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Antimicrobial Activity Induced by A Steroid-brucine Derivative on *S. typhi*, *K. pneumoniae* and *E. coli*

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Abstract: For many years, various drugs have been used for the treatment of infectious diseases but some bacterial microorganisms have induced resistance to several drugs. In the search for an alternative therapy for the infectious diseases, in this study the antibacterial activity of the steroid-brucine derivative against *S. typhi*, *K. pneumoniae* and *E. coli* was evaluated, using as control cefotaxime, gentamicin and ciprofloxacin. The evaluation of antimicrobial effect of the different compounds on the bacterial species was made by the method of microbial minimal inhibitory. The results indicate that bacterial growth of *S. typhi* was inhibited with cefotaxime, gentamicin, ciprofloxacin, brucine-ethylenediamine derivative and brucine-estradiol conjugate. Other results showed that bacterial growth of *E. coli* was inhibited with cefotaxime, gentamicin, ciprofloxacin, brucine-ethylenediamine and brucine-estradiol conjugate. Alternative experimental, indicate that bacterial growth of *K. pneumoniae* was inhibited with cefotaxime, gentamicin, ciprofloxacin, brucine-ethylenediamine derivative and brucine-estradiol conjugate. In conclusion, *S. typhi*, *K. pneumoniae* and *E. coli* were susceptible to gentamicin, cefotaxime, ciprofloxacin, brucine-ethylenediamine derivative and brucine-estradiol conjugate. These phenomenon, involves different molecular mechanism of brucine-ethylenediamine derivative and brucine-estradiol conjugate in comparison with the controls. In addition, the antibacterial activity induced by brucine-estradiol in comparison with the brucine-ethylenediamine derivative can depend of the degree of lipophilicity of these compounds.

Key words: Brucine-ethylenediamine, brucine-estradiol, antibacterial activity

INTRODUCTION

Infectious diseases still remain one of the most important causes of mortality worldwide (Pinner *et al.*, 1996; Crossley and Peterson, 1996; Norman, 1999). There are different causal agents such as *S. aureus* (Henry, 2001), *K. pneumoniae* (Podschun and Ullman, 1998) and *E. coli* (Lautenbach *et al.*, 2001) among others (Rothstein *et al.*, 2003) that can induce various infectious diseases. It is important to mention, that for treatment of the infectious diseases is required many therapeutic agents (Wilson *et al.*, 1995; Yoo *et al.*, 2004; Killgore *et al.*, 2004) unfortunately, prolonged antibiotic therapy may induce bacterial-resistance

(Hackbarth and Chambers, 1989; Maguire *et al.*, 1998), because some bacteria have developed ways to circumvent the effects of antibiotics (Peschel, 2002; Yeaman and Yount, 2003). For example, several studies indicate that β -lactam antibiotics may predispose to patients worldwide for acquisition of resistance to *S. aureus* (Mylotte *et al.*, 1987; Lowy, 1998; Ayliffe, 1997; Merlino *et al.*, 2002). Other reports showed that some antibiotic-resistant strains also have been emerging among Gram-negative bacilli such as *K. pneumoniae* (Podschun and Ullman, 1998) and *E. coli* (Prats *et al.*, 2003). Therefore, antibiotic resistance can be considered a serious threat for the human health. In the search of alternative therapeutics for the treatment of infectious

diseases, new drugs have been developed for control of bacterial resistance (Gordon *et al.*, 1994; Schwab *et al.*, 1999; Patch and Barron, 2003). In this sense, since several years ago, have been evaluated some steroid derivatives as potential therapeutic agents for infectious diseases (Li *et al.*, 1999a). For example, there are data which indicate that the steroid derivative (dihydrotestosterone-ciprofloxacin) induce antibacterial activity against *S. aureus* and *E. coli* (Figueroa-Valverde *et al.*, 2010). In addition, other steroid derivative (squalamine) has been used as antibacterial agent against a broad spectrum of microorganisms (Moore *et al.*, 1993; Kikuchi *et al.*, 1997). Other data indicate that a steroidal heterocyclic derivative showed antibacterial activity against gram positive and gram negative bacteria (Abdelhalim *et al.*, 2007). All these experimental data indicate that several steroid derivatives can exert antibacterial activity on microorganism different. Therefore, in this study the antibacterial effect of the brucine-steroid derivative against *S. typhi*, *K. pneumoniae* and *E. coli* was

evaluated using the method of microbial minimal inhibitory (Chiong and Betancourt, 1985). It is important to mention that the steroid-brucine derivative has a spacer arm in the D-ring of steroid which is bound to the brucine fragment. In addition was used as biological tool a brucine derivative (brucine-ethylenediamine) to assess the molecular mechanism involved in the antibacterial activity of the brucine-steroid conjugate.

