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Short Report

In vitro Evaluation of Methylxanthines and Some Antibiotics: Interaction against *Staphylococcus aureus* and *Pseudomonas aeruginosa*

Hossein Hosseinzadeh[*1], Bibi Sedigheh Fazly Bazzaz[2] and Mojgan Moaddab Sadati[3]

[1]*Pharmaceutical Research Center, Faculty of Pharmacy, Mashhad University of Medical Sciences;* [2]*Biotechnology Research Center, Faculty of Pharmacy, Mashhad University of Medical Sciences;* [3] *Faculty of Pharmacy, Mashhad University of Medical Sciences, Mashhad, Iran*

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ABSTRACT

Background: The development of resistance to antimicrobial agents is a major problem in chemotherapy. Finding agents which potentiate antimicrobial activity could be favorable. There are some reports that methylxanthines changed the inhibitory effect of antibacterial agents. Thus, possible synergistic effect of methylxanthines, aminophylline and caffeine on some antibiotics, carbenicillin, ceftizoxime and gentamicin, which are effective on *P. aeruginosa* and *Staphylococcus aureus*, were studied. **Method:** The interaction of methylxanthines and antibiotics were studied *in vitro* using a checkerboard method. **Results:** At concentrations of 0.25-4 mg/ml, aminophylline and caffeine decreased the MIC of the antibiotics 2-4 times against *P. aeruginosa* and *Staph. aureus*. Both methylxanthines also reduced the minimum bactericidal concentration of the antibiotics by up to 2 times. Caffeine and aminophylline had no antimicrobial effect themselves. **Conclusion:** The results of the present study reveal that aminophylline and caffeine potentiated the antimicrobial action of carbenicillin, ceftizoxime and gentamicin against *Staph. aureus* and *P. aeruginosa*. *Iran. Biomed. J. 10 (3): 163-167, 2006*

Keywords: Aminophylline, Caffeine, Interaction, *Staphylococcus aureus, Pseudomonas aeruginosa*

INTRODUCTION

Methylxanthine drugs such as aminophylline and caffeine block adenosine receptors [1-3], inhibit phosphodiesterases [4] and other enzymes including 5′-nucleotidase and alkaline phosphatase [5]. They also cause the release of calcium from intracellular stores [6, 7]. Adenosine receptor blockade occurs at low micromolar concentrations of some methylxanthines, while other actions occur in the millimolar concentration range [8].

There are some reports [9-12] that methylxanthines changed the inhibitory effect of antibacterial agents. Caffeine increased the inhibitory effect of penicillin G and tetracycline against *Staphylococcus aureus* by a factor of 4 [9]. While in another study, both theophylline and caffeine decreased the antimicrobial effect of tetracycline hydrochloride and chloramphenicol [10]. Also, caffeine increased the efficacy of furazolidone against vibrios [11]. An enzymatic (specific) degradation of caffeine to non-toxic compound is mediated by Pseudomonas and Aspergillus [12].

The development of resistance to antimicrobial agents is a major problem in chemotherapy. Finding agents which potentiate antimicrobial activity could be favorable. Thus, possible synergistic effect of methylxanthines, aminophylline and caffeine on some antibiotics, which are effective on *P. aeruginosa* and *Staph. aureus*, were studied. Three different category antimicrobial agents, penicillin (carbenicillin), cephalosporin (ceftizoxime) and

*Corresponding Author; E-mail: hosseinzadehh@mums.ac.ir

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aminoglycoside (gentamicin), which are effective against *Staph. aureus* and *P. aeruginosa* were evaluated.

MATERIALS AND METHODS

Materials. Carbenicillin was purchased from (Beecham Pharmaceutical), Ceftizoxime from (Jaber ebn Hayyan, Iran), Gentamicin and caffeine from (Daru Phakhsh, Iran), Aminophylline from (Iran Hormone, Iran), Caso Agar from (Merck, Germany) and Muller Hinton Broth (Difco, Germany).

Cultivation of organisms and the preparation of cell suspension for inoculation. The organisms used were *Staph. aureus* (ATCC 29737) and *P. aeruginosa* (ATCC 9027). Prior to the test, the surface of plate of caso agar medium (Merck, Germany) was inoculated from recently grown stock culture of each of the specified microorganisms already on caso agar and incubated at 35-37°C for 18-24 h. Colonies (n = 4-5) of the cells were inoculated into 5 ml Muller Hinton Broth Cation Supplemented (CSMHB), Difco (Germany), and incubated at 37°C for 3-4 h. When turbidity of about 0.5 McFarland was attained, the cells were diluted 1:500 with CSMHB to give a cell suspension of 3×10^5 cfu/ml [13].

