Phytomedicine 20 (2013) 262–269

Contents lists available at SciVerse ScienceDirect

Phytomedicine

journal homepage: www.elsevier.de/phymed

Synergistic activity and mechanism of action of ceftazidime and apigenin combination against ceftazidime-resistant *Enterobacter cloacae*

Griangsak Eumkeb*, Somnuk Chukrathok

School of Pharmacology, Institute of Science, Suranaree University of Technology, Nakhonratchasima, 30000, Thailand

ARTICLE INFO

Keywords:
Apigenin
Naringenin
Ceftazidime
Ceftazidime-resistant *E. cloacae*
Synergistic activity
Mechanism of action

ABSTRACT

The purpose of this investigation was to examine the antibacterial and synergistic effect of naturally occurring flavonoids, apigenin, quercetin, naringenin and ceftazidime when use singly and in combination against ceftazidime-resistant *Enterobacter cloacae* strains by minimum inhibitory concentration (MIC), checkerboard and viable count methods. The mode of actions were also studied by electronmicoscopy, enzyme assay, outer and inner membrane permeabilisation. The results showed that these strains were positive in the ESBL–ampC genes combination by multiplex PCR. These findings were confirmed by MICs that these strains were resistant to ceftazidime, cefepime and flomoxef at >1024, 16–24, >256 μg/ml respectively, while susceptible to imipenem at 1–2 μg/ml. The synergistic activity was observed at ceftazidime plus either apigenin or naringenin combinations with FIC indixes between <0.01 and <0.27 against these strains, whereas ceftazidime plus clavulanic acid or quercetin did not exhibit synergy. The modulation of ceftazidime-resistance by apigenin or narigenin significantly enhanced the activities of ceftazidime. The 5,7-OH group of A ring and one 4′-OH group of the B ring in apigenin and naringenin are important for synergistic activity. Viable counts showed that the killing of ceftazidime-resistant *E. cloacae* DMST 21394 (CREC) cells by 3 μg/ml ceftazidime was potentiated by 3 μg/ml apigenin to low levels (10^3 cfu/ml) over 6 h. Electronmicroscopy clearly showed that ceftazidime 3 μg/ml in combination with 3 μg/ml of apigenin also caused marked morphological damage of cell wall, cell shape and plasma membrane of this strain. Enzymes assays indicated that apigenin showed marked inhibitory activity against penicillinase type IV from *E. cloacae*. The results for outer membrane (OM) permeabilization in both nitrocefin (NCF) assay and crystal violet uptake showed that the combination of ceftazidime plus apigenin significantly altered OM permeabilisation of CREC compared to control or single treatment of these agents. Both o-nitrophenyl-β-D-galactoside (ONPG) uptake and release of UV-absorbing material concentrations results exhibited that ceftazidime and apigenin combination damaged CREC cytoplasmic membrane (CM) and caused subsequent leakage of intracellular constituents. From the results, it can be concluded that apigenin and naringenin have the synergistic effect with ceftazidime to reverse bacterial resistance to this cephalosporin against CREC. This activity may be involved three mechanisms of action by apigenin. The first is on the peptidoglycan synthesis inhibition. The second mechanism is inhibition the activity of certain β-lactamase enzymes. The third mode of action is alteration of OM and CM permeabilization. Apigenin and naringenin have a sufficient margin of safety for therapeutic use. For this reason, apigenin and naringenin offer for the development of a valuable adjunct to ceftazidime against CREC, which currently almost cephalosporins resistance.

Introduction

Enterobacter cloacae are significant causes of nosocomial infections. It is emphasized that *E. cloacae* are mainly responsible for pneumonia, wound infection and urinary tract infection in the most hospital. The three specimen types of sputum, secretions and pus, urine in the past 8 years in the First Bethune Hospital investigation showed that the antimicrobial resistance of *E cloacae* had increased. *E. cloacae* are intrinsically resistant to aminopenicillins, cefazolin, and cefoxitin due to the production of *ampC* β-lactamases (Anggakusuma et al. 2009). Similarly, the resistance to antimicrobial agents in Enterobacteriaceae has become an increasingly relevant problem. International travel and tourism are important modes for the acquisition and spread of antimicrobial-resistant Enterobacteriaceae (Rukayadi et al. 2010). In addition, the *E. cloacae*

* Corresponding author at: School of Pharmacology, Institute of Science, Suranaree University of Technology, 111 University avenue, Suranaree Subdistrict, Muang District, Nakhonratchasima, 30000, Thailand. Tel.: +66 44 224260; fax: +66 44 224633.
E-mail address: griang@sut.ac.th (G. Eumkeb).

0944-7113/$ – see front matter © 2012 Elsevier GmbH. All rights reserved.
http://dx.doi.org/10.1016/j.phymed.2012.10.008