MATERIALS AND METHODS

General methods

Strains: The microorganisms in this study belonged to the strain bank at the Department of Pharmaco-chemistry at the Faculty of Chemical Biological Sciences of the Universidad Autónoma de Campeche. The strains are certified by Center for Disease Control in Atlanta and were as follows: *S. typhi* (ATCC 19430), *K. pneumoniae* (ATCC 700603) and *E. coli* (ATCC 25922). The strains are kept under refrigeration at 4°C in special gel (BBL).

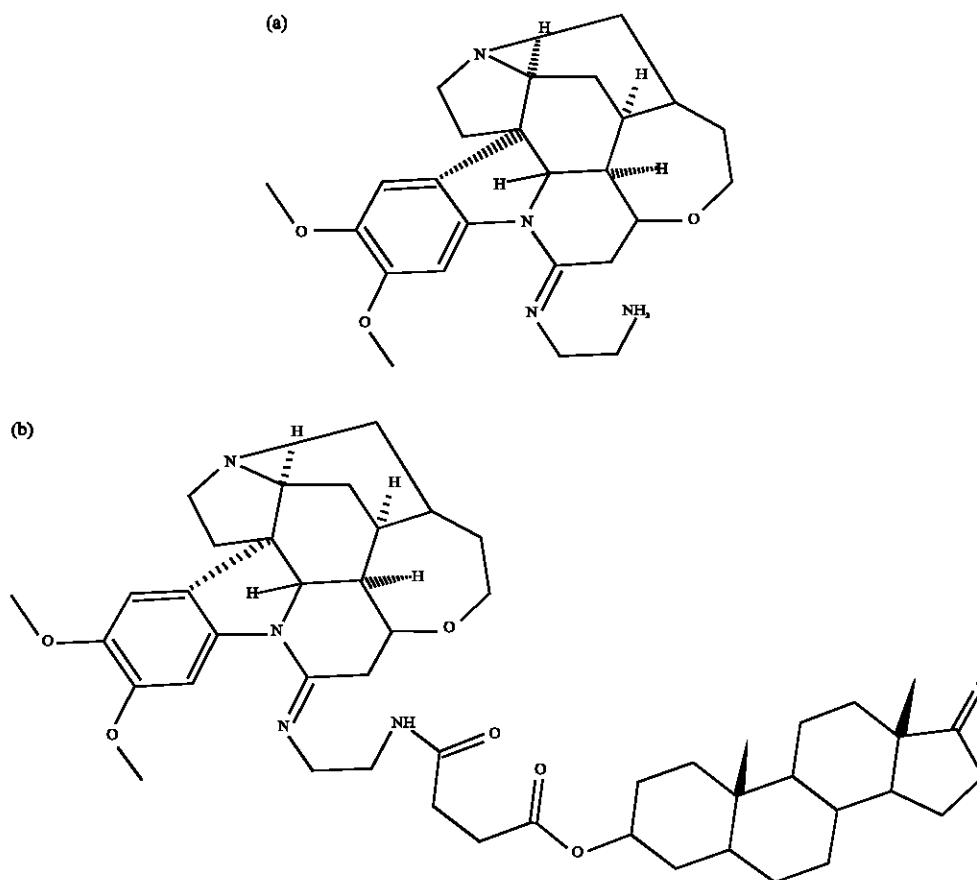


Fig. 1: Chemical structure of brucine-ethylenediamine derivative (N1-(2,3-dimethoxystrychnidin-10-yliden)-ethane-1, 2-diamine) and brucine-steroid conjugate (N-[2-(2,3-dimethoxystrychnidin-10-ylideneamino)-ethyl]-succinamic acid 3-hydroxy-13-methyl-7,8,9,11,12,13,14, 15,16,17-decahydro-6 H-cyclo- penta[a]phenanthren-17-yl ester)

