Histochemical Analysis of Lacrimal Concretions in the Patients using Rebamipide Ophthalmic Suspension

Ken Yamashita
Sapporo Ika Daigaku
miki hiraoka (mikihira@nms.ac.jp)
Sapporo Medical University https://orcid.org/0000-0002-6411-2269
Shintaro Sugita
Sapporo Ika Daigaku
Takatoshi Yotsuyanagi
Sapporo Ika Daigaku

Brief report

Keywords: lacrimal concretion, dacryocystitis, rebamipide, thin layer chromatography, case report

Posted Date: August 28th, 2020

DOI: https://doi.org/10.21203/rs.3.rs-66812/v1

Abstract

Backgroud: Concretions of the lacrimal drainage system can cause dacryocystitis. In the present study, our patient developed dacryocystisis with lacrimal concretions of a white soft mass. Rebamipide ophthalmic suspension had been applied for treatment of dry eye. To evaluate the pathogenic mechanism of the case, histological and chemical studies were performed.

Case presentation: Our case was woman in her seventies. She had a medical history of rheumatoid arthritis. She was referred to our hospital for dacryocystitis after showing a poor response to treatment with antibiotic agents. A head computed tomography (CT) scan showed ductal high-density deposits along the lacrimal sacs. During dacryocystotomy, the hypertrophy of the lacrimal sacs was found replete with pus and cottage cheese-like white substances. The extracts were surgically removed and histological and chemical analysis was performed. The histological examination showed granulation tissues and acellular amorphous material with crystal-like structures. Positive staining by Alcian blue and Kossa was found in crystal-like legions. The extract obtained from the concretions showed the same fluorescence band and UV absorption spectrum in thin layer chromatography (TLC) and spectrometry, respectively, as rebamipide.

Conclusion: Our findings suggest that the obstruction by lacrimal concretions containing rebamipide resulted in dacryocystitis in this case. In addition, it is speculated that in patients who have impaired tear secretion, refractory dacryocystitis may be evoked following the accumulation of rebamipide in the lacrimal sacs.

Introduction

The lacrimal concretions can develop in any lesion of the lacrimal pathway including the canaliculus, lacrimal sac, and nasolacrimal duct. The lacrimal concretions have also been termed as dacryoliths, mucoliths, and/or canaliculolith. It can lead to obstruction of the tear passage and cause dacryocystitis. The region of dacryocystitis is frequently accompanied with infection of the fungus and actinomyces[1].

Tears are indispensable to maintain transparency of the cornea by supplying moisture and nutrients. Insufficient secretion of tears leads to dry eye syndrome. Tears consist of water, proteins, mucins, and lipids. A deficiency of mucins in tears is known to cause dry eye syndrome[2]. Treatment for dry eye syndrome is mainly the administration of eye drops such as a physiological saline solution, sodium hyaluronate solution, diquafosol sodium solution, and lacrimal plugs. Recently, rebamipide ophthalmic suspension (ROS) has been recognized as a new therapeutic eye drop. Rebamipide was originally developed to treat gastric ulcers and gastritis by increasing mucin-like substances and protecting the internal surface of the stomach[3–5]. Rebamipide is hydrophobic particles. ROS has been applied to a large number of dry eye patients with positive outcomes[6]. Among its effect, it increases the number of conjunctival goblet cells, produces a mucin-like substance in corneal epithelial cells, and facilitation of

wound healing at corneal epithelial cells[7–10]. However, complications such as lacrimal passage obstruction and dacryocystitis have been reported[11].

In this report, we describe the case presenting dacryocystitis with a white substance in the lacrimal sac. Histological examination and chemical analysis were carried out to understand the characteristic phenotype and etiology of lacrimal concretions following ROS application.

Case Presentation

The medical records of the patient with dacryocystitis with foreign substances were retrospectively reviewed. The present study protocol was approved by the Ethics Committee of Sapporo Medical University Hospital and conducted in accordance with the Declaration of Helsinki. After a full explanation of the purpose and protocol for this study, patient provided written informed consent for publication.

Our patient was a 77-year-old woman who had developed acute dacryocystitis on her left lid two years prior to presentation. She had been previously diagnosed with dry eye and glaucoma. Additionally, she had undergone ROS for some time. She had initially visited a separate hospital where she received paracentesis and had her lacrimal sac drained, both of which resulting in improvements in her condition. Six months later, the dacryocystitis relapsed and the same treatment was applied, again with a positive outcome. A year later, she developed dacryocystitis on her right lid. Application of an antibacterial agent proved to be effective. However, a fistula between the lacrimal sac and skin was found three months after a right lacrimal tube insertion. She had been diagnosed previously with hypertension and rheumatoid arthritis that was being medically controlled.