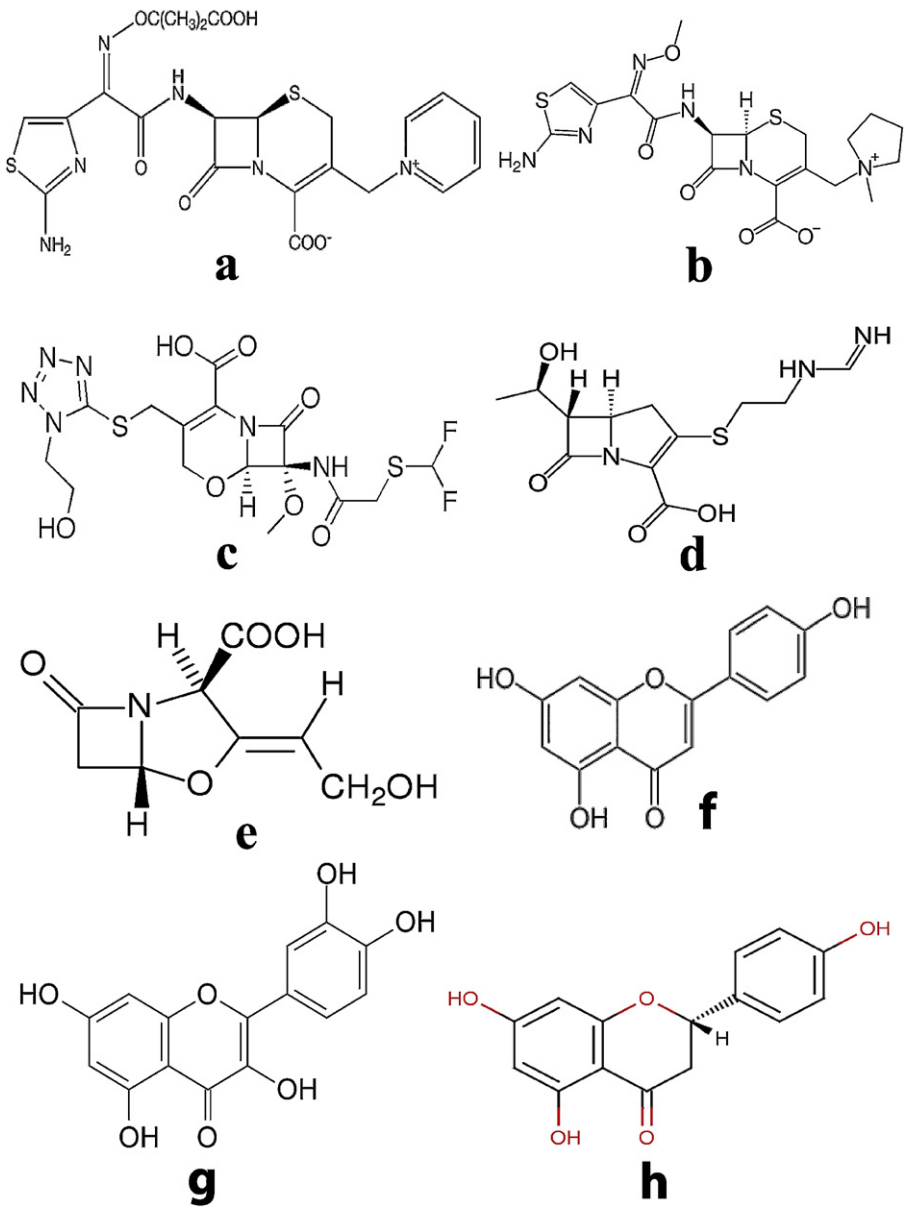

Fig. 1. Structure of drugs and flavone uses; (a) ceftazidime; (b) cefepime; (c) flomoxef; (d) imipenem; (e) clavulanic acid; (f) apigenin; (g) quercetin; (h) naringenin.

from blood of inpatients at an urban public hospital in Berkeley was found that it carried globally-dispersed drug-resistance genes (Yanti et al. 2009b). Furthermore, the SHV-12 β-lactamase of clinical isolates of *E. cloacae* with reduced susceptibility to ceftazidime and cefepime recovered from 2009 to 2010 at the university hospital of Mahdia, Tunisia, was analyzed by PCR analysis (Yoon et al. 2005). Likewise, the prevalence of infection by plasmid mediated *ampC* (p*ampC*), which can hydrolyze penicillins, oxyimino-, 7-α-methoxycephalosporins and monobactams, varies depending on the type of enzyme and geographical location and blaCMY-2 is the most frequently detected worldwide. Typically, p*ampC* producing isolates are associated with resistance to multiple antibiotics making the selection of an effective antibiotic difficult (Rukayadi et al. 2009). For these reasons, antibiotics available for the treatment of multi-drugs resistant *E. cloacae* infection are fairly toxic and their use is frequently associated with unwanted side-effects. Imipenem/cilastatin, often reserved for more serious hospital-acquired infections, is thought to be associated with a higher risk of seizures than other penicillins and carbapenems (Yanti et al. 2009a). Therefore, novel flavonoids or new generation of phytopharmaceuticals approaches that show synergistic effect with antibacterial agents, which have lost their original effectiveness, or enable their use to treat diseases instead of synthetic drugs alone are research objectives of far reaching importance (Eumkeb et al. 2010; Wagner and Ulrich-Merzenich, 2009). In this study, we have investigated the in vitro activity of naturally occurring plant flavone (4′, 5, 7,-trihydroxyflavone), apigenin, which is abundantly present in common fruits such as grapefruit, plant-derived beverages and vegetables such as parsley, onions, oranges, tea, chamomile, wheat sprouts and in some seasonings, against ceftazidime-resistant *E. cloacae* (CREC) when used alone and in combination with ceftazidime. Chamomile is one of the most common sources of apigenin consumed as single ingredient herbal tea, prepared from the dried flowers from *Matricaria chamomilla*. Apigenin has been shown to possess remarkable anti-inflammatory, antioxidant and anti-carcinogenic properties (McKay and Blumberg 2006; Patel