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Title:

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 (tanδ) 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 25ºC and an RH of 84% and at 4ºC (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 4ºC but was noted over time with storage at 25ºC 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 25ºC 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.

Keywords: tetracycline hydrochloride, acyclovir, mixture, ointment, generic drug

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 et al., 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 et al., 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 et al., 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 et al., 1994; Wohlrab et al., 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 physical properties of a semisolid substance, and the viscoelasticity of conditioners and ointments has been assessed (Moji et al., 2002; Hong et al., 2010; Kobayashi et al., 1982). Viscoelasticity is expressed by the loss tangent (tanδ), which is associated with tackiness due to differences in emulsification, water content, viscosity and elasticity, and additives. tanδ 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°’ × R35 cone rotor at 25ºC and 35ºC. 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.

$\tan\delta = G'' / G'$

tanδ: loss tangent

G": loss elastic modulus (Pa)

G': 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 25ºC 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 25ºC). 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 105ºC 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

35ºC, mobile phase of pH3 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-Fisher 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 Fisher 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 dispersibility of mixtures have been reported even when combining preparations with the same ingredients (Ohtani et al., 1997). Differences in the properties of bases may have affected differences in emulsification in the current TC-o/ACV-cr mixtures as well. Thus, differences in dispersibility may have occurred due to the properties of bases in each ACV-cr.

To determine differences in viscosity and elasticity of the mixtures, the viscosity and elasticity of the TC-o, ACV-cr, and TC-o+ACV-cr mixtures were measured. The measured flow curve is shown in Fig. 3. TC-o and ACV-A had a hysteresis loop while ACV-B and ACV-C did not. Thus, the higher water content in ACV-B and ACV-C than in ACV-A led to ACV-B and ACV-C having no structural memory, which may be why ACV-B and ACV-C displayed viscoelastic behavior unlike that of ACV-A. In addition, little memory remained in the TC-o+ACV-cr mixtures. This was evident

because of the differences in water content during mixing and suggested that structural disruption may have occurred.

The storage modulus G’ (the elastic component) and loss modulus G” (the viscous component) are shown in Fig. 4. Results for each of the TC-o+ACV-cr mixtures show that the storage modulus G’ and loss modulus G” behaved not like the elasticity of TC-o but like ACV-cr, which is interesting. In short, this is because the structure of the ointment could not be maintained due to water in the ACV-cr. In addition, looking at the behavior of the storage modulus G’ and loss modulus G” of ACV-cr reveals that there were few disparities between ACV-B and ACV-C but that there were differences between TC-o+ACV-B and ACV-C. Comparison of ACV-B and TC-o+ACV-B indicated that TC-o+ACV-B had a larger storage modulus G’ and loss modulus G”. Comparison of ACV-C and TC-o+ACV-C indicated that TC-o+ACV-C had a smaller storage modulus G’ and loss modulus G”. This was evident from the separation in TC-o+ACV-C as revealed by microscopic examination. The water content in ACV-cr may have had an effect, decreasing its elasticity and viscosity.

The loss tangent tanδ is shown in Fig. 5. tanδ is a ratio of the loss modulus G”, which represents the viscosity component, and the storage modulus G’, which represents the elasticity component (Adeyeye et al., 2002). The tanδ of each of the TC-o+ACV-cr mixtures behaved like that of TC-o. Differences in preparations noted with ACV-cr decreased. Thus, each of the TC-o+ACV-cr mixtures was found to have roughly the same proportion of viscosity and elasticity.

Measurement of viscosity and elasticity revealed differences in the viscosity and elasticity of TC-o+ACV-cr mixtures. These differences were evident because of the differences in water content in the mixtures after mixing and are presumed to have

caused structural disruption and thus affect stability. Thus, each TC-o+ACV-cr mixture was stored away from direct sunlight at 25ºC and an RH of 84% and at 4ºC (in a refrigerator) to examine the mixtures' stability. TC and ACV content were calculated using HPLC. Results of the assays of TC and ACV are shown in Table 2. The ACV content of ACV-cr has been found to be 95% or higher (Inoue et al., 2012). The ACV content in each TC-o+ACV-cr mixture was found to remain at 95–105% for up to 14 days under both sets of storage conditions. Additionally, the TC content in TC-o and each TC-o+ACV-cr mixture was 101.8 ± 1.1%, 95.2 ± 1.9%, 99.0 ± 2.1%, and 97.4 ± 1.6%, so the content was found to be 95% or higher. A decline in TC content in each TC-o+ACV-cr mixture was not noted over 14 days of storage at 4ºC. Nevertheless, a decline in TC content over time was noted with storage at 25ºC and an RH of 84%. This is presumably because molecular motion is excited by a high temperature and humidity, for example, storage at 25ºC and an RH of 84%, in comparison to storage at 4ºC. As a result, the mixture is unstable.

A TC-o is reported to have a decline in TC content and a decline in antibacterial activity when combined with an ointment containing sodium hydroxide (Kawamoto et al., 2008). A closer look at additives contained in the ACV-cr indicates that ACV-A and ACV-C contain additives with Na salts (Table 1). Thus, Na salts contained in additives may have caused the decline in TC content noted in TC-o+ACV-A and TC-o+ACV-C. However, ACV-B contained no Na salts, so other factors may have been at work. Tetracyclines change color and degrade when combined with alkaline drugs, so caution is required in their handling (Kawamoto et al., 2008). ACV-A had a pH of 7.04 ± 0.05, ACV-B had one of 5.17 ± 0.1, and ACV-C had one of 7.29 ± 0.07, so none were basic. In addition, degradation by exposure to light is a possibility.

However, under the current storage conditions, mixtures were stored away from direct sunlight, so light was not a factor. In addition, TC is known to undergo hydrolysis (Kang et al., 2012). Thus, attention was focused on the water content in each ACV-cr.