Examination disclosed an erythematous and swelling area in the right lower lid. Discharge was observed from the right lacrimal point. A head computed tomography (CT) scan demonstrated ductal high-density deposits along the right lacrimal sac (Fig. 1A, B). From the CT image findings, a diagnosis of dacryocystitis with dacryoliths was reached. Under systemic anesthesia, a dacryocystotomy was performed and the incisions were sutured after the contents were extracted. The lacrimal sac was hypertrophic and inundated with pus and calcified white substances. A histological study and chemical analysis were conducted for those extracts. There was no relapse of dacryocystitis after surgery.

Histological analysis

The extracts showed a cottage cheese-like white mass and granular tissues. Extracts were stored in formalin, and then embedded in paraffin. Hematoxylin and eosin (HE) staining, Alcian blue staining, and Kossa staining were then performed on the samples. The findings of HE staining showed inflammatory granulation tissues with severe infiltration of lymphocytes, plasma cells, and neutrophils. Also, actinomyces colonies were observed. Additionally, there was a crystal-like structure (Fig. 2A) surrounded by abscesses in all cases. In the crystal-like structure, a diffuse positive pattern with Alcian blue and

Kossa staining was found (Fig. 2B, C). In granulation tissues, positive staining was partially observed by Alcian blue and Kossa staining (Fig. 2D).

Chemical analysis of lacrimal concretions

According to the chemical structure of rebamipide, it is a lipophilic small molecule. Five µl of the rebamipide suspension (20 mg rebamipide/ml, Otsuka Pharmaceutical Co., Tokyo Japan) was suspended with 1 ml of 0.9% NaCl, mixed with 2 ml of methanol plus 2 ml of chloroform and centrifuged at 800 g for 5 min at room temperature. The resultant organic phase was transferred into another glass tube and dried down under a stream of nitrogen gas. The dried extract was dissolved with 100 µl of chloroform/methanol (2:1, v/v). A part of the extract was applied to an HPTLC plate and developed in a solvent system consisting of chloroform/methanol/water (60:35:8, v/v). To visualize rebamipide, the plate was dried and then exposed under ultraviolet (UV) light. The same extract was then dried again and dissolved with dimethylformamide (DMF). The absorption spectrum of rebamipide in DMF was recorded within the scanning range 270–400 nm using a spectrophotometer (Hitachi U-3010 spectrophotometer, Japan). To identify rebamipide in the concretion, 21 mg (wet weight) of the clinical sample was homogenized with 0.8 ml of cold 0.9% NaCl for 20 strokes using a 1 ml Teflon-glass homogenizer on ice. The homogenates were transferred into a glass tube and mixed with 2 ml of methanol plus 1 ml of chloroform. The homogenates (100 µl) was transferred into a glass tube, mixted 0.7 ml of 0.9% NaCl plus 2 ml of methanol and 1 ml of chloroform, briefly sonicated by a sonic bath and stood for 30 min at room temperature. The suspensions were then mixed with 1 ml of chloroform plus 0.2 ml of 0.9% NaCl and centrifuged at 800 g for 5 min at room temperature. The resultant organic phase was transferred into another glass tube and dried down under a stream of nitrogen gas. The dried materials were examined as the extract obtained from the rebamipide suspension.

The concretion obtained from the patient was a white and water-insoluble soft lump. Rebamipide is a water-insoluble white powder. As shown in the thin layer chromatography (TLC), the rebamipide extracted from the ophthalmic suspension was detected as a single band with fluorescence emission by UV light exposure (Fig. 3A). In addition, the rebamipide in DMF showed a characteristic UV absorption spectrum (Fig. 3B). Intriguingly, the extract obtained from the concretion showed the same fluorescence band and UV spectrum in TLC and spectrometry, respectively, as rebamipide. These results show that the concretion contained water-insoluble ingredient rebamipide.

