physicians and pharmacists have found that combining ointments results in separation or deterioration.

Tetracycline hydrochloride (TC), a tetracycline, blocks the binding of aminoacyl tRNA to the mRNA–ribosome complex. This inhibits protein synthesis in bacteria and is why TC has antibacterial action (Xu et al., 2011). In addition, TC acts specifically on 70S bacterial ribosomes without acting on 80S animal ribosomes, which is why it is reported to have selective toxicity (Weisblum and Davies, 1968). TC ointments (TC-o) have been found to be clinically effective in treating impetigo (Kuniyuki et al., 2005). Impetigo can be caused by an infection due to scratching of the skin. Chickenpox is one such condition that causes scratching, and topical preparations of acyclovir (ACV) that are used to treat herpes-virus infection are also used to treat chickenpox. Thus, in actual practice, topical preparations of ACV are sometimes combined with TC-o. A decline in content and changes in appearance have been reported when combining TC-o with other preparations (Loseva et al., 1978; Kawamoto et al., 2008). However, no studies have described the physical properties and stability of a combination of TC-o and a topical preparation of ACV.

Incompatibility due to mixing is often evident not only in the incompatibility of principal agents but also in the incompatibility of the principal agent and additives (Gao and Li Wan Po, 1994; Wohlrab, 1984). Differences in bases are known to cause different levels of incompatibility and differences in drug penetration when combined. Combining ointments requires selection of bases with similar characteristics (Fukami et al., 2006; Guin et al., 1993). The properties of bases of creams and ointments differ, and combinations of creams and ointments must be studied. ACV cream (ACV-cr) is an o/w emulsion, and Vaseline (petroleum jelly) is used as an oleaginous base. There are both brand-name and generic ACV-cr. The current authors have studied the physicochemical properties of ACV-cr and noted differences in the types of additives and differences in their water content, viscosity, elasticity, and emulsification (Inoue et al., 2012). Viscoelasticity is one reported way to assess the

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Table 1
Additives list for TC-o and ACV-cr.

Formulation	Additives
TC-o	White petrolatum, purified lanolin, propyl-p-hydroxybenzoate, methyl-p-hydroxybenzoate
ACV-A	Liquid paraffin, dimethylpolysiloxane, propylene glycol, white petrolatum, glycerol, cetearyl alcohol, sodium lauryl sulfate, glyceryl stearate, polyoxyethylene(196) polyoxypropylene(67) glycol
ACV-B	Liquid paraffin, dimethylpolysiloxane, propylene glycol, white petrolatum, cetanol, glyceryl monostearate, polyethylene glycol monostearate
ACV-C	Liquid paraffin, dimethylpolysiloxane cetanol, glycerol, stearic acid, glyceryl stearate, polyoxyl stearate, hydrogenated castor oil, 1,3-butanediol, squalane, behenic acid, isopropyl myristate, sodium hydrate

physical properties of a semisolid substance, and the viscoelasticity of conditioners and ointments has been assessed (Adeyeye et al., 2002; Hong et al., 2010; Kobayashi et al., 1982). Viscoelasticity is expressed by the loss tangent (tan δ), which is associated with tackiness due to differences in emulsification, water content, viscosity and elasticity, and additives. tan δ may also affect stability.

Thus, the current study assessed the physical properties and stability when combining TC-o and ACV-cr. This study also examined incompatibility due to that combination in order to provide useful information when combining TC-o and ACV-cr.

2. Materials and methods

2.1. Reagents

TC-o (POLA-Pharma Co., Ltd., Japan) and three different 5% ACVs were used in the present study: the original product, ACV-A (GlaxoSmith Kline K.K.), and the following two generic products: ACV-B (Sandoz Co., Ltd., Japan) and ACV-C (Toko Pharmaceutical Industrial Co., Ltd., Japan). The three products were randomly named ACV-A, ACV-B, or ACV-C (Table 1). All other reagents were of special reagent grade.

2.2. Sample preparation

The mixture of TC and ACV-c (weight ratio of 1:1) was prepared in a mixer (Nanko Neritaro NRB-250: THINKY Co., Ltd., Japan). Conditions for mixing were a mixing time of 30 s and 2000 rpm. Distilled water was added to TC-o at a weight ratio of 5%, 10%, and 15% to serve as a mixture of TC-o and distilled water.

