Potential Activity of the Purine Compounds Caffeine and Aminophylline on Bacteria

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ABSTRACT

Background: Purine compounds are special types of alkaloids. Caffeine and aminophylline are considered the most important members of purines due to their wide use in therapeutics. **Aims:** To detect any potential antibacterial effects on pathogenic bacteria of the widely prescribed members of purines caffeine and aminophylline. **Materials and Methods:** Two species of gram-positive bacteria and five species of gram-negative bacteria were exposed to these purine agents. Antibacterial effects of the tested purines were determined using the spectrophotometer method to assess the minimum inhibition concentrations (MIC). **Results:** Among the strains of bacteria tested, *Bacillus subtilis* showed the most susceptibility to purine agents. *Staphylococcus aureus* and *Bacillus subtilis* were found to be more susceptible to caffeine than the other strains. Aminophylline showed inhibitory action on many isolates, especially at the concentration of 10mg/ml. *Paracoccus yeei* demonstrated resistance to all tested purine compounds up to a concentration of 10.5mg/ml. **Conclusions:** Caffeine and aminophylline had the ability to inhibit many strains of pathogenic bacteria.

Key words: Aminophylline, Antibacterial effect, Caffeine, Purine

INTRODUCTION

Purine compounds of the alkaloid group consist of three main chemical agents: caffeine, theophylline, and theobromine. Some amounts of these compounds can be found in many species of plants, with the amount present varying with the plant's age and the parts tested. Thus, the level of endogenous caffeine in leaves of *Coffea arabica* L. is much higher in buds and young leaves than in developed leaves.[1] Such accumulation of caffeine in young leaves may play a role in protecting the tender tissues of the plant against attack by predators, which include insect larvae and beetles.[2] The leaves of *Thea sinensis* that are commonly used to prepare tea for popular drinking also contain caffeine and small amounts of theophylline.[3]

Aminophylline synthesized from theophylline and ethylenediamine is widely used in pharmaceutical preparations due to its high solubility in water as compared to theophylline.[4] Aminophylline and theophylline have been used in the treatment of asthma and chronic obstructive airway diseases for more than 70 years.[5] Caffeine is formulated in combination with many pharmaceutical drugs; for example, it is used in combination with ergotamine for treatment of migraine headaches and in combination with non-steroidal anti-inflammatory drugs (NSAIDs) for relief of painful conditions.[6]

The action of purine on the metabolic pathways of bacteria may range from harmful to beneficial, depending upon the species of bacteria. Some species of bacteria use caffeine as a major source of carbon for their nutritional requirement, while others are inhibited in the presence of caffeine. *Pseudomonas putida* can degrade 20% of caffeine incorporated in culture media (1 g/L) after 9 hours of incubation, and it uses this purine compound as its sole source of carbon and nitrogen.[7]

Purines can also have an effect on the sporulation rate and morphological development of bacterial cells. The conversion of the rod shape of *Escherichia coli* into a filamentous form has been observed upon addition of 10 mM of theophylline into the culture media.[8] *Aerobacter aerogenes* and *Aerobacter cloaca* developed a long filamentous form in the presence of 1mg/ml of caffeine. In addition,

Access this article online

Quick Response Code:

Website: www.jgid.org

DOI: 10.4103/0974-777X.81689

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the rate of spore production by *Clostridium perfringen*s was increased after culture on casein digest media containing 100–1000 μg/ml of caffeine.[9]

On the other hand, many studies have illustrated no activity of purine compounds on the viability of bacteria. Aminophylline and caffeine (up to 4mg/ml aminophylline and 8mg/ml caffeine) did not show any antibacterial effects on *Staphylococcus aureus* and *Pseudomonas aerogenosa*.[10] Oral microorganisms (including seven strains) were also not affected by caffeine at concentrations of up to 400 μg/ml.[11] Furthermore, theophylline exhibited no action on the persistence of *Serratia marcescens* that was successfully isolated from theophylline bottles given to neonates.[12]

In this study we attepted to detect the potential antibacterial effects of the widely prescribed members of the purine group – caffeine and theophylline (in the form of aminophylline). These compounds were tested against several pathogenic bacteria.