Discussion

In this report, we described the case of dacryocystitis containing concretions. Our case had a history of using ROS. The histological study showed a crystal-like structure in specimens. It was assumed these substances formed crystal-like structure contained unbiological materials that caused the concretions. Therefore, we considered the possibility that the white substance found in the lacrimal path and/or sacs of the patient was formed or developed by accumulation of rebamipide. Results of the chemical analysis of the specimen did in fact reveal that the concretion contained rebamipide.

Dacryocystitis is inflammation of the lacrimal sac. The causes of csytitis vary and include infection, autoimmune diseases, and malignant diseases[12]. The majority is induced by infection. In our study, all three cases showed colonies of acetinomysis. Acetinomysis is a type of indigenous bacterium in the mouth, and is frequently found in dacryocystitis. The histological study in our case showed granular tissues and crystal-like structures. In the granulation tissue area, non-uniform patterns were found through Alcian blue staining. It is difficult to confirm whether the positive staining was caused by rebamipide application or responses to inflammation. The screening of other dacryocystitis specimens without rebamipide application is required to answer this question. There was a faint stain at the marginal region by Kossa staining. However, at the crystal-like structure area, there was prominent staining by Alcian blue and Kossa. It is likely that the positive staining by Alcian blue reflects the mucin augmentation.

Previously, we had experienced other 2 cases of dacryocystitis after showing a poor response to treatment with antibiotic agents [13]. A head CT scan revealed ductal high-density deposits along bilateral lacrimal sac. Both cases had medical history of rheumatoid arthritis with Sjögren syndrome and had been applied ROS for dry eye treatment. Dacryocystotomy was performed and the contents were extracted. Histological study showed granulation tissues with actinomyces colonies and crystal-like structure. Interestingly, all three cases were incidentally affected by rheumatoid arthritis. It is difficult to ascertain whether this systemic background had any influence on the patients contracting dacryocystitis with concretions. It can be speculated that an aggressive autoimmune response following rheumatoid arthritis leads to inflammation and granular tissue formation in the lacrimal pathway. Another possibility is that the immunosuppressive state for the rheumatoid arthritis treatment precedes susceptibility to infections such as actinomyces. We conjecture that the low tear secretion results in a disturbance of the washout system to remove unnecessary substances in the lacrimal pathway.

The content of dacryoliths was found to be organic material with minerals such as calcium and magnesium [14]. In addition, Mano et al. reported that the protein profiles of dacryoliths were different between ROS and non-ROS group [15]. However, the exact composition of organic material is unclear in either with or without ROS application.

The chemical analysis of the specimen in our case revealed that the water-insoluble ingredient contained rebamipide. It is assumed that the crystal-like structure found during histological examination included rebamipide. Rebamipide is water insoluble. When there is stenosis of the lacrimal pathway, the aggregation of rebamipide can obstruct the lacrimal pathway. Then, dacryocystitis can be induced at this site following infection of such as actinomyces.

In light of these results, it can be inferred that the accumulation of rebamipide particles leads to congestion of the lacrimal pathway and complications of actinomycosis can result in the formation of dacryocystitis. In addition, immunosuppressive disorders such as rheumatoid arthritis and Sjögren syndrome may increase the risk of dacryocystitis under ROS application.

Our results suggest that the ROS should be carefully applied to any patient with rheumatoid arthritis and/or Sjögren syndrome. The ROS application to these patients may result in concretions on the lacrimal pathway and induce dacryocystitis.

Abbreviations

CT: computed tomography, TLC: thin layer chromatography, ROS: rebamipide ophthalmic suspension, HE: Hematoxylin and eosin, UV: ultraviolet, DMF: dimethylformamide

Declarations

Ethics approval and consent to participate: The present study protocol was approved by the Ethics Committee of Sapporo Medical University Hospital and conducted in accordance with the Declaration of Helsinki. After a full explanation of the purpose and protocol for this study, patient provided written informed consent.

Consent for publication: Written informed consent was obtained from the patient for publication and any accompanying images.

Availability of data and materials: Not applicable.

Competing interests: The authors report no conflicts of interest in this work.

Funding: None

Authors' contributions: K.Y. and T.Y. managed the patient clinically. S.S. performed and evaluated histological study. M.H. performed and evaluated biochemical study. M.H., K.Y. and S.S. wrote the manuscript. T.Y. revised draft. All authors discussed the results and approved the final manuscript.

Acknowledgments: We are grateful to Dr. Akira Abe for giving us instructions for chemical analysis.

