Evaluation of the in vitro interaction of amoxicillin and cotrimoxazole antibiotics against resistant bacterial strains

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ARTICLE INFO

Article history:
Received on: 06/11/2013
Revised on: 16/12/2013
Accepted on: 09/01/2014
Available online: 30/01/2014

Key words:
Antibacterial combinations, drug-drug interaction, synergy, antibiotics, bacteria.

ABSTRACT

The in vitro combination effects of amoxicillin and cotrimoxazole on clinical isolates was investigated using the agar diffusion and macrobroth dilution methods. The results showed that these organisms had varied susceptibility to the different concentrations of each of these antibiotics and their combinations. The susceptibility of the isolates to the antibacterial combinations showed that they were susceptible in the following order: Streptococcus pyogenes (TD2) > Streptococcus pyogenes (TD10) > Streptococcus pneumoniae (TE10) > Salmonella typhi (TC6) > Salmonella typhi (TC2). The macrobroth assay showed a drastic reduction in the minimum inhibitory concentrations of both antibiotics. While the MIC of amoxicillin ranged between 0.1202 and 0.4808 µg/ml and that of cotrimoxazole ranged between 0.2405 and 0.9619 µg/ml, the MIC of the antibacterial combinations ranged between 0.00305 and 0.0150 µg/ml. A statistical analysis of the zones of inhibitions produced by the antibiotics and their combinations indicated that the mean differences between the zones of inhibitions were significantly diverse. This study showed that there was synergistic interaction between amoxicillin and cotrimoxazole in vitro and could be an alternative choice of therapy for the treatment of streptococcal and gastrointestinal infections in which these organisms have been implicated.

INTRODUCTION

Hospital patients frequently receive more than one antibacterial agent and these agents may interact with each other and with other drugs (Jankel and Speedie, 1990). A drug interaction refers to a change in the magnitude or duration of the pharmacological response of one drug because of the presence of a second drug (Brodey et al., 1998). Drug interactions may result from changes in the pharmacodynamic and pharmacokinetics properties of the drug. While pharmacodynamic drug interactions are relatively common in practice and adverse effects can usually be minimized if interactions are anticipated and appropriate counter measures are taken (Katzung, 2001), combination of drugs may be used to minimize the development of resistant strains, instigate a synergistic effect and reduce toxicity (Tortora et al., 1989). Similarly, these combinations of different drugs are used in empiric therapies to cover a wide spectrum of potential pathogens when the causative agent is unidentified or when infection is likely to be due to mixture of organisms (Andreoli et al., 1997). Combinations of drugs can be additive when both drug acts independently, synergistic when the effect of the two drugs given together is significantly greater than the sum of the individual effect of the two drugs acting separately or antagonistic if the drugs become less effective than when taken alone (Bhatia and Ichhpuijani, 2004). Previously, different interactions between amoxicillin and other drugs have been reported. Adam et al. (1983) reported that the combination of amoxicillin and flucloxacinill was synergistic against beta-lactamase-producing organisms. Cuffini et al. (1998) showed that amoxicillin/clavulanic acid had excellent synergistic antimicrobial activities against Streptococcus pneumoniae. Proton pump inhibitors such as esomeprazole and rabeprazole combined with amoxicillin showed synergistic activity in eradicating Helicobacter pylori (Go, 2002). Although amoxicillin do not affect theophilline clearance (Jonkman, 1986) and antagonism of gentamicin and amoxicillin against Escherichia coli and Enterobacter cloacae strains was reported by Grzybowska et al., (2004), Dogterom et al. (2005) showed that there are no pharmacokinetic interactions between etonogestrol and ethinylestradiol combined with amoxicillin while combining doxycycline and amoxicillin may reduce the effectiveness of amoxicillin (http, 2013).
On account of having successful therapies, polypharmacy has become the usual practice when a patient report in a hospital. However, because of the development of resistance to antibiotics, co-administrations of antibiotics are encouraged as this could result in effective therapeutic outcomes. Since resistance to amoxicillin and cotrimoxazole by many bacteria have been widely reported, this study was designed to investigate the in vitro combination effects of these antibiotics against clinical isolates of *Streptococcus pneumoniae*, *Streptococcus pyogenes* and *Salmonella typhi*.