Determination of minimum inhibitory concentration (MIC). Equal volumes of CSMHB (0.5 ml) were added to sterile capped test tubes (except the first one). A volume of 0.5 ml of the solution of antibiotics (carbenicillin, ceftizoxime or gentamicin) or methylxanthines (aminophylline or caffeine) in CSMHB was added to the first 2 tubes. From the second tube, 0.5 ml was added to the third and continued to the last one, and from that 0.5 ml was discarded. Cell suspension (0.5 ml) was added to all tubes and incubated at 37°C for 18-24 h. After the incubation period, the minimum concentration of antibiotic with no growth on the tube was reported as MIC [13]. Each experiment was done in triplicate.

Determination of minimum bactericidal concentration (MBC). From the above tubes with no growth (no turbidity), 0.1 ml was spread over the surface of agar plate (Caso Agar). After incubation at 37°C for 48 h, the colonies were observed and minimum concentration of antibiotics causing 99.9% death of the bacteria was considered as MBC [13, 14]. Each test was done in triplicate.

Interaction of methylxanthines and antibiotics (checkerboard method). A set of 6 × 6 tubes containing combined concentration of antibiotics (from 0 to MBC) and methylxanthines (from 0 to 4 mg/ml) were prepared (1.5 ml). Cell suspension (0.5 ml) was added to each tube and incubated at 37°C for 18-24 h. After the incubation period, the tubes with no growth (-) and with growth (+) were recorded. From the tubes with no growth, 0.1 ml was spread on the surface of agar plates. Agar plates with no colonies were reported as 99.9% effective, and the MBC of antibiotics in the presence of that concentration of methylxanthines were reported [13]. Each experiment was done in triplicate.

Statistical analysis. Each experiment was done three times and the mode of results reported.

RESULTS

MIC and MBC determination of antibiotics and methylxanthines. Methylxanthines (up to 4 mg/ml aminophylline and 8 mg/ml caffeine) did not show any antibacterial activity against the two microorganisms. The MIC and MBC of ceftizoxime, gentamicin and carbenicillin against *P. aeruginosa* and *Staph. aureus* were determined as shown in Table 1.

Table 1. The MIC and MBC of antimicrobial drugs against *P. aeruginosa* and *Staph. aureus*.

Drug	MIC (µg/ml)		MBC (µg/ml)	
	P. aeruginosa	***Staph. aureus***	***P. aeruginosa***	***Staph. aureus***
Ceftizoxime	64	8.0	128	16
Gentamicin	8	0.5	16	2
Carbenicillin	64	1.0	256	2

For determination of MIC and MBC, broth dilution method was used.

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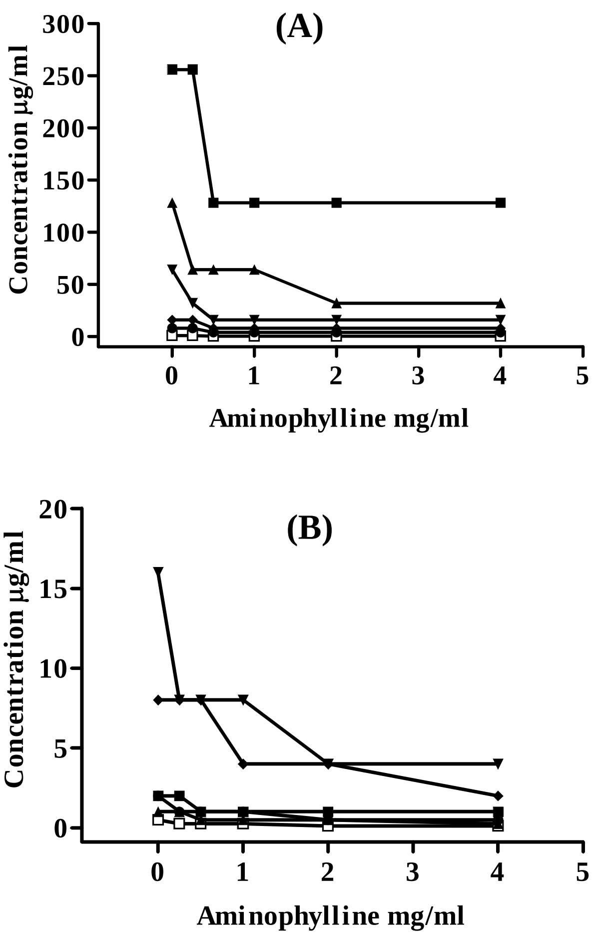
Interaction of methylxanthines and antibiotics. Caffeine and aminophylline had no antimicrobial effect themselves. At concentrations of 0.25-4 mg/ml, aminophylline decreased the MIC and MBC of ceftizoxime, gentamicin and carbenicillin against *P. aeruginosa* 2-4 times (Fig. 1A).

