Evaluation of the Synergistic Effect of Water Soluble Extracts of Green Tea (Camellia sinensis) on the Activity of Ciprofloxacin in Urinary Isolated E. coli

Nima Hosseini Jazani, Minoo Zartoshti, Shahram Shahabi, Zahra Yekta and Shahin Nateghi

Department of Microbiology, Immunology and Genetics,
Department of Community Medicine,
Faculty of Medicine, Urmia Medical Sciences University, Urmia, Iran

Abstract: The aim of this study is the evaluation of the synergistic effect of sub-MIC doses of ciprofloxacin with water soluble green tea extract against urinary tract isolates of E. coli. A total of 18 isolates were collected from urine specimens submitted to a clinical diagnostic Laboratory in Urmia, Iran. The amounts of MIC and MBC for green tea water extract, ciprofloxacin or a mixture of green tea with sub-MIC doses of ciprofloxacin were determined and three groups were compared. The Synergism between ciprofloxacin and water soluble green tea extracts were seen for 93.7% (15 of 16 tested) of bacterial isolates. This study suggests that combination of water soluble green tea extracts and ciprofloxacin has in vitro synergistic effect on urinary tract isolated E. coli.

Key words: Green tea, minimum inhibitory concentration, minimum bactericidal concentration, ciprofloxacin, E. coli, synergistic effect

INTRODUCTION

Tea is the most consumed drink in the world after water. Green tea is a non-fermented tea and has more health beneficial effects than black tea or oolong tea.

Since ancient times, green tea has been considered by the traditional Chinese medicine as a healthful beverage. Recent human studies suggest that green tea may contribute to a reduction in the risk of cardiovascular diseases and some forms of cancer, as well as to the promotion of oral health and other physiological functions such as anti-hypertensive effect, body weight control, antibacterial and antiviral activity, ultraviolet protection, bone mineral density increase, anti-fibrotic properties and neuro-protective power. Increasing interest in its health benefits has led to the inclusion of green tea in the group of beverages with functional properties (Cabrera et al., 2006).

Tea leaves are known for its antibacterial activity against many microorganisms. There are also some reports about synergy between green tea extract and some antibiotics. Isogai et al. (2001) reported synergy between green tea extract and Levofloxacin against enterohaemorrhagic E. coli. Tiwari et al. (2005) showed that tea extracts has synergistic activity with chloramphenicol and other antibiotics like gentamicin, methicillin and nalidixic acid against enteropathogenic bacteria. However there are only limited studies on synergistic activity of green tea extracts with antibiotics.

Urinary tract infections are one of the most common urological diseases in adults (Lee et al., 2005) and E. coli is the main agent of this disease. Antibiotic therapy is the gold standard of treatment. However, long-term therapy may result in many side-effects and cause selection of resistant bacteria. So, we need new treatments that could replace antibiotic therapy. Green tea consumption resulted in highest intakes in urine (He and Kies, 1994), also green tea has antimicrobial effect against various bacteria and synergistic activity with some of antibiotics. Fluoroquinolones is one of the best antibiotics for treatment of urinary tract infections (Gagliotti et al., 2007).

The synergistic effect of catechin, an extract of green tea, with ciprofloxacin has been proven on E. coli Z17, O2:K1: H in a rat model of Chronic bacterial prostatitis (Lee et al., 2005), however there is no studies on the synergistic effects on green tea and ciprofloxacin on several isolates of uropathogenic E. coli.

The aim of this study is the evaluation of the synergistic effect of sub-MIC doses of ciprofloxacin with different concentrations of water soluble green tea extract against 18 isolates of E. coli from urinary tract infections.

Corresponding Author: Nima Hosseini Jazani, Department of Microbiology, Immunology and Genetics, Faculty of Medicine, Urmia Medical Sciences University, Urmia, Iran Tel: +98-441-3449548, +98-914-3464234
MATERIALS AND METHODS

Bacterial isolates and culture media: A total of 18 isolates were collected from urine specimens submitted to a clinical diagnostic laboratory in Urmia, Iran during a two months period throughout October and November 2006. The isolates were further processed by the standard methods to identify as the *Escherichia coli* isolates. Isolated bacteria were maintained for long storage on skimmed milk medium (BBL) by adding 10% glycerol in 60°C, cultures were maintained for daily use on Nutrient agar slants on 4°C.

Preparation of tea leaves and ciprofloxacin powder: Commercial green tea was used for doing experiments. Tea samples were stored in plastic bags at 4°C. Crude green tea extracts were prepared by the method described by Tiwari *et al.* (2005) with some modifications. Ciprofloxacin powder were kindly provided by Exir Pharmaceutical Company, Tehran, Iran.

Determination of antimicrobial activity of green tea extracts: Antibacterial activity of water soluble green tea extracts and ciprofloxacin were measured by determining MIC and MBC for each bacterial isolate described previously else where (Jazani *et al.*, 2007; Tiwari *et al.*, 2005; Sahm and Weisfled, 2002).

Determination of synergetic activity of green tea water extract with ciprofloxacin: Nutrient broth medium with green tea extract (initial concentration equivalent to MIC of each test isolate) prepared by two-fold serial dilution, ciprofloxacin were added to all tubes with sub-MIC concentrations. Each dilution was inoculated with 3×10⁶ of test isolate and incubated at 37°C. After overnight incubation, MIC and MBC of green tea in the presence of sub-MIC concentrations of ciprofloxacin were determined. (Tiwari *et al.*, 2005). Synergy was present when there was a reduction in MIC of green tea extract in the presence of sub-MIC doses of ciprofloxacin for each strain (Gombert and Aulicino, 1983).