Antimicrobial agents: Brucine-ethylenediamine [N1-(2,3-dimethoxystrychnidin-10-yliden)-ethane-1, 2-diamine], brucine-estradiol conjugate [N-[2-(2, 3-dimethoxystrychnidin-10-ylideneamino)-ethyl]-succinamic acid 3-hydroxy-13-methyl-7, 8, 9, 11, 12, 13, 14, 15, 16, 17-decahydro-6 H-cyclopenta (a) phenanthren-17-yl ester] (Fig. 1) were synthesized by the previously method reported (Figuroa-Valverde *et al.*, 2013). The compounds were dissolved in methanol and diluted with distilled water. Cefotaxime, gentamicin and ciprofloxacin were used as the standard drugs.

Antimicrobial activity: The evaluation of antimicrobial effect of the different compounds on the bacterial species was made by a method described previously (Chiong and Betancourt, 1985). The bacterial species were incubated on Mc Conkey (*E. coli*, *S. typhi* and *K. pneumonia*) agar for 24 h at 37°C. After such time, it was determined whether growth had taken place or not. In addition, a series of tubes were prepared, the first of which contained 2 mL of culture medium (tripticase soye) at double concentration and the remainder (11 tubes), contained the same quantity of medium at single concentrations. From the first tube (double concentration) an aliquot of 2 mL of the studied compound (1 mg mL⁻¹) was added and stirred, from this tube an aliquot of 2 mL was taken and added to the following tube (simple concentration) and the process was successively repeated until the last 2 mL of dissolution had been used up. After this process, each tube was inoculated with 0.1 mL of the bacterial suspension, whose concentration corresponded to Mc-Farland scale (9×10⁸ cells mL⁻¹) and all the tubes were incubated at 37°C for 24 h. Subsequently, a loop was taken from each of them and inoculated into the appropriate cultures for different bacterial organisms and were incubated for 24 h at 37°C. After such time, the Minimum Inhibitory Concentration (MIC) was evaluated to consider the antimicrobial effect of the different compounds. In order to discard the effect of methanol (solvent) on the bacterial species studied, a series of the same number of tubes was prepared in parallel, to which 2 mL of methanol at 60% was added to the first and corresponding successive dilutions were added in the same way as before. In addition a control series was also performed using distilled water to pH 7.0.

Statistical analysis: All the values obtained were expressed as Mean±SD error. The differences were considered significant when p-value was equal or smaller than 0.05.

RESULTS

The bacterial activity of brucine-estradiol conjugate was compared with the antibacterial effect of cefotaxime, gentamicin and ciprofloxacin (controls) in such bacterial microorganism studied. The results obtained (Fig. 2) indicate that bacterial growth of *S. typhi* was inhibited by cefotaxime (MIC = 5.23×10⁻⁴ mmol), gentamicin (MIC = 1.34×10⁻⁵ mmol) and ciprofloxacin (MIC = 3.01×10⁻³ mmol). In addition, the bacterial growth was significantly blocked (p = 0.05) in presence of the brucine-ethylenediamine derivative (MIC = 2.68×10⁻³ mmol) in comparison with the brucine-estradiol conjugate (MIC = 2.29×10⁻³ mmol).

On the other hand, alternative experimental were made in *K. pneumoniae* and *E. coli* using the same controls to evaluate the antibacterial effect of brucine-ethylenediamine derivative and brucine-estradiol conjugate. The results indicate that bacterial growth of *E. coli* was inhibited (Fig. 3) in presence of cefotaxime (MIC = 5.23×10⁻⁴ mmol), gentamicin (MIC = 1.34×10⁻⁵ mmol), ciprofloxacin (MIC = 3.01×10⁻³ mmol). Additionally, the growth of *E. coli* was significantly inhibited (p = 0.06) by brucine-estradiol conjugate (MIC = 2.68×10⁻³ mmol) in comparison with brucine-ethylenediamine derivative (MIC = 2.29×10⁻³ mmol).

