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Research Article

In vitro interaction between caffeine and some penicillin antibiotics against *Staphylococcus aureus*

CO Esimone[1], FBC Okoye[2]*, CS Nworu[3], CO Agubata[4]

[1]Department of Pharmaceutics, [2]Department of Pharmaceutical and Medicinal Chemistry, [3]Department of Pharmacology and Toxicology, Faculty of Pharmaceutical Sciences, University of Nigeria, Nsukka, Enugu State, Nigeria, [4]Department of Pharmacy, Ministry of Health, Anambra State, Awka, Anambra State, Nigeria.

Abstract

***Purpose**: The aim of this study is to evaluate the in vitro interaction of some penicillins (amoxicillin, ampicillin and benzylpenicillin) and caffeine against Staphylococcus aureus.*
***Method**: The interaction between the penicillins and caffeine was studied using the Overlay Inoculum Susceptibility Disc (OLISD) method. Minimum inhibitory concentrations (MIC) of the drugs were determined separately and in combination with caffeine (5 and 10 mg/ml).*
***Result**: At 5 and 10 mg/ml, caffeine decreased the MIC of amoxicillin by 22 and 25 times respectively, while that of ampicillin was decreased by 6 and 8 times. The MIC of benzylpenicillin against Staphylococcus aureus was, however, increased by 59 and 40 times at caffeine concentrations of 5 and 10 mg/ml respectively. The inhibition zone diameter increment above 19 % (index of synergism in OLISD method) was recorded only for amoxicillin at amoxicillin concentrations of 7.81, 15.3, 31.25 and 62.5 mg/ml.*
***Conclusion**: The results of this study revealed that the concomitant use of caffeine and the studied antibiotics may potentiate the antibacterial effect of amoxicillin against Staphylococcus aureus, decrease that of benzylpenicillin and has virtually no effect on that of ampicillin. This implies that the intake of caffeine in form of analgesic combination or as tea, coffee, beverages or from other food sources may affect the effectiveness of a co – administered amoxicillin and bezylpenicillin.*

Keywords: *antimicrobial interactions, caffeine, ampicillin, amoxicillin, bezylpenicillins, penicillins*

***Corresponding author** :Email: basdenc@yahoo.com Tel: +234-8033607639*

INTRODUCTION

Caffeine (1, 3, 7 – trimethylxanthine) is a member of methylxanthines, a class of compounds that is widely distributed in nature. Caffeine is regularly consumed from dietary sources including coffee, tea, cola beverages and chocolate[1,2]. Caffeine is the most widely used behavioral active substance in the world[3,4] and as such has been a subject of intensive study. Various pharmacological effects have been observed for caffeine. These include: antagonistic effect on adenosine receptors[5], inhibition of phosphodiesterase[6,7], stimulation of muscle contraction[8] and alteration in glucose metabolism[9,10]. Caffeine is included in a number of analgesic medications in common use.

A number of drug – drug and drug – food interactions involving caffeine are well known and has been documented. Some of these interactions are attributed to the ability of caffeine to form complexes with these drug molecules[11]. Others may be due to the influence caffeine on certain cellular enzymes[12,13,14]. There are reports that methylxanthines in general affect inhibitory effects of some antibacterial agents[15]. Caffeine was shown to increase the inhibitory effect of tetracycline and penicillin G against *Staphylococcus aureus*[16]. In another study, caffeine was found to decrease the antibacterial effect of tetracycline hydrochloride and chloramphenicol[17]. Interaction involving food sources of caffeine and some antibacterial agents are well documented[18].