**MATERIALS AND METHODS**

**Bacteria used and inoculum preparation**

The bacteria used in this study included *Salmonella typhi* (TC2), *Salmonella typhi* (TC6), *Streptococcus pyogenes* (TD2) *Streptococcus pyogenes* (TD10), and *Streptococcus pneumoniae* (TE10) which are clinical strains obtained from Lagos State University Teaching Hospitals, Lagos State, Nigeria. They were identified and confirmed using morphological, microscopy and biochemical tests following standard procedures described by Cowan and Steel (1974) and Cheesbrough (2006).

**Drug Preparations**

Pure powders of amoxicillin and cotrimoxazole were used. Stock antibiotic solutions were prepared and dilutions made according to the CLSI (Clinical Laboratory Standardization Institute) method or manufacturer’s recommendations (NCCLS, 1997; Richard, 2007).

Here, 0.0197 g of Trimethoprim and 0.0985 g of Sulfamethoxazole were combined in the ratio 1:5 as cotrimoxazole. This mixture was dissolved in 10 ml of absolute acetone while 0.0985 g of amoxicillin was dissolved in 10 ml of sterile distilled water to form their (w/v) stock solution. Different concentrations (15.38, 30.78, 61.56, 123.12, 246.3, and 492.5 µg/ml) of both antibiotics were prepared and used during this study while the stock solutions were stored in a freezer at -20°C until use.

**Agar Diffusion Susceptibility Testing with Cotrimoxazole and Amoxicillin**

Each of the isolates was standardized using colony suspension method. Each strain's suspension was matched with 0.5 McFarland standards to give a resultant concentration of $1.5 \times 10^8$ cfu/ml. The antibiotic susceptibility testing was determined using the modified Kirby–Bauer diffusion technique (Cheesbrough, 1987) by swabbing the Mueller-Hinton agar (MHA) (Oxoids UK) plates with the resultant saline suspension of each strain. Wells were then bored into the agar medium with heat sterilized 6 mm cork borer.

The wells were filled with 100 µL of different concentrations prepared for the amoxicillin alone, cotrimoxazole alone and their combinations taking care not to allow spillage of the solutions onto the surface of the agar. The plates were allowed to stand for at least 1 h before being incubated at 37°C for 24 h (BSAC, 2002). The determinations were done in duplicate. After 24 h of incubation, the plates were examined for zones of inhibition (Bauer et al., 1966). The diameter of the zones of inhibition produced by the amoxicillin alone, cotrimoxazole alone and their combinations were measured and interpreted using the CLSI zone diameter interpretative standards (CLSI, 2008).

**Determination of MIC by Broth Dilution Methods**

The minimum inhibitory concentrations (MICs) for the amoxicillin alone, cotrimoxazole alone and their combinations were determined in duplicate by the macrobroth dilution method in Mueller-Hinton broth according to CLSI (Clinical Laboratory Standardization Institute) (Richard, 2007). Different concentrations of each of the antibiotic and their combinations ranging from 1.202 to 2462.5 µg/ml were prepared. One milliliter (1 ml) of each working antibiotic concentration was serially diluted in Mueller Hinton broth.

After the serial dilution, 100 µl of each of the adjusted bacterial strains was dispensed into each tube containing each antibiotic or their combinations and incubated at 37°C for 24 h. The minimum inhibitory concentration (MIC) was expressed as the lowest concentrations which inhibited growth as judged by the lack of turbidity in the tube. As a control, a tube containing antibiotic alone and a tube containing inoculums alone, in each rack, was incubated simultaneously along with other tubes containing inoculums for MIC determination. The MIC was defined as the lowest dilution that showed no growth in the Mueller Hinton broth.

**Determination of Minimum Bactericidal Concentration (MBC)**

The MBC was determined by sampling all the macroscopically clear tubes and the first turbid tube in the series. Before being sampled, the tubes were gently mixed by flushing them with a sterile pipette before being subcultured on nutrient agar and incubated at 37°C overnight. After the incubation periods, the lowest concentrations of the antibacterial agents that did not produce any bacterial growth on the solid medium were regarded as their MBC values (Irkin and Korukluoglu, 2007). This observation was matched with the MIC test tube that did not show evidence of growth after 48 h of incubation.

