Journal of Global Antimicrobial Resistance 10 (2017) 228–237

Contents lists available at ScienceDirect

Journal of Global Antimicrobial Resistance

journal homepage: www.elsevier.com/locate/jgar

Sesamol exhibits potent antimycobacterial activity: Underlying mechanisms and impact on virulence traits

Sandeep Hans, Sharda Sharma, Saif Hameed*, Zeeshan Fatima*

Amity Institute of Biotechnology, Amity University Haryana, Gurgaon (Manesar) 122413, India

ARTICLE INFO

Article history:
Received 7 March 2017
Received in revised form 31 May 2017
Accepted 12 June 2017
Available online 19 July 2017

Keywords:
Ses
Mycobacterium
Colony morphology
Sliding motility
Passive diffusion
Lipase
Biofilm
Cell adherence
Oxidative stress
Iron
DNA damage

ABSTRACT

Objectives: Novel strategies to overcome multidrug resistance (MDR) in Tuberculosis (TB) still remain a concern. Usage of natural compounds nowadays to surmount the increasing burden of MDR-TB has shown promising results. The aim of this study was to evaluate the antimycobacterial potential of sesamol (Ses) a natural phenolic compound against *Mycobacterium smegmatis*, a surrogate for MTB and its underlying mechanism of action along with its effect on mycobacterial virulence traits.

Methods: Cell surface phenotypes were estimated microscopically and spectrophotometrically respectively. Membrane parameters were assessed using propidium iodide (PI) uptake, passive diffusion of drug with substrate EtBr and phenotypic susceptibility assay. Changes in lipid profiles were estimated by lipase assay. Oxidative and genotoxic damage were studied using fluorescent probes DCFDA and DAPI. Biofilm formation was studied using crystal violet and calcoflour white staining probes along with biomass measurement. Cell adherence was estimated using buccal epithelial cells.

Results: We observed that antimycobacterial activity of Ses was 6 mM and it enhances the efficiency of known anti-TB drugs. Ses affects cell surface phenotypes as displayed by altered colony morphology, impaired sliding motility and enhanced cell sedimentation rate. Membrane perturbation was revealed by hypersensitivity against SDS, reduced PI uptake, enhanced passive diffusion and lipase activity. In addition, Ses leads to oxidative and DNA damage along with abrogated iron homeostasis. Furthermore, we uncover phenotypes related to virulence like inhibited biofilm formation and cell adherence to buccal epithelial cells.

Conclusion: This study for the first time establishes the anti-mycobacterial potential of Ses that may be further exploited for improving the therapeutic strategies and warrants further attention.

1. Introduction

Tuberculosis (TB) caused by *Mycobacterium tuberculosis* (MTB) continues to be a major cause of mortality and morbidity worldwide. TB has been consistently claiming a larger number of lives than any other infectious disease [1]. Present arsenal of available anti-TB drugs and vaccine has no significant impact on TB control. In addition they also suffer from disadvantages of side effects and cost effectiveness [2]. Under such compelling circumstances, to look for novel drugs and adopt novel strategies to conquer TB, researchers are continuously working for alternative options.

Natural compounds have attracted attention nowadays in search for anti-TB drugs because of their richness in chemical diversity and inherent privileged antimicrobial activity [3]. Previously, we have deciphered the antifungal potential of Sesamol (Ses), a natural phenolic compound isolated from sesame oil with its possible mode of action in prevalent human pathogen, *Candida albicans* [4,5]. Ses is already known for its beneficial properties and is non toxic in nature [6]. In this study, we have extended to elucidate the antimycobacterial potential of Ses against *Mycobacterium smegmatis*, a surrogate of MTB. We explored that Ses acts on multiple targets responsible to contribute development of MDR along with its effect on virulence traits of mycobacteria.

2. Material and methods

All Media chemicals Middlebrook 7H9 broth, Middlebrook 7H10 agar, albumin/dextrose/catalase (ADC), oleic acid/albumin/dextrose/catalase (OADC) supplements was purchased from BD

* Corresponding authors.
E-mail addresses: saifhameed@yahoo.co.in (S. Hameed), drzeeshanfatima@gmail.com (Z. Fatima).

http://dx.doi.org/10.1016/j.jgar.2017.06.007

Biosciences (USA). Tween-80, sesamol (Ses) INT (iodonitrotetrazolium), Isoniazid (INH), Ethambutol (EMB), DAPI (4′6-diamidino-2-phenylindole dihydrochloride) and DCFDA (dichlorodihydrofluorescein diacetate) were obtained from Sigma Chemical Co. (St. Louis, MO, USA). Ethidium Bromide (EtBr), Dinitrophenol (2,4, DNP), Propidium iodide (PI), Methanol (CH_3OH), Triton X crystal violet, ferrozine and calcofluor white were purchased from Himedia (Mumbai, India). Dimethyl sulfoxide (DMSO), Potassium chloride (KCl), Sodium chloride (NaCl), di-Sodium Hydrogen Orthophosphate (Na_2HPO_4), Potassium Di-hydrogen Orthophosphate (KH_2PO4), Glycerol, Sodium Dodecyl Sulphate (SDS), Boric acid (H_3BO_3), ferrous sulphate ($FeSO_4$) and ascorbic acid (AA) were obtained from Fischer Scientific. Agarose was purchased from CDH, India. Sodium acetate was purchased from Qualigens Fine Chemicals (Mumbai, India).