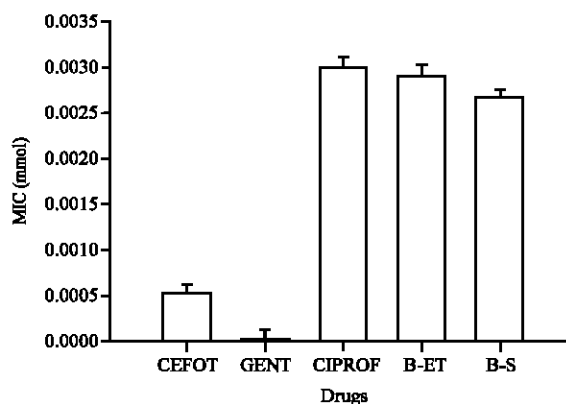


Fig. 2: Antibacterial effects induced by brucine-estradiol derivative (B-S), brucine-ethylenediamine (B-ET) and controls (cefotaxime, CEFOT; gentamicin, GENT and ciprofloxacin, CIPROF) on *S. typhi*. The results showed that *S. typhi* was susceptible to cefotaxime (MIC = 5.23×10⁻⁴ mmol), gentamicin (MIC = 1.34×10⁻⁵ mmol) and ciprofloxacin (MIC = 3.01×10⁻³ mmol). In addition, the bacterial growth in the presence of the brucine-ethylenediamine (MIC = 2.68×10⁻³ mmol) was significantly inhibited (p = 0.05) in comparison with the brucine-estradiol derivative (MIC = 2.29×10⁻³ mmol).

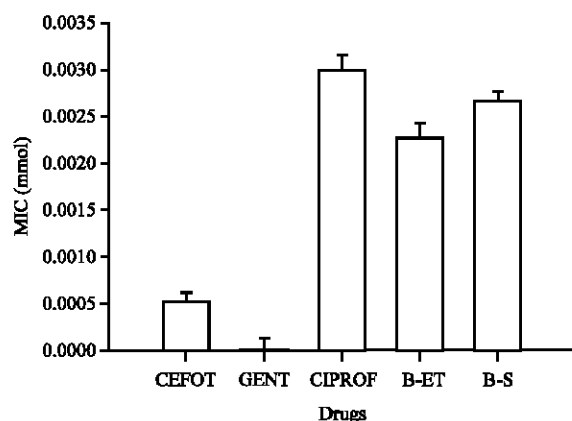


Fig. 3: Antibacterial activity induced by brucine-estradiol derivative (B-S), brucine-ethylenediamine (B-ET) and controls (cefotaxime, CEFOT; gentamicin, GENT and ciprofloxacin, CIPROF) on *E. coli*. Experimental data showed that *E. coli* was susceptible to CEFOT (MIC = 5.23×10^{-4} mmol), GENT (MIC = 1.34×10^{-5} mmol) and CIPROF (MIC = 3.01×10^{-3} mmol). Additionally, the growth bacterial was significantly blocked ($p = 0.06$) in the presence of B-S (MIC = 2.68×10^{-3} mmol) in comparison with B-ET (MIC = 2.29×10^{-3} mmol)

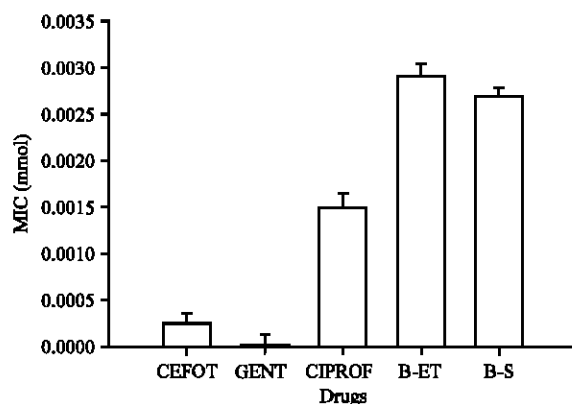


Fig. 4: Effects induced by brucine-estradiol derivative (B-S), brucine-ethylenediamine (B-ET) and controls (cefotaxime, CEFOT; gentamicin, GENT and ciprofloxacin, CIPROF) on *K. pneumoniae*. The results showed that bacterial growth of *K. pneumoniae* in the presence of CEFOT (MIC = 2.61×10^{-4} mmol), GENT (MIC = 2.68×10^{-5} mmol), CIPROF (1.50×10^{-3} mmol) was inhibited. In addition, the growth of *K. pneumoniae* was significantly inhibited ($p = 0.05$) by the brucine-estradiol derivative (MIC = 2.68×10^{-3} mmol) and brucine-ethylenediamine (2.29×10^{-3} mmol)