References

1. Hawes MJ (1988) The dacryolithiasis syndrome. Ophthalmic Plast Reconstr Surg 4:87–90
2. Mantelli F, Argueso P (2008) Functions of ocular surface mucins in health and disease. Curr Opin Allergy Clin Immunol 8:477–483.ã
3. Kleine A, Kluge S, Peskar BM (1993) Stimulation of prostaglandin biosynthesis mediates gastroprotective effect of rebamipide in rats. Dig Dis Sci 38:1441–1449
4. Higuchi K, Arakawa T, Nebiki H, Uchida T, Fujiwara Y, Ando K, Yamasaki K, Takaishi O, Fukuda T, Kobayashi K, Kuroki T (1998) Rebamipide prevents recurrence of gastric ulcers without affecting Helicobacter pylori status. Dig Dis Sci 43 9:99S–106S

5. Naito Y, Yoshikawa T, Iinuma S, Yagi N, Matsuyama K, Boku Y, Fujii T, Yoshida N, Kondo M, Sasaki E (1998) Rebamipide protects against indomethacin-induced gastric mucosal injury in healthy volunteers in a double-blind, placebo-controlled study. Dig Dis Sci 43(Suppl 9):83S–89S
6. Kinoshita S, Oshiden K, Awamura S, Suzuki H, Nakamichi N, Yokoi N (2013) Rebamipide ophthalmic suspension phase 3 study group. A randomized, multicenter phase 3 study comparing 2% rebamipide (OPC-12759) with 0.1% sodium hyaluronate in the treatment of dry eye. Ophthalmology 120:1158–1165
7. Urashima H, Okamoto T, Takeji Y, Shinohara H, Fujisawa S (2004) Rebamipide increases the amount of mucin-like substances on the conjunctiva and cornea in the N-acetylcysteine-treated in vivo model. Cornea 23:613–619
8. Tanaka H, Fukuda K, Ishida W, Harada Y, Sumi T, Fukushima A (2013) Rebamipide increases barrier function and attenuates TNFα-induced barrier disruption and cytokine expression in human corneal epithelial cells. Br J Ophthalmol 97:912–916
9. Kimura K, Morita Y, Orita T, Haruta J, Takeji Y, Sonoda KH (2013) Protection of human corneal epithelial cells from TNF-α-induced disruption of barrier function by rebamipide. Invest Ophthalmol Vis Sci 54:2572–2560
10. Kase S, Shinohara T, Kase M, Ishida S (2017) Effect of topical rebamipide on goblet cells in the lid wiper of human conjunctiva. Exp Ther Med 13:3516–3522
11. Sugimoto Y, Fukuta Y, Tsubota K, Ohashi Y, Kinoshita S (2015) [Retrospective review of dacryostenosis, dacryocystitis and foreign body in eye or lacrimal duct under administration of rebamipide ophthalmic susupension (Mucosta ophthalmic suspension UD 2%)]. Journal of the eye 32:1741–1747. Japanese
12. Anderson NG, Wojno TH, Grossniklaus HE (2003) Clinicopathologic findings from lacrimal sac biopsy specimens obtained during dacryocystorhinostomy. Ophthalmic Plast Reconstr Surg 19:173–176
13. Ikeda T, Hiraoka M, Inatomi S, Funabashi K, Yamashita K, Sugita S, Ohguro H (2017) Bilateral dacryolith in two cases of Sjogren syndrome. Japanese J Clin Ophthalmol 71:593–598. Japanese
14. Iliadelis ED, Karabatakis VE, Sofoniou MK (1999) Dacryoliths in chronic dacryocystitis and their compositin (spectrophotometric analysis). Eur J Ophthalmol 9:266–268
15. Mano F, Takimoto H, Oe M, Chang K, Mano T, Yoshida Y (2018) Proteomic analysis of dacryoliths from patients with or without topical rebamipide treatment. Biomed Hub 3:1–11
16. **Titles and legends to figures**