2.3. Microscopy

Polarization microscopy was performed with an Olympus model BX51 microscope (Olympus Co., Ltd., Japan). In addition, a polarizing plate with a wavelength of 488 nm was used.

2.4. Measurement of viscosity and viscoelasticity

Measurement of viscosity or viscoelasticity was done using a Rheometer (HAAKE MARS; Thermo Scientific Co.) with a $1^{\circ\prime} \times$ R35 cone rotor at 25 °C and 35 °C. The conditions for measurement of viscosity were a sample amount of 0.2 mL and a gap of 0.051 mm. A flow test was used to determine the relative viscosity of all formulations with the following parameters: for the upcurve, a continuous ramp with shear rate as controlled variable (0–1000 s^{-1}), log mode, and a 1-min ramp duration were used. The same procedure was used for the downcurve with reversed shear rate (1000–0 s^{-1}) to measure thixotropy and yield stress. The conditions for measurement of viscoelasticity were a sample amount of 2 mL and a gap of 1 mm. Stress was increased gradually from 1 Pa to 10 Pa.

$$\tan\delta = \frac{G''}{G'}$$

where tan δ is the loss tangent, G'' is the loss elastic modulus (Pa) and G' is the storage elastic modulus (Pa).

2.5. Preparation of humidification samples

Each TC-o/ACV-cr mixture and each TC-o and distilled water mixture were stored in a thermostated bath at 25 °C for 0 days, 3 days, 7 days, and 14 days in a desiccator (relative humidity, 84%) in the presence of a KCl-saturated aqueous solution.

2.6. HPLC assay

For the assay, 0.5 g of each TC-o/ACV-cr mixture and 0.25 g of TC-o and each TC-o and distilled water mixture was weighed accurately and placed in a stoppered centrifuge tube. Then 40 mL of distilled water was added and the solution was shaken and then centrifuged (10,000 rpm for 30 min, at 25 °C). The portion of the lower layer was filtered with a 0.45-μm filter, and the filtrate served as the sample solution. A calibration curve was prepared using TC and ACV that had separately been dried at 105 °C for 24 h. TC and ACV were assayed using high-performance liquid chromatography (HPLC: LC-20ADvp, Shimadzu). TC and ACV assay conditions were a column of Inertsil ODS-3 (4.6 mm × 250 mm, φ5 μm), column temperature of 35 °C, mobile phase of pH 3 phosphate buffer/methanol = 95/5, and detection wavelength of 254 nm; conditions were tailored for TC to produce a peak at 9 min. Multiple groups were then analyzed with Tukey's test using the statistical program R 2.1.1.

2.7. Measurement of water content

The titrimetric determinations of water were performed at room temperature using a CA-06 Karl-Fischer moisture content meter (Mitsubishi Chemical Co., Ltd., Japan) equipped with a coulometric titration system (n = 3). Karl-Fischer reagents (AQUAMICRON®AX RS as the catholite and AQUAMICRON®CNU as the anolyte) were purchased from Mitsubishi Chemical Co. For measurement, 5–10 mg of sample was dissolved in Karl-Fischer reagent using a glass rod to yield a paste.

2.8. Measurement of sample pH

The pH was measured directly in each ACV-cr using a Docu-pH Meter (Sartorius Co., USA).

3. Results and discussion

The compatibility of bases is crucial when combining ointments, and incompatibility of bases can lead to separation and disruption of emulsification (Ohtani et al., 1997). Thus, TC-o + ACV-cr mixtures were observed here using polarization microscopy to determine the dispersion of the mixtures (Fig. 1). Results revealed the precipitation of crystals in each TC-o + ACV-cr mixture. Separation was noted in TC-o + ACV-C, so emulsification was not uniform, and there were differences in dispersibility. In addition, TC-o and ACV-cr were also observed (Fig. 2). Results revealed crystals in each preparation. The crystals observed in TC-o were found to be larger than the crystals found in ACV-cr. In addition, droplets were noted in ACV-C, and emulsification was found to be non-uniform. Additives in the TC-o and ACV-cr are shown in Table 1. As indicated, there were differences in additives in the ACV-cr. Instances of differences in the