**Statistical analysis**

All the data were subjected to one way analysis of variance (ANOVA) and the mean values were separated at \( p<0.05 \) using Duncan’s Multiple Range Test. The one way ANOVA test was used to determine if there was any statistically significant difference in the diameter of the zones of inhibition obtained from the different concentrations of the extract tested against the microorganisms. All statistical analyses were done using SAS software (1999) model.
Fig. 1: *In vitro* susceptibility of *Salmonella typhi* (TC2) (Mean ± Std. dev) to Amoxicillin alone (A), Cotrimoxazole alone (C) and their combinations (AC).

Fig. 2: *In vitro* susceptibility of *Salmonella typhi* (TC6) (Mean ± St. dev) to Amoxicillin alone (A), Cotrimoxazole alone (C) and their combinations (AC).

Fig. 3: *In vitro* susceptibility of *Streptococcus pyogenes* (TD2) (Mean ± St. dev) to Amoxicillin alone (A), Cotrimoxazole alone (C) and their combinations (AC).
Fig. 4: In vitro susceptibility of *Streptococcus pyogenes* (TD10) (Mean ± Std. dev) to Amoxicillin alone, Cotrimoxazole alone and their combinations.

Fig. 5: In vitro susceptibility of *Streptococcus pneumoniae* (TE10) (Mean ± St. dev) to Amoxicillin alone (A), Cotrimoxazole alone (C) and their combinations (AC).

Table 1: *In vitro* effects of amoxicillin, cotrimoxazole and their combinations against the test isolates

<table>
<thead>
<tr>
<th></th>
<th>MIC range (µg/ml)</th>
<th>MBC range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amoxicillin</td>
<td>0.1202 - 0.4808</td>
<td>0.0241 - 0.0962</td>
</tr>
<tr>
<td>Cotrimoxazole</td>
<td>0.2405 - 0.9619</td>
<td>0.4804 - 1.924</td>
</tr>
<tr>
<td>Cotrimoxazole + Amoxicillin</td>
<td>0.0035 - 0.0150</td>
<td>0.0035 - 0.0301</td>
</tr>
</tbody>
</table>
RESULTS

The effects of antibacterial activities of amoxicillin alone, cotrimoxazole alone and their combinations at different concentrations were investigated against clinical isolates of Salmonella typhi (TC2), Salmonella typhi (TC6), Streptococcus pyogenes (TD2), Streptococcus pyogenes (TD10), and Streptococcus pneumoniae (TE10). In this study, the susceptibility of these isolates was concentration dependent. Cotrimoxazole was more effective against the two strains of Salmonella typhi (TC2 and TC6) than amoxicillin. While the antibacterial combination showed synergy against Salmonella typhi (TC6), antagonistic effect was observed in Salmonella typhi (TC2) although the combination effects at lower concentrations ranging between 15.39 and 123.12 µg/ml showed synergy and indicated higher antibacterial effect than what was obtained in amoxicillin. In the S. pyogenes strains (TD2 and TD10), amoxicillin exhibited higher antibacterial effects than cotrimoxazole. Their combinations indicated synergy when compare with the antibacterial effects of cotrimoxazole and amoxicillin alone against the two strains of S. pyogenes. In S. pneumoniae (TE10), cotrimoxazole was more effective at concentrations ranging between 61.56 and 492.5 µg/ml than amoxicillin. Comparatively, the combination of the two antibiotics was consistently synergistic.

