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Short communication

In vitro activity of (-)-deoxypergularinine, on its own and in combination with anti-tubercular drugs, against resistant strains of *Mycobacterium tuberculosis*

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ABSTRACT

Background: The increasing incidence of multidrug-resistant tuberculosis (MDR-TB) infections has created a need for new effective drugs that also target extensively drug-resistant tuberculosis (XDR-TB) and/or augment the activities of existing drugs against tuberculosis.
Aim: This study searched natural products for a new lead compound that targets MDR/XDR-TB.
Methods: An active compound was purified from the roots of *Cynanchum atratum* Bunge (*Asclepiadaceae*) after screening 1640 plant extracts, and its inhibitory effects against MDR/XDR strains and synergistic effects with existing anti-TB drugs were assessed using the resazurin, MGIT, and checkboard assays.
Results: (-)-Deoxypergularinine, purified from the roots of *C. atratum*, inhibited not only *M. tuberculosis* but also MDR/XDR strains. The minimum inhibitory concentrations (MICs) of (-)-deoxypergularinine for H37Ra, H37Rv, MDR, and XDR strains were all about 12.5 μg/ml. Moreover, combinations of (-)-deoxypergularinine with the first-line standard drugs rifampicin or isoniazid afforded six- and eight-fold reductions in drug MIC values, respectively, against strain H37Ra.
Conclusions: (-)-Deoxypergularinine exerts anti-tubercular activities not only against normal tuberculosis strains but also MDR/XDR strains, and synergic effects with rifampicin and isoniazid for the H37Ra strain. The alkaloid may be valuable for targeting M/XDR *M. tuberculosis*.

Introduction

Tuberculosis (TB) is a widespread infectious disease usually caused by *Mycobacterium tuberculosis*. The disease causes an estimated 1.7 million deaths annually and the current annual number of new cases worldwide (over 9 million) is higher than at any other time in history (Lawn and Zumla 2011). The two antibiotics most commonly prescribed are rifampicin and isoniazid (INH), but treatment can be prolonged, often requiring several months. Active TB is best treated with a combination of several antibiotics to reduce the risk of development of antibiotic-resistance. However, such resistance is a growing problem when multiple drug-resistant tuberculosis (MDR-TB) infections are to be treated; the bacteria are not killed by first-line standard treatments (i.e. the strains are resistant to [at least] rifampicin and INH). Inappropriate treatment is the principal risk factor for development of MDR-TB. The association between TB and HIV, and the increasing emergence of MDR-TB and extensively drug-resistant TB (XDR-TB; such strains are resistant to second-line drugs, for example capreomycin, kanamycin, and/or amikacin), have worsened the situation; the threat to health is serious (Matteelli et al. 2014). Therefore, new anti-TB agents active against MDR/XDR-TB are urgently required.

The dried roots of *Cynanchum atratum Bunge (Asclepiadaceae)*, commonly termed “Bai Wei” in Chinese, are rich in C-21 steroidal glycosides (Zhao et al. 2009), cynatratosides, pregnan glycosides (Bai et al., 2009), seco-pregnan steroidal glycosides

Abbreviations: (-)-DPG, (-)-deoxypergularinine; TB, tuberculosis; MDR-TB, multidrug-resistant tuberculosis; XDR-TB, extensively drug-resistant tuberculosis; MGIT, mycobacteria growth indicator tube; MICs, minimum inhibitory concentrations; INH, isoniazid; TMV, tobacco mosaic virus; MIC, minimum inhibitory concentration; FICIs, fractional inhibitory concentration indices.

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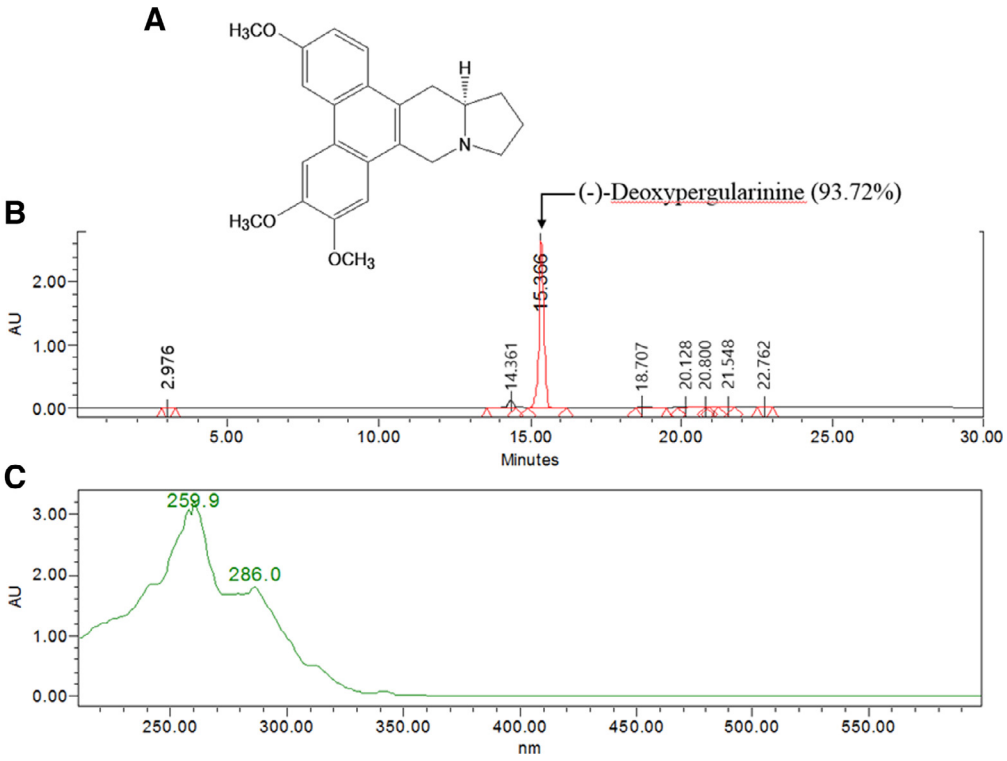

