Phytomedicine 21 (2014) 1015–1020

Contents lists available at ScienceDirect

Phytomedicine

journal homepage: www.elsevier.de/phymed

Efficacy and safety of *Indigo naturalis* extract in oil (Lindioil) in treating nail psoriasis: A randomized, observer-blind, vehicle-controlled trial

Yin-Ku Lin[a,b,*,1], Lai-Chu See[c,1], Yu-Huei Huang[d,e], Ya-Ching Chang[d,e], Teng-Cheng Tsou[a], Tung-Yi Lin[a], Na-Ling Lin[c]

[a] *Department of Traditional Chinese Medicine, Chang Gung Memorial Hospital at Keelung, Taiwan*
[b] *School of Traditional Chinese Medicine, Chang Gung University, Taoyuan, Taiwan*
[c] *Biostatistics Consultation Center, Department of Public Health, and Biostatistics Core Laboratory, Molecular Medicine Research Center, Chang Gung University, Taoyuan, Taiwan*
[d] *Department of Dermatology, Chang Gung Memorial Hospital at Taipei, Taiwan*
[e] *College of Medicine, Chang Gung University, Taoyuan, Taiwan*

ARTICLE INFO

Article history:
Received 16 October 2013
Received in revised form 20 January 2014
Accepted 24 February 2014

Keywords:
Traditional Chinese medicine
Indigo naturalis
Indirubin
Psoriasis
Nail psoriasis

ABSTRACT

Treating nail psoriasis is notoriously difficult and lacks standardized therapeutic regimens. Indigo naturalis has been demonstrated to be safe and effective in treating skin psoriasis. This trial was conducted to evaluate the efficacy and safety of refined indigo naturalis extract in oil (Lindioil) in treating nail psoriasis. Thirty-one outpatients with symmetrically comparable psoriatic nails were enrolled. Lindioil (experimental group) or olive oil (control group) was applied topically to the same subjects' two bilaterally symmetrical psoriatic nails twice daily for the first 12 weeks and then subjects applied Lindioil to both hands for 12 additional weeks. Outcomes were measured using Nail Psoriasis Severity Index (NAPSI) for five nails on one hand and for the single most severely affected nail from either hand. The results show a reduction of NAPSI scores for the 12-week treatment for the Lindioil group (49.8% for one hand and 59.3% for single nail) was superior to the reduction in the scores for the control group (22.9%, 16.3%, respectively). There were no adverse events during the 24 weeks of treatment. This trial demonstrates that Lindioil is a novel, safe and effective therapy for treating nail psoriasis.

Introduction

Nail psoriasis is a notoriously difficult disease to treat and is common among patients with psoriasis, occurring in 50% of patients with the disease (Reich 2009). Psoriasis with nail involvement is a relevant manifestation of psoriasis and the estimated lifetime incidence is 80–90% (Reich 2009). The unsightly appearance of affected nails has a marked impact on the patient's quality of life (de Jong et al. 1996).

To date, there are no standardized therapies for the treatment of nail psoriasis. The most common therapies in treating nail psoriasis are topical steroid, vitamin A (Tazarotene) or vitamin D analog (Calcipotriol). These therapies may have a negative impact on patient's compliance because nail psoriasis requires prolonged treatment and continuity. Occasionally these therapies are associated with side effects, such as skin atrophy and irritation, and/or disappointing results. Therefore, it is necessary to establish safety profiles for treatments intended for long-term use.

Indigo naturalis has been used for centuries in traditional Chinese medicine because of its antipyretic, anti-inflammatory, antiviral, antimicrobial, and detoxifying effects. Indigo naturalis and its active component, indirubin, have been used for decades in China to treat systemic psoriasis and leukemia (Xiao et al. 2002; Koo and Arain 1998).

Previous studies have shown that topical application of indigo naturalis significantly improved skin psoriatic symptoms (Lin et al. 2007; Lin et al. 2008). However, compliance is affected because the ointment is difficult to wash off and causes esthetically unappealing dark blue stains on the applied area.