Other results obtained (Fig. 4) showed that bacterial growth of *E. coli* was significantly inhibited ($p = 0.05$) in presence brucine-ethylenediamine derivative (MIC = 2.29×10^{-3} mmol) in comparison with the brucine-estradiol conjugate (MIC = 2.68×10^{-3} mmol). This results were compared with the antibacterial effect of controls, in this case, the cefotaxime showed a MIC of 2.61×10^{-4} mmol, the MIC for gentamicin was of 2.68×10^{-5} mmol and the MIC for ciprofloxacin was MIC = 1.50×10^{-3} mmol.

DISCUSSION

The experimental data obtained indicate that the brucine-estradiol conjugate had different antibacterial activity against *S. typhi*, *K. pneumoniae* and *E. coli*, in comparison with the controls (cefotaxime, gentamicin and ciprofloxacin). This phenomenon may be due mainly to the different molecular mechanisms involved in the antibacterial effect induced by the compounds studied. To evaluate the mechanism involved in the antibacterial activity induced by the brucine-estradiol conjugate against *S. typhi*, *K. pneumoniae* and *E. coli*, in this study the brucine-ethylenediamine compound was used as a biological tool. The result showed that bacterial growth of *S. typhi*, *K. pneumoniae* and *E. coli* in presence of brucine-ethylenediamine was blocked. It is important to mention that this compound contains in the chemical structure an arm with a free amine group. Therefore, the antibacterial activity of this compound may depend on the nature of the free amino group contained in their chemical structure (Fig. 1) which is a membrane-perturbing agent whose antibacterial activity can be possibly by the interaction with the positively charged phosphate groups contained in the Lipid A that are essential polymers that plays a vital role in the growth and development of the gram-negative bacteria (Fisher, 1990). It is important to mention, that some authors proposed a compelling model of complex formation involving ionic interactions between the phosphates on lipid A and the amine groups on polymyxin B. This phenomenon may increase the permeability of the outer membrane and induce bacterial growth inhibition on this gram-negative microorganism (Li *et al.*, 1999b; Figueroa-Valverde *et al.*, 2009). Nevertheless, also it is important to mention that when the brucine-ethylenediamine compound is bound with steroid to form the brucine-steroid conjugate, the antibacterial activity is increasing. This phenomenon, suggest that brucine-steroid conjugado requires of a hydrophobic region in order to interact with some components of bacterial cell, disturbing the bacterial growth and to cause cell death.

On the other hand, also possibly the antibacterial activity involve the intramolecular interaction of brucine-steroid with divalent cations (Mg^{2+} and Ca^{2+}), involved in the membrane cell providing a substantial increase the permeability of the outer membrane of Gram-negative bacteria include bactericidal/permeability increasing protein. In conclusion, in this study the bacterial microorganism such as *S. typhi*, *K. pneumoniae* and *E. coli* were more susceptible to brucine-steroid in comparison with brucine ethylenediamine. Possibly the molecular mechanism could be by mimic the actions of several steroid-derivates with antibacterial characteristics (Ding *et al.*, 2004). The molecular mechanism suggest that brucine-steroid conjugate can adopt cationic, facially amphiphilic conformations and involved the nature of functional groups contained in the chemical structure, which appears to be key requirement for antibacterial activity. This chemistry structure allows them to disrupt bacterial membranes at relatively different concentrations by the interaction with some factors in the bacterial membrane as effective targets of the brucine-steroid conjugate.

CONCLUSION

The antibacterial activity of the steroid-brucine derivative possibly involves, (1) Differences in the molecular mechanism involved in the antibacterial activity exerted by the brucine-ethylenediamine derivative and the brucine-estradiol conjugate in comparison with the controls and (2) The antibacterial activity induced by the brucine-estradiol derivative in comparison with the brucine-ethylenediamine conjugate can depend of the lipophilicity degree of this compound.

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