Fig. 1. (A) Chemical structure of (-)-deoxypergularinine. (B) HPLC chromatogram showing the active compound (AB6163) detected at 254 nm. Based on the peak area, the active compound contained 93.72 ± 0.13% (-)-deoxypergularinine. (C) UV absorption spectrum of the major peak of (-)-deoxypergularinine.

(Yan et al., 2014), and 2,4-dihydroxyacetophenone (Yuan et al., 2007). *C. atratum* exerts anti-osteoporotic effects (Mukudai et al., 2014), anti-acetylcholinesterase, anti-amnesic, (Lee et al., 2005), anti-tobacco mosaic virus (TMV) (Yan et al., 2014), and anti-parasitic effects (Fu et al., 2014). In the present study, we isolated (-)-deoxypergularinine from the roots of *C. atratum* and tested it against *M. tuberculosis* H37Rv, MDR/XDR-TB, pyrazinamide-resistant-TB, INH-resistant-TB, rifampicin-resistant-TB, and streptomycin-resistant-TB strains. The alkaloid was evaluated both alone and in combination with existing drugs.

Materials and methods

Preparation of crude plant extract

Air-dried roots of *C. atratum* were purchased from the medicinal herbs market of Jegi-dong, Dongdaemun-gu, South Korea. A voucher specimen was deposited in the Herbarium of the College of Life Science, Soonchunhyang University (SCH-2013-0017). The roots (1.0 kg) were extracted three times with EtOH (9 L × 7 days) at room temperature and the extract concentrated under reduced pressure. The dried extract (238.1 g) was suspended in water (1 L) and partitioned with *n*-hexane (fr. A, 33.2 g), dichloromethane (fr. B, 17.3 g), ethyl acetate (fr. C, 5.5 g), and water (fr. D, 180.2 g).

Bioassay-guided fractionation and isolation of (-)-deoxypergularinine

Bioassay-guided fractionation was performed to isolate and identify compounds with anti-tubercular effects. Frs. A and B (44.0 g) were subjected to column chromatography using a step-wise gradient of dichloromethane/ethanol (10:1, 5:1, 1:1, and 1:5, v/v), and ethanol, to yield seven sub-fractions (fr. AB1-fr. AB7). The most active fraction (fr. AB6) was further subfractionated on a silica gel column via elution with dichloromethane/ethanol (20:1, 10:1, 5:1, 1:1, 1:5, and 0:10, v/v) to obtain new fractions AB61-AB66. The active fraction AB61 was further fractionated on Sephadex LH-20, using ethanol, to obtain fractions AB611-AB616. The active fraction AB616 was further fractionated on an RP silica gel column eluted with ethanol/water (80:20, v/v) to yield compounds AB6161, AB6162, and AB6163. The active compound AB6163 (93.72%) was separated by high performance liquid chromatography (HPLC) on an RP-18, 5 μm (particle size), 4.6 mm × 250 mm (length) column. A gradient system of aqueous acetonitrile (0.1% TFA) from 30% (0 min) to 100% (30 min) was applied at a flow rate of 1 ml/min (Fig. 1B and C). The compound was recrystallised from pyridine and identified with the aid of various spectroscopic techniques, including IR absorption, UV absorption, specific rotation, ESI–MS, HR-ESI–MS, 1D NMR (^{1}H,^{13}C NMR), DEPT, and 2D NMR (^{1}H-^{1}H COSY, HSQC, HMBC). The compound was (-)-deoxypergularinine and its optical rotation is given in the Results (Lebrun et al., 1999; Han et al., 2013).

Bacterial strains

The H37Rv strain of *M. tuberculosis* subsp. *tuberculosis* (ATCC® 27294™) was obtained from the American Type Culture Collection (Rockville, MD, USA). MDR-TB (KMRC 00116-00250), XDR-TB (KMRC 00203-00197), pyrazinamide-resistant TB (KMRC 00130-00064), INH-resistant TB (KMRC 00120-00137), rifampicin-resistant TB (KMRC 00121-00341), and streptomycin-resistant TB (KMRC 00122-00123) were purchased from the Korean Mycobacterium Resource Centre (KMRC).

Reagents and antibiotics

INH, rifampicin, and streptomycin were purchased from Sigma. A stock solution of INH was prepared in sterilized distilled water and that of rifampicin in DMSO (Sigma, St Louis, Mo, USA). In all experiments, the final concentration of DMSO (Sigma) in culture medium was 0.5% (v/v).