MATERIALS AND METHODS

Organisms

Seven strains of bacteria were clinically isolated from patients (30–38 years of age) with respiratory and gastrointestinal tract infections at AL-Hussein General Hospital of Karbala province. Throat swabs and stool samples were cultured on Mueller-Hinton agar (HiMedia, Mumbai, India) and incubated at 35ºC for 24 hours. In addition to gram-staining and morphological criteria, we used the API-20 system (Biomérieux, Netherlands-France) to identify the bacterial species. For further confirmation, the isolates were sent to the bacteriological bank of the College of Science, University of Basraha, Iraq.

The isolated strains (one strain for each species) included two species of gram-positive bacteria (*S aureus* and *Bacillus subtilis*) and five species of gram-negative bacteria (*E coli, Enterobacter aerogenes, Enterobacter cloacae, Salmonella typhi, and Paracoccus yeei*).

Chemical agents

Caffeine and aminophylline were purchased from HiMedia, Mumbai, India. Ampicillin sodium and cefotaxime sodium were supplied by KonTam Pharmaceuticals Co. (Zhongshan, China).

Antibacterial assays

For antibacterial assay, total count of isolated strains were standardized to equivalent a 0.5 MacFarland Nephelometer standard (1×10^8 cfu/ml) by Mueller-Hinton broth (HiMedia, Mumbai, India) and then diluted as 1:10.

The spectrophotometer technique was applied to determine the antibacterial activities of purine agents. Briefly, various concentrations of caffeine and aminophylline (0.312, 0.625, 1.25, 2.5, 5, and 10mg/ml) were singly mixed in tubes containing Mueller-Hinton broth. These tubes were inoculated with previously prepared cultures of each strain (50 μl to each milliliter of broth). The tubes were then incubated at 35ºC for 24 hours. The optical density of bacterial growth was measured using a spectrophotometer (Optima-SP-300; Karzma Co. Tokyo, Japan) at a wavelength of 450 nm.[13]

Ampicillin sodium and cefotaxime sodium were dissolved in sterile distilled water to prepare concentrations of 20, 39, and 78 μg/ml. Both of these drugs and media without any added chemicals were used as controls.

Determination of minimum inhibitory concentration

Determination of minimum inhibitory concentration (MICs) were determined by the microdilution method recommended by the NCCLS.[14] Briefly, the tested chemical agents were two-fold diluted in Mueller-Hinton broth. A volume of 100 μl of each dilution was dispensed into the wells of 96-well microdilution plates. The filled well was inoculated with 50 μl of previously prepared of standardized count of bacteria. The inoculated trays were incubated at 35ºC for 24 hours and examined for visible growth in order to determine the MIC. Three previous controls were also included.

Statistical analysis

Each experiment was repeated three times with triplicates of each concentration. The data were statistically analyzed using two-way analysis of variance (ANOVA) with least significant difference (LSD) at $P<0.05$.

RESULTS

The purine compounds caffeine and aminophylline were investigated at various concentrations for antibacterial effects against seven strains of bacteria using the spectrophotometer method.

B subtilis was found to be more susceptible to the tested purines than the other species of bacteria. The growth rate of six of the seven strains of bacteria (the exception

being *Staphylococcus aureus*) significantly decreased in the presence of a high level of aminophylline (10mg/ml). *S typhi* and *B subtilis* needed only 5mg/ml of aminophylline to reduce their ability to grow in culture media [Figures 1 and 2] [Table 1].

As Figure 3 shows, bacterial strains exhibited more variable susceptibility to different concentrations of caffeine. The gram-positive bacteria, *S aureus* and *B subtilis*, were more susceptible to the action of caffeine than the gram-negative bacteria. The turbidity of the broth culture of *Paracoccus yeei*, and the elevated optical density ratio, pointed to a very clear resistance to the toxic action of caffeine. Statistical analysis showed that the purine compounds had significant effect ($P<0.05$) in reducing the growth of *Paraccocus yeei* [Figures 2 and 3]. The efficacy of aminophylline in decreasing bacterial growth (as compared to growth in the control media) was significantly more than that of caffeine.

Caffeine and aminophylline, at concentrations of 10 mg/ml, revealed greater ability to inhibit most of tested strains (especially *S aureus, E cloacea,* and *E aerogenes*) than the standard antibiotic ampicillin, which was used as a control. However, cefotaxime showed greater effect on the isolated bacteria than the purine compounds [Figures 2 and 3].