Ampicillin, amoxicillin and benzyl penicillin are commonly used β–lactam antibiotics with known activity against *Staphylococcus aureus*[19]. The clinical usefulness of these drugs is, however, limited by their susceptibility to β–lactamase hydrolysis. Several strains of *Staphylococcus aureus* are known to produce β–lactamase and this has resulted to the development of resistance to some commonly used penicillin antibiotics[19]. The penicillins included in this study are sometimes consumed with food and drug sources of caffeine. It is a common practice, for instance, for a patient to take a capsule of ampicillin or amoxicillin with a cup of caffeine beverages. More so, caffeine/paracetamol analgesic is frequently taken concurrently with these drugs. In this study, therefore, we intend to investigate the possibility of drug – drug interaction between caffeine and the selected antibiotics. Caffeine has been shown to inhibit *Staphylococcus* β–lactamase[20]. It is expected that this inhibition will potentiate the antibacterial effect of the selected antibiotics against *Staphylococcus aureus* when used in combination with caffeine. Thus, possible synergistic effects of the selected antibiotics with caffeine against *Staphylococcus aureus* were evaluated.

MATERIALS AND METHODS

Materials

Ampicillin sodium and amoxicillin sodium were purchased from Juhel Pharmaceuticals, Enugu, Nigeria, Benzyl penicillin sodium from Doyin Pharmaceutical, Lagos, Nigeria and caffeine from SKG Pharma Lagos, Nigeria. Other materials used were Bacto® Agar (Difco, Germany) and Nutrient broth (Biotec, Italy)

Test microorganisms

Staphylococcus aureus was obtained from the Pharmaceutical Microbiology Unit of Department of Pharmaceutics, University of Nigeria, Nsukka, Enugu State, Nigeria. The organism was maintained by weekly subculturing on nutrient agar slant stored at 4 ^{0}C after previous 24 h incubation at 37 ^{0}C. Before each experiment, the organism was activated by successive subculturing and incubation.

Standardization of test microorganisms

A 10 ml volume of sterile water was added to the agar slant containing a 24 h old culture of the purified test microorganism and shaken carefully to harvest the organism. Subsequently, dilutions were carried out to get microbial population of 10^5 cfu/ml by comparing with Mcfarland 0.5 standard.

Preparation of drug stock solutions and discs

Stock solutions containing 1000 mg/ml of each of the drugs were prepared by weighing out accurately 10 g each of amoxicillin, ampicillin and benzylpenicillin and dissolving in 10 ml sterile water. Two fold serial dilutions were carried out to obtain 500, 250, 125, 62.5, 31.25, 15, 15.23 and 7.81 mg/ml of the drug solutions. These solutions were used to prepare the antibiotic discs using Whatmann No 1 filter paper in accordance with the NCCL standards[21].

Determination of MIC of the antibiotics

The sensitivities of the test microorganism to ampicillin, amoxicillin and benzyl penicillin were evaluated by determining their MIC as previously reported[22,23].

Interaction of caffeine and the antibiotics

Molten agar (9 ml) was inoculated with 0.1 ml of standard *Staphylococcus aureus* suspension. The mixture was poured into sterile Petri dishes, shaken slowly for uniform distribution and allowed to solidify. The plates were each divided into four sections using permanent marker. The antibiotic discs of various concentrations prepared as described above were placed on the solidified surface of the *S. aureus*-seeded agar and the plates incubated at 37 ^{0}C for 24 h. The Petri dishes so treated were regarded as control plates. For interactions, the test antibiotic discs were prepared as already described using serially diluted drug solutions containing 5 mg/ml and 10 mg/ml of caffeine. The susceptibility of the test microorganism to the antibiotic discs was assessed by measuring the inhibition zone diameter (IZD). The mean % increases in IZD of the test over the control groups were obtained and the interaction results determined as previously described[24].

Statistical Analysis

Each experiment was done in triplicates and the results reported as mean ± SEM.

RESULTS

The results of the combined activity of the antibiotics with caffeine are shown in Tables 1 – 4. At concentrations of 5 and 10 mg/ml, caffeine decreased the MIC of amoxicillin against *Staphylococcus aureus* by 22 and 25 times respectively while that of ampicillin was decreased by 6 and 8 times respectively. The MIC of benzylpenicillin against *Staphylococcus aureus* was, however, increased by 59 and 40 times at caffeine concentrations of 5 and 10 mg/ml respectively. Caffeine did not show any inhibition of the growth of *S. aureus* at concentrations up to 10 mg/ml (Table 1).