The water content in each TC-o+ACV-cr mixture was measured. TC-o+ACV-A had a water content of 6.0 ± 1.4, TC-o+ACV-B had one of 14.3 ± 0.8, and TC-o+ACV-C had one of 12.1 ± 0.6% before storage. Given the fact that water content can affect stability, distilled water was mixed with TC-o to a weight ratio of 5%, 10%, and 15% and the TC content in each mixture was examined. Results revealed no changes in content with storage at 4ºC. In contrast, a decline in TC content was noted with storage at 25ºC and an RH of 84% (Table 3). Significant differences in the decline in TC content were not noted in any of the mixtures stored at 25ºC and an RH of 84%. Thus, water presumably caused a decline in TC content, as did differences in the storage temperature and additives.

In addition, significant differences in the decline in TC content over time were noted in each TC-o+ACV-cr mixture stored at 25ºC and an RH of 84%. TC-o+ACV-A and TC-o+ACV-B had a greater percent decline in TC content than TC-o+ACV-C (Table 2). One cause of this may have been differences in separation as revealed by microscopic examination. Separation was noted in TC-o+ACV-C though not in TC-o+ACV-A and TC-o+ACV-B. Thus, the TC-o in TC-o+ACV-A and TC-o+ACV-B was dispersed in ACV-cr in an o/w manner. The TC-o in TC-o+ACV-A and TC-o+ACV-B was in contact with the additives and water in the ACV-cr. Thus, TC-o+ACV-A and TC-o+ACV-B had greater incompatibility of TC-o and ACV-cr than did TC-o+ACV-C, facilitating a decline in TC content.

The current study noted differences in separation, viscosity, and elasticity as a result

of combining TC-o and ACV-cr. Differences in physical properties may lead to differences in feel and can affect patient compliance and adherence as well. With regard to the stability of each TC-o+ACV-cr mixture, a decline in TC content over time was noted due to differences in storage conditions. The decline in TC content in the TC-o+ACV-cr mixtures is attributed to storage conditions, suggesting the importance of how such mixtures are stored.

Differences in the physical properties of and storage conditions for preparations may lead to differences in clinical effectiveness. In addition, differences in the types and ratios of additives are reported to affect skin penetration (Trottet et al., 2005). Differences in the types and ratios of additives may also lead to differences in clinical effectiveness. Detailed information on the physical properties and content of brand-name and generic drugs can be a useful piece of information when determining what drugs are safe and efficacious to combine.

Conclusion

The current study found differences in separation, viscosity, and elasticity in combinations of a TC-o and ACV-cr. With regard to the stability of the TC-o+ACV-cr mixtures, a decline in TC content over time was noted due to differences in storage conditions. In addition, the decline in TC content in TC-o+ACV-cr mixtures is attributed to storage conditions. Thus, differences in physical properties may occur when combining brand-name and generic drugs, and water content and humidity may be the cause of the TC-o’s incompatibility.

Conflict of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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330 Figure legends

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

332

333 Table 2. The mean ACV and TC content in TC-o+ACV-cr mixtures according to HPLC

334 (±SD, n = 3)

335

336 Table 3. The mean TC content in TC-o according to HPLC (±SD, n = 3)

337

338 Fig. 1 Light microscopy of TC-o/ACV-cr mixtures. Scale bars indicate 50 μm.

339 a) TC-o+ACV-A, 0 days after mixing; b) TC-o+ACV-B, 0 days after mixing; c)

340 TC-o+ACV-C, 0 days after mixing; d) TC-o+ACV-A, 14 days after mixing at 25ºC

341 RH84%; e) TC-o+ACV-B, 14 days after mixing at 25ºC RH84%; f) TC-o+ACV-C, 14

342 days after mixing at 25ºC RH84%; g) TC-o+ACV-A, 14 days after mixing at 4ºC; h)

343 TC-o+ACV-B, 14 days after mixing at 4ºC; i) TC-o+ACV-C, 14 days after mixing at

344 4ºC

345

346 Fig. 2 Light microscopy of TC ointment and ACV creams respectively. Scale bars

347 indicate 50 μm.

348 a) TC-o, b) ACV-A, c) ACV-B, d) ACV-C

349

350 Fig. 3 Shear stress versus shear speed curves for TC-o, ACV-cr, and TC-o+ACV-cr

351 mixtures at 25ºC

352 a) Shear stress versus shear speed curves for TC-o and ACV-cr

353 ○: TC-o, ◆: ACV-A, □: ACV-B, △: ACV-C

b） Shear stress versus shear speed curves for TC-o+ACV-cr mixtures

◆: TC-o+ACV-A, □: TC-o+ACV-B, △: TC-o+ACV-C

Fig. 4 G’ and G” versus Tau for TC-o, ACV-cr, and TC-o+ACV-cr mixtures

a) G’ versus Tau for TC-o and ACV-cr, b) G” versus Tau for TC-o and ACV-cr

○: TC-o, ◆: ACV-A, □: ACV-B, △: ACV-C

c) G’ versus Tau for TC-o+ACV-cr mixtures, d) G” versus Tau for TC-o+ACV-cr mixtures

◆: TC-o+ACV-A, □: TC-o+ACV-B, △: TC-o+ACV-C

Fig. 5 tanδ versus Tau for TC-o, ACV-cr, and TC-o+ACV-cr mixtures

a) tanδ versus Tau for TC-o and ACV-cr

○: TC-o, ◆: ACV-A, □: ACV-B, △: ACV-C

b) tanδ versus Tau for TC-o+ACV-cr mixtures

◆: TC-o+ACV-A, □: TC-o+ACV-B, △: TC-o+ACV-C