DISCUSSION

Caffeine and theophylline are important purine compounds for human beings as large amounts are consumed daily in foods, beverages, and drugs.

Caffeine is the most widely consumed behaviorally active substance in the world. In the USA alone, the daily intake of caffeine among adults approaches an average of approximately 200mg.[15] Dietary surveys in North America indicate that 80%–90% of children and adults have weekly or more frequent consumption of caffeine-containing foods.[16]

Table 1: MICs of caffeine and aminophylline in the bacterial isolates

Strains	Aminophylline concentrations (mg/ml)					Caffeine concentrations (mg/ml)				
	10.5	10	7	5	2.5	10.5	10	7	5	2.5
S aureus	–	+	+	+	+	–	–	+	+	+
S typhi	–	–	–	–	+	–	+	+	+	+
B subtilis	–	–	–	-	+	–	–	–	+	+
E cloacae	–	–	–	+	+	–	+	+	+	+
E aerogenes	–	–	+	+	+	–	+	+	+	+
E coli	–	–	–	+	+	–	–	+	+	+
P yeei	–	–	+	+	+	+	+	+	+	+

+ = clear growth; – = No growth

Theophylline is still commonly prescribed in the treatment of asthma and chronic obstructive airway disease because it is inexpensive.[5] The similarities between purine alkaloids and nucleic acids in structure encourage the use of these compounds as antimicrobial agents.

The results of this study revealed that high concentrations are required for an antibacterial action, with aminophylline

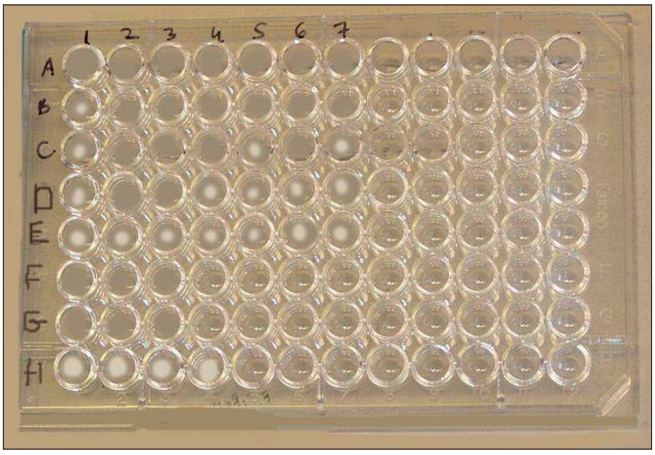

Figure 1: Microdilution plate for MIC of aminophylline in tested bacteria. (A) 10.5mg/ml, (B) 10mg/ml, (C) 7mg/ml, (D) 5mg/ml, (E) 2.5mg/ml, (F) Ampicillin, (G) Cefotaxime, and (H) Control (free medium). Bacterial strains are (1) *S aureus*, (2) *S typhi,* (3) *B subtilis*, (4) *E cloacae*, (5) *E aerogenes*, (*6) E coli,* and (7) *P yeei*

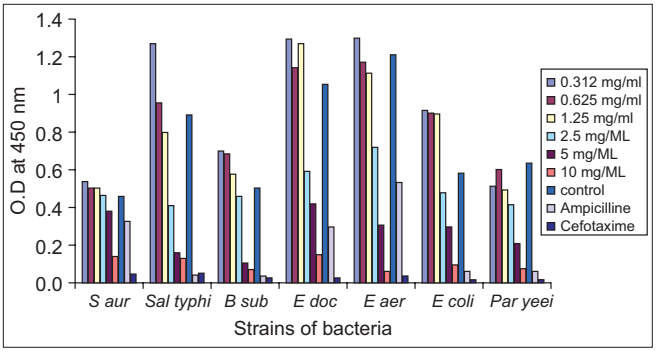

Figure 2: Effects of different concentrations of aminophylline on the growth of bacteria