At concentrations of 0.5-4 and 1-8 mg/ml, caffeine decreased the MIC and MBC of ceftizoxime, gentamicin and carbenicillin against *P. aeruginosa* 2-4 times, respectively (Fig. 1B).

At concentrations of 0.25-4 mg/ml, aminophylline decreased the MIC and MBC of ceftizoxime, gentamicin and carbenicillin against *Staph. aureus* 2-4 times (Fig. 2A).



`**Fig. 1.** Interaction of aminophylline **(A)** and caffeine **(B)** on the inhibitory and bactericidal effects of carbenicillin (CBN), ceftizoxime (CFZ) and gentamicin (GNN) against *P. aeruginosa*. MIC, minimum inhibitory concentration; MBC, minimum bactericidal concentration, each point is the mode of 3 experiments. ■ CBN (MBC), ▲ CBN (MIC), ▼ CFZ (MBC), ◆ CFZ (MIC), ● GNN (MBC), □ GNN (MIC), each point is the mode of 3 experiments.

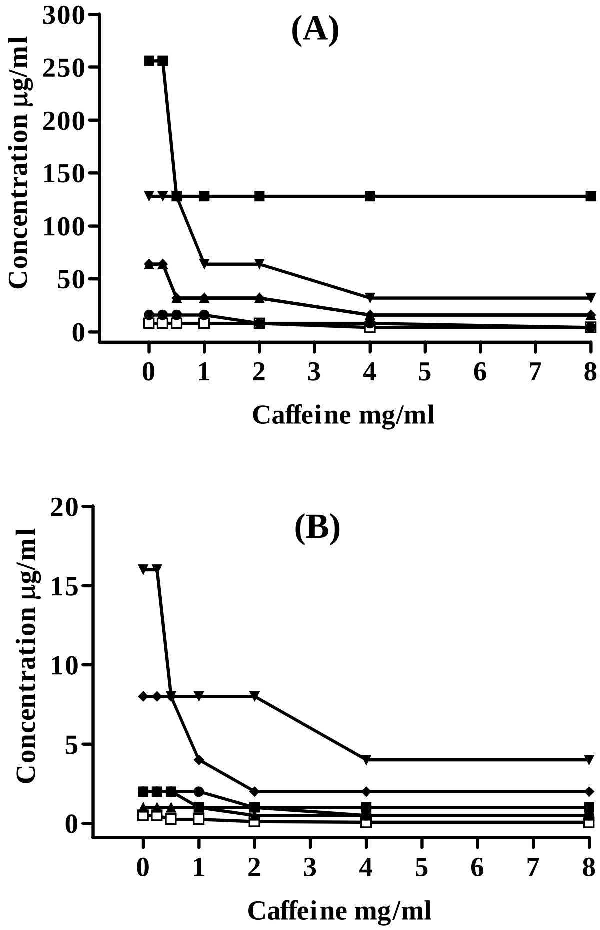


Fig. 2. Interaction of aminophylline **(A)** and caffeine **(B)** on the inhibitory and bactericidal effects of carbenicillin (CBN), ceftizoxime (CFZ) and gentamicin (GNN) against *Staph. aureus.* MIC: minimum inhibitory concentration, MBC: minimum bactericidal concentration, each point is the mode of 3 experiments.

At concentrations of 0.5-4 mg/ml, caffeine decreased the MIC and MBC of ceftizoxime, gentamicin and carbenicillin against *Staph. aureus* 2-8 times (Fig. 2B).