In 2008, we optimized the formulation for indigo naturalis and named it "Lindioil". Lindioil is a trademark product name created by

Abbreviations: AE, adverse event; CI, confidence interval; NAPSI, Nail Psoriasis Severity Index; mtNAPSI, modified target Nail Psoriasis Severity Index; PASI, psoriasis area and severity index; PGA, physician global assessment; SD, standard deviation; SGA, subject global assessment; shNAPSI, single hand Nail Psoriasis Severity Index.

* Corresponding author. Tel.: +886 2 24313131x2777; fax: +886 2 25462083.
E-mail addresses: lin1266@adm.cgmh.org.tw, lin1266@hotmail.com (Y.-K. Lin).
[1] Yin-Ku Lin and Lai-Chu See are co-first authors.

http://dx.doi.org/10.1016/j.phymed.2014.02.013
0944-7113/

the author using a proprietary extraction and formulation process which meets consistent commercial pharmaceutical Chemistry, Manufactory and Control (CMC) standards. The extract process for indigo naturalis was changed by the use of olive oil, which would be more acceptable to the patients because it could be easily washed off and would not leave visible stains on the skin or nails (Lin 2011).

To test the formulation change, a clinical trial was performed to compare Lindioil ointment to crude indigo naturalis ointment (Lin et al. 2012a). It was proven that Lindioil was equally effective in treating skin psoriasis. A concurrent non-controlled clinical trial also demonstrated that Lindioil was efficacious and safe in treating nail psoriasis; however, this pilot trial lacked a control arm (Lin et al. 2011). Consequently we conducted a randomized, observer-blind, vehicle-controlled, intra-subject trial to evaluate the efficacy and safety of Lindioil in treating nail psoriasis.

Materials and methods

Patients

Subjects with nail psoriasis were recruited from the Department of Traditional Chinese Medicine and Dermatology at the Chang Gung Memorial Hospital (CGMH), Taiwan, from September 2009 to May 2011. Subjects aged 20–65 years with symmetrically comparable psoriatic nails on each hand were eligible. Females of childbearing age were instructed to use birth control measures during the study period. Subjects were excluded if they had a known allergy to indigo naturalis, had any other obvious fingernail infection signs (such as redness, swelling, pus excretion), were pregnant or planning to become pregnant, were breast feeding or were currently using concomitant topical or systemic treatments that could affect nail psoriasis.

This study was approved by the Institutional Review Board of CGMH (approval number 98-2184C) and was registered in ClinicalTrial.gov (Identifier: NCT00999687, http://register.clinicaltrials.gov). Written informed consent was obtained from each subject.

Plant material and drug preparation

The powdered form of indigo naturalis used in this study was prepared from the leaves of *Baphicacanthus cusia* (Nees) Bremek and purchased from Shufeng Shangrchia Indigo Farmer Cooperative, Xianyou county, Fujian province, China. The CMC report was created by Chuang Song Zong Pharmaceutical Co., Ltd. (CSZ), Taiwan. The material was identified and authenticated by Ms. Shu-Tuan Chiang, Research and Quality Control Department Manager of CSZ, with sample vouchers stored for reference under the code number M0813. The fingerprints and quantity analysis of standard samples, indirubin and indigo blue, were established. Pure indigo blue was purchased from Fluka (Buchs, Switzerland), indirubin was obtained from Alexis (Lausen, Switzerland). Indigo naturalis powder used in this trail contained 3.15% indigo blue and 0.15% indirubin as determined by the HPLC.

In this trial, olive oil was added to extract the powdered indigo naturalis from *B. cusia* (Nees) Bremek. Previous analyses of the leaves and the powdered form of this indigo naturalis plant detected indigo, indirubin, tryptanthrin and isatin (Chiang et al. 2013). According to the Guidance for Industry Botanical Drug Products, the CMC report isolated indirubin and indigo blue only.