Figures

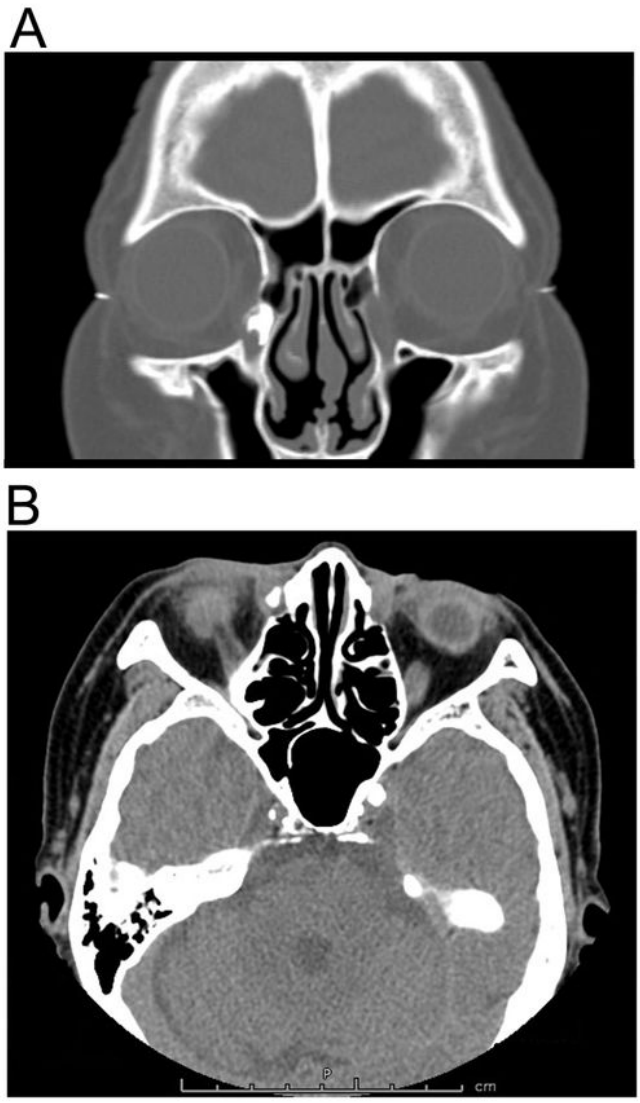

Figure 1

A head computed tomography (CT) scan. A. The born window image on the coronal section (A) and the contrast-enhanced image on the axial section (B) demonstrated high-density deposits in right lacrimal sac.