DISCUSSION

The present study shows that methylxanthines, i.e. aminophylline and caffeine, reduce the MIC and MBC of antibiotics: ceftizoxime, gentamicin and carbenicillin against *P. aeruginosa* and *Staph. aureus.*

The antibiotics used in this study are effective against *P. aeruginosa* and/or *Staph. aureus* [15, 16]. Gentamicin has a bactericidal effect against aerobic

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Gram-negative micro-organisms and some *Staphylococcus* species [15]. In this study, the MIC and MBC of gentamicin against *Staph. aureus* were 0.5 and 2 µg/ml and against *P. aeruginosa* were 8 and 16 µg/ml, respectively. As effective plasma concentration for gentamicin is 8 µg/ml [14], thus, the micro-organisms may be sensitive to gentamicin *in vivo*. Theophylline and caffeine had a synergistic effect on antimicrobial effect of neomycin against *P. aeruginosa* and *Staph. aureus* [9]. These activities of methylxanthines were similar to our results about gentamicin.

Carbenicillin, a carboxypenicillin, has a bactericidal effect against some *Pseudomonas* species [16]. In this study, the MIC and MBC of carbenicillin against *Staph. aureus* were 1 and 2 µg/ml and against *P. aeruginosa* were 64 and 256 µg/ml, respectively. Intravenous injection of carbenicillin (1 g) induces a plasma concentration of about 70-140 µg/ml [17], thus, the micro-organisms may be sensitive to this penicillin *in vivo*.

Ceftizoxime, a third generation cephalosporin, has a broad spectrum of activity against Gram-negative and Gram-positive bacteria [16]. In this study, the MIC and MBC of ceftizoxime against *Staph. aureus* were 8 and 16 µg/ml and against *P. aeruginosa* were 64 and 128 µg/ml, respectively. Intramuscullar injection of ceftizoxime (0.5 and 1 g) induces a plasma concentration of about 14-39 µg/ml [17], thus, the micro-organisms may be sensitive to *Staph. aureus in vivo* but resistance to *P. aeruginosa*. Both aminophylline and caffeine when used with ceftizoxime reduced the MIC and MBC of this antibiotic against the resistant micro-organisms, thus the use of the methylxanthines with ceftizoxime may cause *Pseudomonas* become sensitive to ceftizoxime *in vivo*.

The reduction of MIC and MBC of the antibiotics by methylxanthines may also decrease adverse effects of the antimicrobial drugs. For example, Gentamicin as aminoglycosides has nephrotoxicity and outoxicity [13, 18]. Thus, co-administration of these drugs may reduce their adverse reactions. The exact mechanism of action of this synergistic effect is not clear. Caffeine and theophylline inhibit Staphylococcus penicillinase enzyme [19]. This activity will potentiate the effect of antibiotics sensitive to penicillinase and reduce resistance. Caffeine inhibits incorporation of adenine and thymidine in the synthesis of DNA [20], with inhibition of thymidine kinase. Caffeine also inhibits the synthesis of DNA [21] or enhances genotoxicity after DNA damage [22]. It may be possible that penicillin and cephalosporin cause lyses of the cell wall, facilitating the diffusion of methylxanthines into micro-organisms and affect on DNA. The synergistic effect may be also due to the fact that aminoglycosides and caffeine inhibit syntheses of proteins and DNA, respectively. The therapeutical blood levels for methylxanthines such as aminophylline are in the range of 10-20 µg/ml [23] but the concentration of these drugs for the above interactions is higher than normal values. Thus, further experiments such as structure activity relationship experiments for finding methylxanthines with the higher potency should be done.

This study shows that there is a potentiation effect between the methylxanthines and some antibiotics. Clinically, this might be very important for the treatment of infective diseases that are resistance to antibiotics. It is suggested to study the effect of topical preparations which consists of methyl-lxanthines and some antimicrobial agents against some local infections such as burns.

REFERENCES

1. Daly, J.W., Bruns, R.F. and Snyder, S.H. (1981). Adenosine receptors in the central nervous system: relationship to the central actions of methylxanthines. *Life Sci. 28: 2083-2097*.
2. Fredholm, B.B. and Persson, C.G.A. (1982). Xanthine derivatives as adenosine receptor antagonists. *Eur. J. Pharmacol. 81: 673-76*.
3. Fredholm, B.B., Chen, J.-F., Masino, S.A. and Vaugeois, J.-M. (2005). Actions of adenosine at its receptors in the CNS: Insights from knockouts and drugs. *Ann. Rev. Pharmacol*. Toxicol. *45: 385-412*.
4. Butcher, R.W. and Therland, E.W. (1962). Adenosine 3 ',5 '-phosphate in biological materials. *J. Biol. Chem. 237: 1244-1250*.
5. Fredholm, B.B., Hedqvist, P. and Vernet, L. (1978). Effect of theophylline and other drugs on rabbit renal nucleotide phosphodiestrase, 5 '-nucleotidase and adenosine deaminase. *Biochem. Pharmacol. 27: 2845-2850*.
6. Lee, H.C. (1993) Potentiation of calcium and caffeine-induced calcium release by cyclic ADP-ribose. *J. Biol. Chem. 268: 647-456*.
7. Tanaka, Y. and Tashjian, Jr. A.H. (1993) Functional identification and quantitation of three intracellular calcium pools in OH4C1 cells: evidence that the caffeine-responsive pool is coupled to a thapsigargin-resistant, ATP-dependent process. *Biochemistry 32: 12062-12073*.