Lindioil was prepared by the CSZ using powdered indigo naturalis mixed with olive oil (1:10), Kirkland Signature Pure Olive Oil (Italian, item 71008), then heated at 120 °C for 1 h and filtered by 2-μm filter (Millipore, France). The oil extract was tested using HPLC analysis and placed in 5-ml eye drop bottles for clinical use.

Analysis of indigo naturalis and Lindioil using HPLC

The HPLC analysis was performed on a Waters 2690 Separations Module/Waters 2996 Photodiode Array Detector and detected at $\lambda = 289$ nm. The chromatographic separation was carried out on a Cosmosil C18 MS-II column (4.6 mm × 250 mm i.d.; 5 μm particle size) eluted with the mixture of 0.2% phosphoric acid (A) and methanol (B). The linear gradient program was set from 50% of 0.2% phosphoric acid plus 50% of methanol to 30% of 0.2% phosphoric acid plus 70% of methanol for 40 min. The flow rate was 1.0 ml/min. The sample injecting volume for each program was 20 μl.

A standard solution was prepared using indirubin from Sigma. The calibration curve was constructed with dilutions of 2.5, 5, 10, 20 and 40 μg/ml in DMSO. A volume of 20 μl was injected and the calibration curve was based on the peak areas of the HPLC chromatograms. The calibration curve showed an $R^2 = 1.0$.

Study design

This study was a randomized, observer-blind, vehicle-controlled, intra-subject trial. Lindioil was applied to the fingernails of one bilateral hand (Lindioil group) and olive oil was applied to the fingernails of the contra-lateral hand (control group) twice daily for 12 weeks. The randomization process was conducted by block randomization method with 1:1 ratio. Beginning with week 13, Lindioil was applied to all affected nails on both hands twice daily for another 12 weeks.

All subjects were instructed to apply one drop (0.05 ml) of Lindioil or olive oil onto the nail plate folds and hyponychium of affected nails twice daily (morning and before bed). Subjects were asked to avoid washing their hands during the first 30 min after application, to avoid activities that might injure the nails and to avoid cross-contamination between the two hands. Subjects were treated for a maximum of 24 weeks unless there was complete clearing of nail psoriasis or they developed obvious local or systemic adverse events (AEs) possibly related to treatment. Subjects who did not comply with the study protocol were terminated from the study. In addition, subjects were allowed to drop out of the study for any reason at any time.

Outcome measures

The primary efficacy measures used were single hand Nail Psoriasis Severity Index (shNAPSI) for all of the nails on one hand and modified target NAPSI (mtNAPSI) for the single most severely affected nail on both hands. Photos of the affected fingernails were taken at baseline and at 4-week intervals for 24 weeks. At each visit, all subjects were requested to clean their fingernails thoroughly so no staining was visible before photographs were taken. Two physicians, who were blinded to the treatment modality, rated the shNAPSI and mtNAPSI based on the photographs of the fingernails. Prior to the study, these two physicians underwent a special training course for the assessment of shNAPSI and mtNAPSI to minimize inter and intra-rater discrepancies.

The NAPSI is a reproducible, objective, and simple tool for clinical assessment of nail psoriasis and is the only clinical scale that has been validated. The nail is divided by imaginary horizontal and longitudinal lines into quadrants. Each nail is given a score for nail bed psoriasis (0–4) and nail matrix psoriasis (0–4) depending on the presence of any of the features of nail psoriasis in that quadrant. The NAPSI evaluates presence of signs in the nail bed (onycholysis, splinter hemorrhages, nail bed discoloration, and subungual hyperkeratosis) and on the nail matrix (pitting, leukonychia, red spots in the lunula and nail plate crumbling) in all 10 fingernails, providing a maximum score of 80 (Rich and Scher 2003). Since this study is an intra-subject hand-to-hand comparison and all 5 fingers on each