2.1. Strains and growth culture

M. smegmatis mc^2155 was grown in Middlebrook 7H9 (BD Biosciences) broth supplemented with 0.05% Tween-80 (Sigma–Aldrich), 10% ADC (BD Biosciences), and 0.2% glycerol (Thermo Fischer Scientific) in 100-mL flasks and incubated at 37 °C. Cultures were subsequently grown on Middlebrook 7H10 (BD Biosciences) agar media supplemented with 10% (v/v) OADC (BD Biosciences) for solid agar allowing growth for 48 h at 37 °C. Stock cultures of log-phase cells were maintained in 30% glycerol and stored at −80 °C.

2.2. Drug-susceptibility testing

2.2.1. Minimum inhibitory concentration

Minimum inhibitory concentration (MIC) was determined using the broth-dilution method described previously [7,8] according to Clinical and Laboratory Standards Institute guidelines. Briefly, 100 μL of Middlebrook 7H9 broth was placed in each well of a 96-well plate, followed by the addition of the drug with the remaining media and subsequent serial dilution. Cell suspension (100 μL in normal saline at an optical density (OD_{600}) of 0.1) was added to each well, followed by incubation at 37 °C for 48 h. The MIC (Genetix, Biotech Asia Pvt. Ltd.) values were evaluated by observing the OD_{600} in a microplate reader. The value was considered as MIC_{80} which showed atleast 80% inhibition in OD_{600} compared to positive control without any drug.

2.2.2. Spot assay

Spot assays for the strains were determined using a method described previously [7,8]. Briefly, 5 μl of fivefold serial dilutions of each *M. smegmatis* culture (each with cells suspended in normal saline to an OD_{600} of 0.1) was spotted onto Middlebrook 7H10 agar plates in the absence (control) or presence of Ses. Growth difference was measured after incubation at 37 °C for 48 h.

2.3. Colony morphology and sliding motility

Colony morphology and sliding motility were determined as described elsewhere [8,9]. Briefly, cells were plated on MB7H10 agar plates supplemented with 10% OADC (BD Difco) and incubated at 37 °C for 2 to 4 days in presence of Ses. Post incubation, images of the individual colonies were taken at 10× magnification. To score for sliding motility, cells were grown until stationary phase in MB7H9 medium supplemented with 10% OADC (BD Difco) in the absence (control) and presence of Ses at its sub-inhibitory concentration (2 mM). 3 μl of the culture was spotted in the middle of MB7H10 plates solidified with 0.3% agarose without any carbon source. The plates were incubated at 37 °C for 4 days.

2.4. Cell sedimentation assay

Cell sedimentation assay was performed as described elsewhere [8,9]. Cultures at $OD_{600} \sim 1.0–1.4$ of the control and cells treated with Ses at its sub-inhibitory concentration (2 mM) in Middlebrook media supplemented with ADC were adjusted in triplicate to $OD_{590} \sim 1.0$ and kept unshaken at 37 °C. At 3 and 22 h, the upper 1 ml was removed for OD_{590} measurements.

2.5. Propidium iodide uptake assay

Propidium iodide (PI) uptake assay was performed as described elsewhere with modification [10]. Briefly, cells were grown at 37 °C for 24 h in Middlebrook broth supplemented with 10% ADC with Ses. The cells were harvested by centrifugation and suspended in PBS to 0.5 OD at 600 nm. 50 μg PI added to 1 ml bacterial suspension was placed in each tube and kept at room temperature for 15 min in the dark. 10 μl bacterial suspension was transferred to a glass slide, covered with cover-slip and examined under fluorescence microscope at 40×.

2.6. Passive diffusion of EtBr

The efflux of EtBr was determined by using protocol described previously [8,11]. Briefly, approximately 1×10^6 cells were incubated until exponential phase in the absence (control) and presence of Ses at its sub-inhibitory concentration (2 mM). Cells were pelleted, washed twice with phosphate-buffered saline (PBS) (without glucose), and resuspended as a 2% cell suspension. The cells were then de-energized with an efflux pump inhibitor 2,4 DNP (20 μg/ml) in PBS (without glucose). The de-energized cells were pelleted, washed, and then resuspended as a 2% cell suspension (w/v) in PBS without glucose, to which EtBr was added at a final concentration of 4 μg/ml and incubated for 45 min at 30 °C. The equilibrated cells with EtBr were then washed and resuspended as a 2% cell suspension (w/v) in PBS (with glucose 0.4%) for EtBr efflux and PBS (w/o glucose) for passive diffusion respectively. Samples with a volume of 2 ml were withdrawn at different time interval and centrifuged at 10,000 rpm for 1 min. The supernatant was collected, and absorption was measured at 285 nm. Glucose-free controls were included in all the experiments.

2.7. Lipase assay

M. smegmatis cells were grown in middlebrook 7H9 broth in the absence (control) and presence of Ses. Whole cell protein was extracted and protein concentration was determined by Lowry method as previously described [12]. Lipase activity was performed by measuring the amount of *p*-nitrophenol (*p*-NP) released from *p*-NP ester substrate with varying lengths of fatty acids. The total lipase activity was assayed as using protein extract of *M. smegmatis*. The standard lipase activity assays were performed in 100 μl reaction system consisting of a final concentration of 0.5 mM *p*-NP esters substrate and the buffer (pH 8.0) of 80 mM H_3BO_3, 80 mM H_3PO_4, 300 mM NaCl, 0.3% Triton X-100 and 20% glycerol. The reaction mixture of purified protein was incubated at 37 °C for 40 min and the release of *p*-nitrophenol was determined by measuring spectrophotometrically at 405 nm [13,14].

2.8. ROS estimation

M. smegmatis cells were grown in middlebrook 7H9 broth in the absence (control) and presence of Ses and allowed to grow until it reaches 0.8–0.9 OD. The cells were harvested at 13,000 rpm for