RESULTS

A total of 18 *E. coli* isolates were collected from urine samples submitted to the clinical microbiology laboratories of selected hospitals in Urmia, Iran.

Sensitivity of bacterial isolates to green tea water soluble extracts: The average MICs and MBCs of the water soluble green tea extract against all isolates of *E. coli* were 122.9±40.3 mg mL⁻¹. Also the MIC and MBC amounts were showed for each tested isolate (Table 1).

<table>
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<tr>
<th>Isolates</th>
<th>MIC of ciprofloxacin for each isolate (μg mL⁻¹)</th>
<th>MBC of ciprofloxacin for each isolate (μg mL⁻¹)</th>
<th>MIC or MBC of green tea extract for each isolate (mg mL⁻¹)</th>
<th>MBC or MBC of green tea extract for each isolate (mg mL⁻¹)</th>
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<td>122.9±40.3</td>
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*: MIC amount for each strain was equivalent to MBC, R = Resistant, S = Sensitive, NG = No Growth, NT = No Tested

Sensitivity of bacterial isolates to ciprofloxacin: As shown 4 isolates were resistant even to very high concentrations of ciprofloxacin investigated in this study, also there was 2 isolates that were sensitive to even very low concentrations (Table 1).

Synergism between water soluble green tea extract and ciprofloxacin: 43.7% of isolates showed decrease in MIC of green tea in the presence of sub-MIC concentrations of ciprofloxacin and 50% of *E. coli* isolates did not show growth in the presence of the sub-MIC concentrations of ciprofloxacin plus different concentrations of green tea extract under the MIC and for one of the isolates there is no difference in MIC of green tea extract in the presence or absence of sub-MIC concentration of ciprofloxacin, however 2 isolates did not tested in synergy experiments, because of their sensitivity to even very low concentrations of ciprofloxacin, there is synergistic activity between water soluble green tea extracts and ciprofloxacin between 93.7% of urinary tract *E. coli* isolates.

DISCUSSION

*Escherichia coli* is the most common cause of uncomplicated urinary tract infection and accounts for approximately 75 to 95% of all infections. Although the incidence of urinary tract infection has not changed
substantially over the last 10 years, the diagnostic criteria, bacterial resistance patterns and recommended treatment have changed. Trimethoprim-sulfamethoxazole has been the standard therapy for urinary tract infection; however, \textit{E. coli} is becoming increasingly resistant to medications. Many experts support using ciprofloxacin as an alternative and, in some cases, as the preferred first-line agent. However, others caution that widespread use of ciprofloxacin will promote increased resistance (Mehnert-Kay, 2005). Because of these reasons, we need new treatment modality that could replace traditional antibiotic therapy. Green tea components show antiviral and antifungal activities in addition to their inhibitory effects on exotoxins (Taguri et al., 2004). Various studies have shown significant suppressive effects of green tea against many microorganisms, for example \textit{Salmonella typhimurium} (Shetty et al., 1994), \textit{Salmonella typhi}, \textit{Shigella dysenteriae}, \textit{Yersinia enterocolitica}, \textit{Escherichia coli}, \textit{Staphylococcus aureus}, \textit{Vibrio cholerae}, \textit{Campylobacter jejuni}, \textit{Plesiomonas shigelloides}, \textit{P. aeruginosa} and many other species of bacteria (Toda et al., 1989; Taguri et al., 2004; Yam et al., 1997, 1998; Kim et al., 2004; Stapleton et al., 2004; Lee et al., 2003). There is several evidence that high levels of green tea polyphenols were found in urine after drinking tea in human or experimental animal models (Kim et al., 2000; Yang et al., 1998). So using of green tea seems reasonable for treatment of urinary tract infections.

It has been shown that green tea has synergistic activity with some of antibiotics on different bacterial species especially \textit{E. coli} and \textit{Staphylococcus aureus}. Green tea catechins acting in synergy with beta-lactams, tetracycline and fluoroquinolones (Roccaro et al., 2004; Isogai et al., 2001).

In this study urinary tract isolates of \textit{E. coli} were sensitive to green tea water soluble extracts, present results showed that green tea extracts had bacteriostatic as well as bactericidal effects on urinary tract isolates of \textit{E. coli} (Table 1).

It has been shown that catechin (an extract of green tea) is an effective material in chronic bacterial prostatitis treatment in rat model. Synergistic effects between catechin and ciprofloxacin on chronic bacterial prostatitis rat model formerly has been proven by Lee et al. (2005). They create an experimental chronic bacterial prostatitis model in 70 male rats by instillation of 0.2 mL bacterial suspension of only one strain of \textit{E. coli} (Z17. O2:K1:H). However in the present study we used different urinary tract isolates of \textit{E. coli} obtained from patients. In this study \textit{in vitro} synergistic effect of a combination of water soluble green tea extract and ciprofloxacin has been showed (Table 1), so present results confirms the data obtain from the study has been done by Lee et al. (2005) about Synergistic effects between green tea and ciprofloxacin on different urinary isolates of \textit{E. coli} \textit{in vitro}. Therefore, we suggest that the combination of green tea and ciprofloxacin may be effective in treating of urinary tract infections. However more studies on numerous strains of urinary tract isolates of \textit{E. coli} as well as \textit{in vivo} studies not only in experimental animals but also in humans should be done for confirmation of the synergistic antibacterial effects of water soluble green tea extracts in combination with ciprofloxacin for treatment of urinary tract infections caused by \textit{E. coli}.

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REFERENCES


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