The combination of amoxicillin and caffeine (5 and 10 mg/ml) resulted in an increase in IZD at all the tested concentrations (Table 2). The combination of ampicillin and caffeine (5 and 10 mg/ml) resulted only in a slight increase in IZD at 7.81 and 15.63 mg/ml and a slight decrease at 250, 500 and 1000 mg/ml (Table 3) while that of benzylpenicillin resulted in total decrease in IZD at all the tested concentrations (Table 4).

DISCUSSION

Ampicillin, amoxicillin and benzylpenicillin have been shown, in several reports, to be active against some strains of *Staphylococcus aureus*[19,22]. The sensitivity of the strain used in the study was, however, ascertained by determining the MIC of the selected antibiotics (Table 1). These antibiotics are frequently consumed with food and drug sources of caffeine and this could lead to possible drug – drug or drug – food interactions. The results of the combined antimicrobial activities indicate that caffeine exhibited varied antimicrobial interaction with the tested antibiotics. In the study of antimicrobial interaction using Overlay Inoculum Susceptibility Disc (OLISD) a 19 % increase in IZD is usually taken as index of synergism[24]. Thus, the combined antimicrobial activity of amoxicillin and caffeine (5 and 10 mg/ml) produced synergistic effect at amoxicillin concentrations of 7.81, 15.3, 31.25 and 62.5 mg/ml. That of ampicillin produced indifferent effect while complete antagonism was observed with benzylpenicillin.

Table 1: MIC (mg/ml ± SEM) of the antibiotics against *Staphylococcus aureus*

	Antibiotic alone	Antibiotic + 5 mg/ml caffeine	Antibiotic + 10 mg/ml caffeine
Amoxicillin	2.0178	0.0910	0.0792
Ampicillin	0.0355	0.0060	0.0019
Benzyl penicillin	1.9002	112.61	75.514

NB: Caffeine showed no inhibition of the growth of S. aureus at concentrations up to 10 mg/ml.

Table 2: Combined activity (IZD [mm] ± SEM) of amoxicillin and caffeine against *Staphylococcus aureus*

Concentration (mg/ml)	Amoxicillin alone	Amoxicillin + 5 mg/ml caffeine	Amoxicillin + 10 mg/ml
7.81	13.5 ± 0.5	18.8 ± 0.5 (39.3)	19 ± 0.5 (40.7)
15.63	14.7 ± 0.6	19.7 ± 0.3 (34.0)	20 ± 0.0 (36.1)
31.25	16.3 ± 0.6	21.0 ± 0.0 (28.8)	21.0 ± 0.0 (28.8)
62.5	18.2 ± 0.3	22.07 ± 0.7 (21.3)	22.2 ± 0.3 (22.0)
125.0	20.5 ±0.0	23.0 ± 0.0 (12.2)	23.5 ± 0.5 (14.6)
250	22.0 ± 0.0	24.0 ± 0.5 (9.1)	24.5 ± 0.5 (11.4)
500	24.0 ± 0.6	24.7 ± 0.7 (2.9)	25.0 ± 0.0 (4.2)
1000	26.5 ± 0.5	27.4 ± 0.5 (3.4)	27.4 ± 0.5 (3.4)

Values in parenthesis represent percent increase in IZD.

Table 3: Combined activity (IZD [mm] ± SEM) of ampicillin and caffeine against *Staphylococccus aureus*