A comparative analysis of the susceptibility of these isolates to amoxicillin alone showed that TD10 was the most susceptible, followed by TD2 > TC6 > TC2 > TE10. The susceptibility of the isolates to the cotrimoxazole showed that TD2 was the most susceptible, followed by TC2 > TC6 > TD10 > TE10. The susceptibility of the isolates to the antibacterial combinations showed that they were susceptible in the following order: TD2 > TD10 > TE10 > TC6 > TC2. Although these organisms had varied susceptibility to the different concentrations of each of these antibiotics and their combinations, the antibacterial combinations showed synergistic interactions that were dependent on the susceptibility of each of the isolates (Table 1 – 5). The macrobroth assay of the interaction between the two antibiotics showed a drastic reduction in the minimum inhibitory concentrations of both antibiotics. While the MIC of amoxicillin ranged between 0.1202 and 0.4808 µg/ml and that of cotrimoxazole ranged between 0.2405 and 0.9619 µg/ml, the MIC of the antibacterial combination ranged between 0.00305 and 0.0150 µg/ml (Table 1). A statistical analysis of the zones of inhibition produced by different concentrations of amoxicillin, cotrimoxazole and their combinations indicated that the mean difference between the zones of inhibitions were significantly different as shown in Tables 2. A p < 0.05 was considered significant.

DISCUSSION

A number of therapeutic agents with different structures and mechanisms of action have been combined and implicated in drug-drug interactions (Yamreudeewong et al., 2003; Niami et al., 2003; Zou et al., 2005). Many drugs used in combination with antibiotics have shown different interactions of clinical significance. While clinically important interactions occur occasionally, most drug combinations do not result in significant adverse interactions and interactions severe enough to warrant reducing dosages are rare (Greenblatt, 2001). Although the incidence of clinically important adverse drug interactions remain unknown (Bianco, 1992) and adverse effects of drug interactions account for only a small fraction of all adverse effects (Fuhr, 2000), several drug combinations have resulted in positive interactions, negative interactions and interactions in which neither of the drugs had any effects on each other in vivo when the
bioavailability of each drug combined was considered. However, to increase the antimicrobial spectrum of these drugs (Chait et al., 2007), antibiotic combinations could be used in combating the dramatic increase in the number of bacteria pathogens which are resistant to conventional antibiotics (Service, 1995; Davies, 1996).

Although there is dearth of information on the interactions between amoxicillin and cotrimoxazole, interactions of amoxicillin and other therapeutic agents had been reported (Derras-Jolly et al., 1996; Chan et al., 2007; Olajuyigbe, 2012). Since the antibacterial combination of antibiotics has gained interest because it often resulted in a synergistic antibacterial effect enabling the dose of the individual drugs to be reduced (Barriere, 1992), this study gave credence to synergistic interactions that would be able to prevent the development of drug-resistance (Wu et al., 1999; Steenbergen et al., 2009) to amoxicillin and cotrimoxazole in vitro. In agreement with Lorian (1991) who showed that the bactericidal activity could best be achieved by the combination of two different antibiotics rather than the effect produced by an individual antibiotic, the mechanism of synergistic action that resulted in high bactericidal effect may involve the penetration of amoxicillin into the peptidoglycan layer to prevent of cross-links, inhibit cell wall synthesis and, therefore, increase the permeability of the different bacterial strains to cotrimoxazole that acts sequentially in preventing folic acid synthesis (Kutty et al., 1998). The synergistic effect may, also, be due to the formation of certain complexes which became more effective in inhibiting these clinical isolates either by inhibiting the cell synthesis or by causing its lyses or death.

In conclusion, the steady increase in bacterial resistance to existing drugs is a serious problem. As resistance to old antibiotics spreads, there is a dire need to search for new classes of antibacterial substances if the problem is to be contained. Consequently, investigating newer drugs to which there is lesser resistance and combining old antibiotics for synergistic interactions against clinical bacterial pathogens becomes essential in an era where high toxicity are associated with newer antibacterial agents and funding for discovery of new therapeutic agent has been retracted. Since the need of the moment is to develop newer, useful and important antimicrobial agents capable of overcoming bacterial resistance, the resultant synergy in the combination of amoxicillin and cotrimoxazole is a novel concept as such combinations will have different mechanism of action which may lead to new choices of therapeutic agents for the treatment of streptococcal and gastrointestinal infections in which these organisms have been implicated. These combinations can enhance the efficacy of amoxicillin and cotrimoxazole and could be used effectively in treating respiratory infections as well as gastrointestinal infections caused by multidrug resistant microorganisms having no effective therapy available.

REFERENCES

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SAS, Proprietary software release 8.2. SAS institute Inc. NC., USA.