8. Barry, S.R. (1988) Dual effects of theophylline on spontaneous transmitter release from frog motor nerve terminals. *J. Neurosci. 8: 4427-4433*.
9. Charles, B.G. and Rawal, B.D. (1973) Synergistic effect of methyl substituted xanthines and neomycin sulphate on *Staph. aureus* and *P. aeruginosa* in vitro. *Lancet 1: 971-973*.
10. Charles, B.O. and Rawal, B.D. (1979) The combined action of methylxanthines with erythromycin, chloramphenicol and tetracycline on *Staph. aureus. Microb. Lett. 10: 143-147*.
11. Banerjee, S.K. and Chatterjee, S.N. (1981) Radiomimetic property of furazolidone and caffeine enhancement of its lethal action on the vibrios. *Chem. Biol. Interact. 37: 321-335*.
12. Gokulakrishnan, S., Chandraraj, K. and Gummadi, S.N. (2005). Microbial and enzymatic methods for the removal of caffeine. *Enz. Microb. Technol. 37: 225-232*.
13. Anhalt, J. P. and Washington, J.A. (1995) Laboratory procedures. In: *Clinical Microbiology.* Washington, J.A. ed.), New York, Springer-Verlag, pp. 283-91, 731-745.
14. Finegold, S.M. and Baron, E.J. (1990). *Bailey and Scott's Diagnostic Microbiology.* 8th ed. The C.V. Mosby Company, St. Louis. pp. 171-178.
15. Chambers, H.F. and Sande, M.A. (1996). Antimicrobial agents: penicillins, cephalosporins and other β-lactam. In: *The Goodman and Gilman's, the Pharmacological Basis of Therapeutics.* (Hardman, J.G. and Limbird, L.E. eds.), 9th ed. McGraw-Hill, New York. pp. 1073-1101.
16. Mandell, G.L. and Petri, Jr. W.A. (1996). Antimicrobial agents: the aminoglycosides. In: *The Goodman and Gilman's, the Pharmacological Basis of Therapeutics*. (Hardman, J.G. and Limbird, L.E. eds.), 9th ed. McGraw-Hill, New York. pp. 1103-1121.
17. Reynold, J.E.F. (1996). *Martindale, the Extrapharmacopoeia*. 31st ed. Royal Pharmaceutical Society, London. pp. 183-238.
18. Lanzoni, I., Corbacella, E., Ding, D., Previati, M. and Salvi, R. (2005). MDL 28170 attenuates gentamicin ototoxicity. *Audiol*. Med. *3: 82-89*.
19. Charles, B.G. and Rawal, B.D. (1977) Action of caffeine and theophylline on Staphylococcal penicillinase synthesis. *Chemotherapy 23: 452-457*.
20. Labbe, R.G. and Nolan, L.L. (1978) Inhibition of macromolecular synthesis by caffeine in *Clostridium perfringes*. *Can. J. Microbiol. 33: 589-592*.
21. Sandlie, I., Solberg, K. and Kleppe, K. (1980) The effect of caffeine on cell growth and metabolism of thymidine in *Escherichia coli. Mutat. Res. 73: 29-41*.
22. Kaufmann, W.K., Heffernan, T.P., Beaulieu, L.M., Doherty, S., Frank, A.R., Zhou, Y., Bryant, M.F. and Cordeiro-Stone, M. (2003) Caffeine and human DNA metabolism: The magic and the mystery. *Mut. Res. Fundam. Mol. Mech. Mutagen. 532: 85-102*.
23. Serafin, W.E. (1996). Drugs used in the treatment of asthma. In: *The Goodman and Gilman's, the Pharmacological Basis of Therapeutics.* (Hardman, J.G. and Limbird, L.E. eds.), 9th ed. McGraw-Hill, New York. pp. 659-682.

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