International Journal of Antimicrobial Agents 47 (2016) 195–201

Contents lists available at ScienceDirect

International Journal of Antimicrobial Agents

journal homepage: http://www.elsevier.com/locate/ijantimicag

Repurposing auranofin for the treatment of cutaneous staphylococcal infections

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ARTICLE INFO

Article history:
Received 21 August 2015
Accepted 27 December 2015

Keywords:
Repurposing
Auranofin
Multidrug resistance
Topical antimicrobials
Inflammatory cytokines

ABSTRACT

The scourge of multidrug-resistant bacterial infections necessitates the urgent development of novel antimicrobials to address this public health challenge. Drug repurposing is a proven strategy to discover new antimicrobial agents; given that these agents have undergone extensive toxicological and pharmacological analysis, repurposing is an effective method to reduce the time, cost and risk associated with traditional antibiotic innovation. In this study, the in vitro and in vivo antibacterial activities of an antirheumatic drug, auranofin, was investigated against multidrug-resistant *Staphylococcus aureus*. The results indicated that auranofin possesses potent antibacterial activity against all tested strains of *S. aureus*, including meticillin-resistant *S. aureus* (MRSA), vancomycin-intermediate *S. aureus* (VISA) and vancomycin-resistant *S. aureus* (VRSA), with minimum inhibitory concentrations (MICs) ranging from 0.0625 μg/mL to 0.125 μg/mL. In vivo, topical auranofin proved superior to conventional antimicrobials, including fusidic acid and mupirocin, in reducing the mean bacterial load in infected wounds in a murine model of MRSA skin infection. In addition to reducing the bacterial load, topical treatment of auranofin greatly reduced the production of inflammatory cytokines, including tumour necrosis factor-α (TNFα), interleukin-6 (IL-6), interleukin-1 beta (IL-1β) and monocyte chemoattractant protein-1 (MCP-1), in infected skin lesions. Moreover, auranofin significantly disrupted established in vitro biofilms of *S. aureus* and *Staphylococcus epidermidis*, more so than the traditional antimicrobials linezolid and vancomycin. Taken together, these results support that auranofin has potential to be repurposed as a topical antimicrobial agent for the treatment of staphylococcal skin and wound infections.

1. Introduction

Staphylococcus aureus is the most frequently isolated pathogen from human skin infections and is the leading cause of nosocomial wound infections [1–4]. Virulence factors and toxins (such as α-haemolysin and Panton–Valentine leukocidin) secreted by drug-resistant strains of *S. aureus* allow this pathogen to evade the host immune system, leading to recurring/chronic infections, prolonged inflammation and delayed healing of infected wounds [3,4]. Furthermore, cutaneous staphylococcal skin infections can develop into invasive infections that ultimately result in septicaemia [5,6]. Recently, skin infections with biofilm-producing staphylococci have become an emerging clinical problem; treatment failure is occurring more frequently with the topical drugs of choice, including mupirocin and fusidic acid, indicating that new treatment options are urgently required [2,7,8]. The recent US Food and Drug Administration (FDA) approval of drugs such as tedizolid phosphate and dalbavancin to combat skin infections caused by Gram-positive pathogens [9,10] further highlights the pressing need for the identification of new antibacterials capable of treating cutaneous meticillin-resistant *S. aureus* (MRSA) infections.

Most current antibiotics were discovered by screening libraries of chemical compounds to find new lead 'hits' that could be subsequently modified to enhance potency/physicochemical properties and to mitigate toxicity [11]. However, this process is a risky venture given the significant financial and time investment required by researchers and the limited success rate of translating these compounds to the clinical setting. An alternative approach to unearthing new antibacterials that has received more attention recently is evaluating the repository of approved drugs

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http://dx.doi.org/10.1016/j.ijantimicag.2015.12.016
0924-8579/

(or drugs that made it to clinical trials but failed to receive regulatory approval) in order to identify candidates that can be repurposed as antimicrobials [11]. Recently, we assembled and screened one-half of all commercially available drugs (ca. 2200 drugs) and small molecules used in human clinical trials [2,12] and identified three drugs (auranofin, ebselen and 5-fluoro-2′-deoxyuridine) [2,13,14] that exhibited potent antibacterial activity against important clinical pathogens. One of these drugs, auranofin, was found to inhibit the growth of clinical isolates of MRSA at sub-microgram/mL concentrations in vitro.

Auranofin is an oral gold-containing drug initially approved for the treatment of rheumatoid arthritis [15]. Recent studies have demonstrated that auranofin also possesses potent antiparasitic [15] and antibacterial activities [16,17], including against MRSA and *Streptococcus pneumoniae* [16,18–20]. Recent studies by Harbut et al. [16] and Aguinagalde et al. [18] demonstrated that auranofin is efficacious in the treatment of invasive staphylococcal infections. However, the efficacy of auranofin for the treatment of cutaneous MRSA infections remains unexplored.

Building upon these recent reports, the present study investigated the in vitro antibacterial and antibiofilm activities of auranofin against multidrug-resistant clinical isolates of *S. aureus* and tested the efficacy of auranofin in a mouse model of MRSA skin infection. In addition, this study aimed to examine the immunomodulatory activity of auranofin in MRSA-infected skin lesions. The findings presented in this study lay the foundation for repurposing auranofin as a novel topical antibacterial agent for treatment of cutaneous MRSA infections in humans.