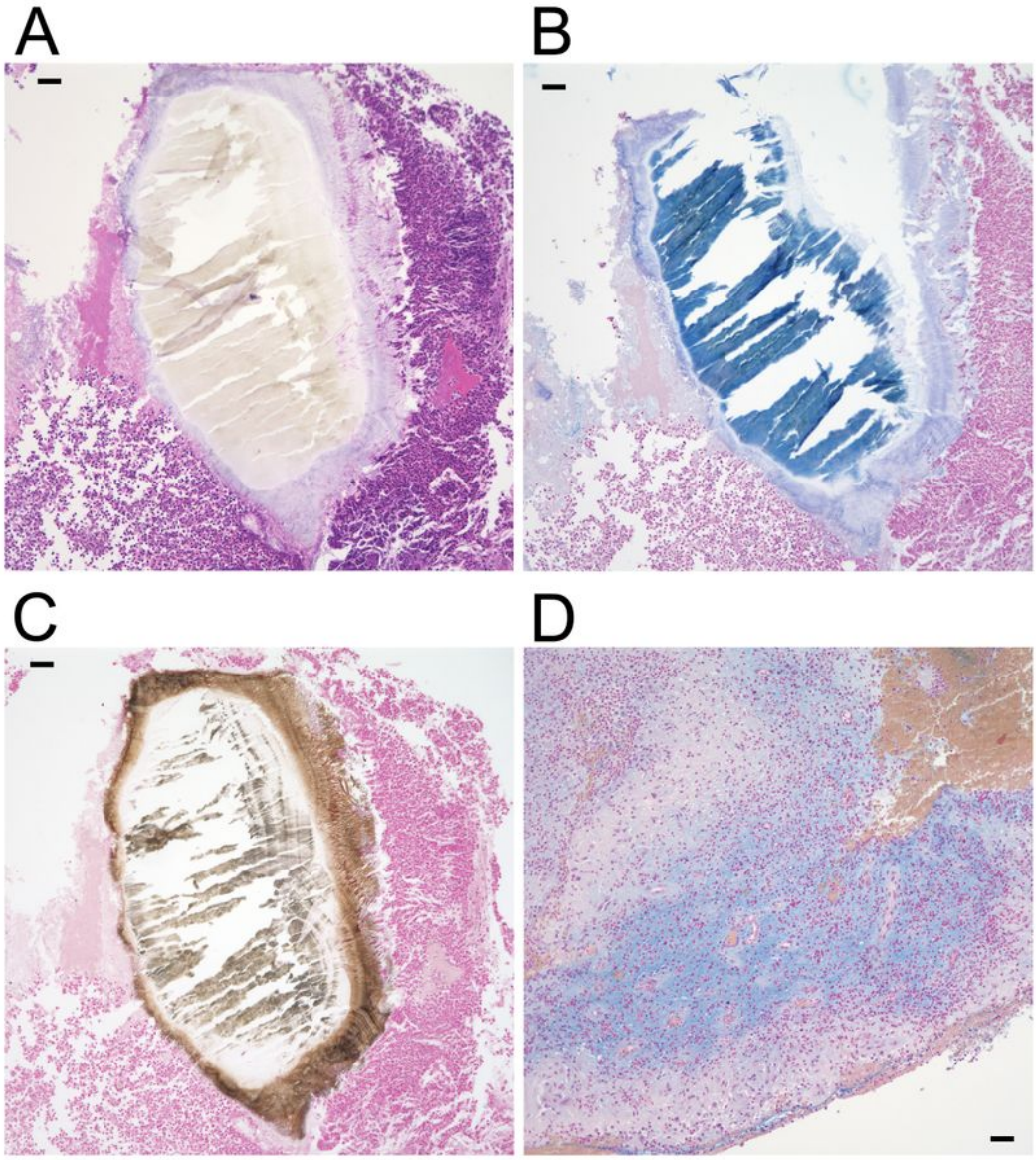

Figure 2

Histology of surgically removed specimen. It showed crystal-like structure and granulation tissues. A. Hematoxylin-eosin staining. B. D. Alcian blue staining. C. Kossa staining. A. B. C. The lesion of the crystal-like structure area. D. The lesion of the granulation area. Scale bar indicates 50 μm.

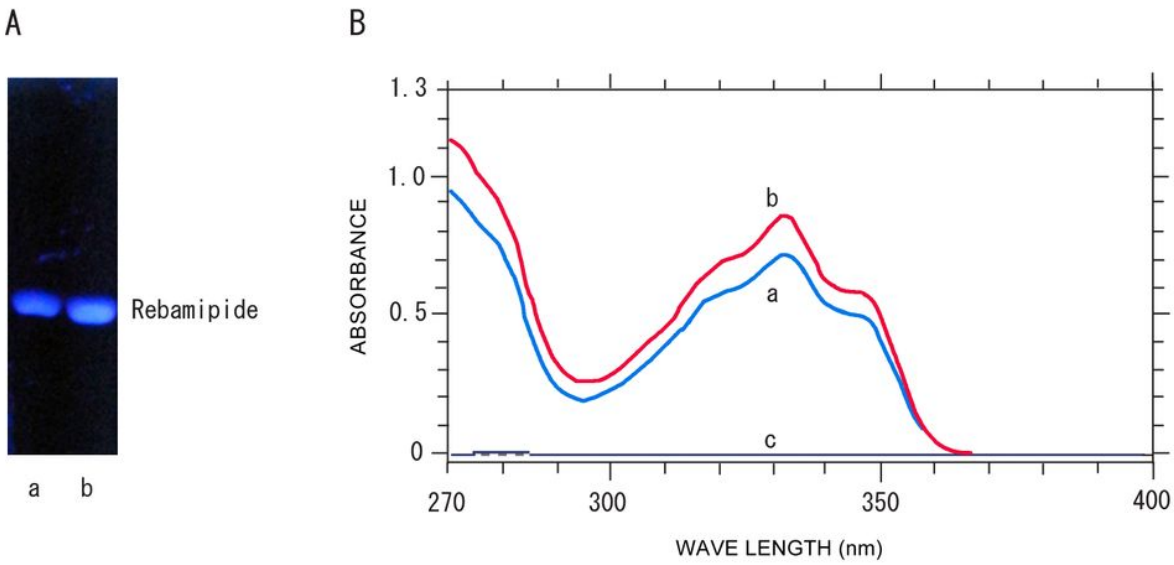

Figure 3

Chemical analysis of white mass. A. In thin layer chromatography (TLC), white mass extracts (b) showed the same mobility as rebamipide (a). B. In UV spectrometry, white mass extracts (b) showed the same spectrum pattern as rebamipide (a). Negative control (DMF) did not show any peak (c).

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- JOISMikiHiraokaMember2020.pdf