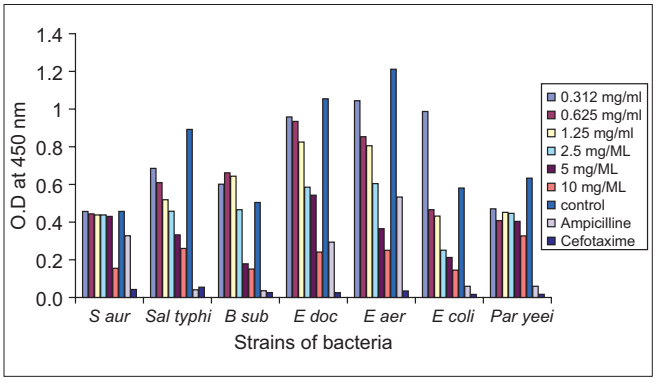

Figure 3: Effects of different concentrations of caffeine on the growth of bacteria

proving to be much more effective than caffeine against the bacterial isolates. In contrast, Raj and Dhala[17] reported that caffeine at concentrations up to 5mg/mL had more antibacterial activity than theophylline.

Among the seven strains used in this study, the spore-forming bacteria (*B subtilis*) was most susceptible to the action of the tested purine substances. *Salmonella typhi* was susceptible to aminophylline only. Concordant with our findings, a previous study also found that *B subtilis* and *Salmonella typhi* were inhibited by 5mg/ml of theophylline and caffeine.[17]

In the present study, some strains of bacteria, including *S aureus* and *P yeei,* exhibited resistance to both purine compounds. Hosseinzadeh *et al*.[10] also recorded such resistance to caffeine and aminophylline after culturing *S aureus* and *P aerogenosa* in media containing these compounds.

Antimicrobial drugs act in one of several ways: by selective toxicity, by inhibition of cell membrane synthesis and function, by inhibition of protein synthesis, or by inhibition of nucleic acid synthesis. Resistance to cephalosporins had been observed in many strains of bacteria due to the production of a stable cephalosporinase.[18] This enzyme is related to the β-lactamase group that is expressed by plasmids.[19] It is considered the most important mechanism of development of drug resistance in *Enterobacter* strains.[20] However, the *E cloacea* and *E aerogenes* that showed resistance to ampicillin in the present study were found to be susceptible to the purine compounds.

The main action of caffeine in bacterial cells can be explained by many mechanisms: for example, interaction of caffeine with bacterial nucleic acid[9] or inhibition of the synthesis of DNA (as noted in *E coli* K2).[21] Caffeine also can interact with the enzyme responsible for repair of bacterial DNA damage by inhibition of the ATP-dependent enzyme.[22]

Three important mechanisms of theophylline action in mammalian cells are proposed: inhibition of cAMP phosphodiesterase enzyme,[23] adenosine receptor antagonism,[24] and inhibition of histone deacetylase (HDACs).[25] In bacterial cells, the inhibition of cAMP phosphodiesterase resembles the accepted mechanism of theophylline.[8]

A previous study has shown that purine compounds have both *in vitro* and *in vivo* efficacy in inhibiting a special group of skin pathogenic fungi (dermatophytes).[26] Recent data suggests that these compounds also have inhibitory activity on other groups of microorganisms such as bacteria. These results indicate that purine agents, with their ability to destroy different types of pathogenic organisms, may be promising drugs for treatment of many diseases.

Judging on the basis of their pharmaceutical characteristics, caffeine and aminophylline have the potential to be useful drugs for treatment of bacterial infection. The beneficial characteristics include the following: these compounds are toxic to humans only at relatively high concentrations;[4] the mode of action in microorganisms is suspected to be an interaction with the nucleic acids of the pathogen;[8,9,21] development of resistance in microorganism to these purine agents arises slowly because of usage of high concentrations of the drug; few adverse effects are associated with administration of these agents;[3] caffeine and aminophylline are inexpensive drugs, so large numbers of patients can be benefitted.[5] Most of the known antibacterial drugs lack one or more of these characteristics. Thus, caffeine and aminophylline have the potential to become one of the prefered drugs for treatment of bacterial infection.

CONCLUSIONS

The purine compounds demonstrated potential antibacterial activities in high concentrations, with aminophylline showing greater ability than caffeine to decrease bacterial growth. However, more studies are needed to investigate the antimicrobial activity of purine compounds and their potential for use in clinical situations. Certainly, present knowledge regarding the characteristics of purine compounds suggest that they could find use as antimicrobials.

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How to cite this article: AL-Janabi AS. Potential activity of the purine compounds caffeine and aminophylline on bacteria. J Global Infect Dis 2011;3:133-7.

Source of Support: Nil. **Conflict of Interest:** None declared.