2. Materials and methods

2.1. Bacterial strains and reagents

The bacterial strains used in this study are presented in Table 1. Auranofin (Enzo Life Sciences, Farmingdale, NY), mupirocin (AppliChem, Maryland Heights, MO), clindamycin (Sigma-Aldrich, St Louis, MO), vancomycin hydrochloride (Gold Biotechnology, St Louis, MO), linezolid (Selleck Chemicals, Houston, TX), retapamulin (Oxchem Corporation, Irwindale, CA), crystal violet (Sigma-Aldrich), 95% ethanol (Fisher Scientific, Pittsburgh, PA), MTS assay reagent (Promega Corp., Madison, WI), dimethyl sulphoxide (DMSO) and fusidic acid (Sigma-Aldrich) were all purchased from commercial vendors. Mueller–Hinton broth was purchased from Sigma-Aldrich, and trypticase soy broth (TSB), trypticase soy agar (TSA) and mannitol salt agar (MSA) were purchased from Becton Dickinson & Co. (Cockeysville, MD).

2.2. Antibacterial assays

To examine the antibacterial activity of auranofin against *S. aureus*, the broth microdilution method was utilised to determine the minimum inhibitory concentration (MIC) of each drug (tested in triplicate) following the guidelines outlined by the Clinical and Laboratory Standards Institute (CLSI) [21]. Each drug was incubated with the respective strain of *S. aureus* for 16 h at 37 °C before the MIC was confirmed. The MIC was classified as the lowest concentration of each test agent where no bacterial growth was visible.

2.3. Mice infection

Eight-week-old female BALB/c mice (Harlan Laboratories, Indianapolis, IN) were used in this study. All animal procedures were approved by the Purdue University Animal Care and Use Committee (West Lafayette, IN). An in vivo murine MRSA skin infection study was conducted as described elsewhere [2,22–24]. Briefly, mice (five mice per group) received an intradermal injection (40 μL) of MRSA USA300 containing 1.65×10^8 CFU. Approximately 2 days later, an open wound/abscess formed at the site of injection. Five groups of mice were then treated topically with a suspension containing 2% fusidic acid, 2% mupirocin, or 0.5%, 1% or 2% auranofin in petroleum jelly. Another two groups were treated orally with 25 mg/kg of either linezolid or clindamycin. The control group was treated with petroleum jelly (vehicle). Mice were treated twice daily for 5 days. Then, 24 h after the last dose was administered, mice were humanely euthanised via CO_2 asphyxiation. The region around the skin wound was slightly swabbed with 70% ethanol and the wound (1 cm^2) was precisely excised, homogenised, serially diluted in phosphate-buffered saline (PBS) and then transferred to MSA plates. Plates were incubated at 37 °C for 24 h prior to enumeration of MRSA.

2.4. Detection of cytokines from the MRSA murine skin infection experiment

Skin homogenates obtained from the murine skin infection experiment described above were centrifuged. The supernatant was collected and was used to quantify the levels of inflammatory cytokines, including tumour necrosis factor-a (TNFα), interleukin-6 (IL-6), interleukin-1 beta (IL-1β) and monocyte chemoattractant protein-1 (MCP-1). Duo-set ELISA Kits (R&D Systems, Inc., Minneapolis, MN) were used for cytokine detection according to the manufacturer's protocol.

2.5. Combination testing of auranofin with commercial antibiotics

The additive activity of auranofin with conventional topical antibiotics (mupirocin, fusidic acid and retapamulin) was evaluated as described previously [25,26]. Briefly, MRSA USA300 was incubated with auranofin, control antibiotics, or a combination of auranofin plus a control antibiotic at different concentrations for 16 h. Next, the optical density at 600 nm was measured using a spectrophotometer. The percent bacterial growth for each treatment regimen was calculated.

2.6. Biofilm assay

The ability of auranofin to disrupt adherent staphylococcal biofilm was analysed using the microtitre dish biofilm formation assay [2,27]. *Staphylococcus aureus* ATCC 6538 and *Staphylococcus epidermidis* ATCC 35984 were inoculated in TSB supplemented with 1% glucose and were transferred to the wells of a 96-well tissue culture treated plate (CELLTREAT Scientific, Shirley, MA). Bacteria were incubated at 37 °C for 24 h to allow the formation of an adherent biofilm. The medium was removed and the wells were carefully washed with PBS four times to remove planktonic bacteria. TSB was transferred to all wells of the 96-well plate prior to addition of auranofin and control antibiotics (linezolid and vancomycin). Drugs were added at the indicated concentrations and were incubated again at 37 °C for 24 h. Afterward, plates were washed by submerging in tap water. Biofilms were stained with 0.1% (w/v) crystal violet for 30 min at room temperature before subsequently being washed four times with water. Plates were air dried for 1 h prior to the addition of 95% ethanol to solubilise dye bound to the biofilm. The biofilm mass was quantified by measuring the optical density of wells at 595 nm using a microplate reader (BioTek Instruments Inc., Winooski, VT). Data are presented as the mean percent biofilm mass reduction of each test agent (tested in triplicate) in relation to untreated wells.