Concentration (mg/ml)	Ampicillin alone	Ampicillin + 5 mg/ml caffeine	Ampicillin + 10 mg/ml caffeine
7.81	21.8 ± 0.3	22.3 ± .6 (2.3)	22.7 ± 0.6 (4.1)
15.63	22.5 ± 0.5	23.3 ± 0.6 (3.6)	23.5 ± 0.5 (4.4)
31.25	24.7 ± 0.6	24.7 ±0.6 (0.0)	24.7 ± 0.6 (0.0)
62.5	25.5 ± 0.5	25.3 ± 0.6 (-0.8)	25.3 ± 0.6 (-0.8)
125.0	26.5± 0.5	26.2 ± 0.3 (-1.1)	26.2 ± 0.3 (-1.1)
250	27.8 ± 0.3	27.2 ± 0.3 (-2.2)	26.7 ± 0.3 (-3.4)
500	29.0 ± 0.7	28.0 ± 0.0 (-3.4)	27.7 ± 0.3 (-4.5)
1000	29.7 ± 0.6	29.0 ± 0.5 (-2.3)	28.7 ± 0.3 (-3.4)

Values in parenthesis represent percent increase in IZD. Negative sign (-) indicates percentage decrease.

Caffeine is known to mediate its effects through the inhibition of some cellular enzymes. It has been shown to inhibit the enzymes required for DNA synthesis, for example, it inhibits the incorporation of adenine and thymidine during the synthesis of DNA[25,12,13,14]. The exact mechanism of the antimicrobial synergistic interaction observed between caffeine and amoxicillin is not yet clear. However, it is possible that the inhibition of bacterial cell wall by penicillins, which leads to lyses of the cells, might facilitate the influx of caffeine into the bacterial cells. Such higher concentration in the cells will enhance the damage on DNA caused by caffeine[12,26]. The synergistic effect may also be as a result of the inhibition of *Staphylococcus* penicillinase enzyme[20], which will potentiate the activity of the penicillinase sensitive antibiotics.

Table 4: Combined activity (IZD [mm] ± SEM) of benzylpenicillin and caffeine against *Staphylococcus aureus.*

Concentration (mg/ml)	Benzyl Penicillin alone	Benzyl Penicillin + 5 mg/ml caffeine	Benzyl Penicillin + 10 mg/ml caffeine
7.81	15.0 ± 0.7	0.0 (-100)	0.0 (-100)
15.63	16.8 ± 0.8	0.0 (-100)	0.0 (-100)
31.25	19.0 ± 0.0	0.0 (-100)	0.0 (-100)
62.50	21.3 ± 0.6	0.0 (-100)	0.0 (-100)
125.00	24.3 ± 0.6	0.0 (-100)	0.0 (-100)
250.00	26.5 ± 0.9	11.3 ± 0.6 (-57.4)	11.3 ± 0.6 (-57.4)
500.00	27.8 ± 0.8	14.3 ± 0.6 (-48.6)	14.3 ± 0.6 (-48.6)
1000.00	29.3 ± 0.6	18.3 ± 0.6 (-37.5)	16.7 ± 0.6 (-43.0)

Values in parenthesis represent percent increase in IZD. Negative sign (-) indicates percentage decrease

The observed correlation between the polarity of the selected antibiotics and the effects of caffeine on their antibacterial activities is of interest. Amoxicillin is the most polar of the three antibiotics followed by ampicillin and then bezylpenicillin. It appears that caffeine enhances the antibacterial activities of the more polar antibiotics while decreasing that of the less polar. It is not clear whether this is a mere coincidence. However, a plausible explanation may be the influence of the physicochemical interaction between caffeine and the drugs in vitro. Complexation with caffeine via hydrophobic van der Waals interaction[1,27,28,29] may have reduced the in vitro antibacterial activity of the least polar antibiotic. Whether this has an in vivo correlation or not will be a subject for further investigation.

CONCLUSION

Our results show that concomitant intake of the antibiotics and caffeine will lead to the potentiation of the antibacterial effect of amoxicillin, reduction in antibacterial effect of benzylpenicillin and virtually no effect on that of ampicillin. The former is a clear beneficial interaction and may imply using a lower dose of amoxicillin to achieve the same therapeutic effect when given in combination with caffeine. It also implies that intake of caffeine in form of analgesic combination or as tea, coffee, beverages or from other food sources may affect the effectiveness of a co–administered amoxicillin and bezylpenicillin.

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