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Medicinal plant extracts variously modulate susceptibility of *Escherichia coli* to different antibiotics☆

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ABSTRACT

Antioxidant activity of green and black tea and extracts of medicinal plants and their ability to modulate antibiotic susceptibility in *Escherichia coli* were studied. Among a number of extracts tested the maximal capacity to scavenge DPPH radicals and chelate iron in chemical tests was found in green and black tea, *Arctostaphylos uva-ursi* and *Vaccinium vitis-idaea*. These extracts contained high level of polyphenols and in aerobic conditions exhibited prooxidant features, producing H_2O_2 and inducing expression of the *katG* gene encoding catalase HPI in *E. coli* cells. A good correlation between the polyphenol content and the ability of extracts to protect bacteria against peroxide stress was observed ($r = 0.88$). Polyphenol-rich extracts and iron chelators demonstrated the highest modulating effect on the antibiotic susceptibility by changing the time period before lysis started and by influencing the colony-forming ability of bacteria. The direction of the modulating effect was dependent on nature of antibiotic applied: under treatment with ciprofloxacin and ampicillin the extracts predominantly provided protective effects, while under treatment with kanamycin a bactericidal action was enhanced. Mechanism of modulating action of extracts on bacterial antibiotic susceptibility probably involves antioxidant, preferentially iron-chelating, or prooxidant properties of polyphenols.

1. Introduction

Over the last decade intense investigation revealed that oxidative stress is involved in lethality of antibiotics with different mechanisms of action. In the presence of certain antibiotics, an increase in intracellular oxidants, disruption of iron and Fe–S cluster homeostasis, DNA damage, and upregulation of many oxidative stress defense and DNA repair genes were observed (Albesa et al. 2004; Dwyer et al. 2007; Kohanski et al. 2007, 2008; Wang and Zhao 2009; Yeom et al. 2010). Collectively, these data suggest that antibiotics promote Fenton-mediated hydroxyl radical formation via H_2O_2 reduction by Fe^{2+} ions. Hydroxyl radicals are extremely toxic and readily damage proteins, membrane lipids, and DNA. Recent data suggest, however, that specific oxidation of the guanine nucleotide pool rather than the cumulative effect of oxidizing various classes of cellular molecules and macromolecules is responsible for much of the death caused by bactericidal antibiotics (Foti et al. 2012). Catalase induction and cotreatment with iron chelator dipyridyl or hydroxyl radical scavenging substances significantly decreased susceptibility of *Escherichia coli* to norfloxacin, ampicillin and kanamycin (Wang and Zhao 2009). Antioxidants glutathione and ascorbic acid confer protection against fluoroquinolones and aminoglycosides (Goswami et al. 2006, 2007; Dhamdhere et al. 2010) but augment antibacterial activity of β-lactams against *E. coli* (Goswami and Jawali 2007). Sensitivity of resistant strain *Staphylococcus aureus* 22 to ciprofloxacin and gentamicin significantly increased in the presence of glutathione. However, in the sensitive *S. aureus* ATCC 29213, the association of glutathione with these antibiotics did not induce significant variations of MIC (Paez et al. 2010).

These findings reveal the possibility of the modification of antibiotic action in the presence of compounds with chelating and pro- and antioxidant activity. Among them, polyphenols may be compounds of particular interest. Plant polyphenols (flavonoids and tannins) occur ubiquitously in foods of plant origin, medicinal and cosmetic products and have attracted great interest since the 1990s owing to growing evidence of their beneficial effects on human health (Crozier et al. 2009). These compounds are known to have antioxidant properties due to their ability to scavenge radicals and chelate iron (Ferrali et al. 1997; Rice-Evans et al. 1995; Perron and Brumaghim 2009). At the same time it has been demonstrated that in certain conditions polyphenols may take part in generation of reactive oxygen species (ROS) and acts as prooxidants (Smith

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et al. 2003; Tang and Halliwell 2010). The mechanism of antioxidant action of polyphenols on living cells is not limited to ROS scavenging, but includes upregulation of antioxidant and detoxification enzymes, modulation of cellular redox thiols, and modulation of cell signaling and gene expression (Eberhardt and Jeffery 2006). Earlier we have reported that pretreatment of *E. coli* with flavonol quercetin and tannic acid upregulated antioxidant genes *katG* and *sodA* owing to prooxidant and chelating activity and resulted in remarkable protective effects against oxidative stress (Smirnova et al. 2009).

Medicinal plant extracts, widely applied in traditional and modern medicine, demonstrate antioxidant activity which may, in part, be associated with polyphenol compounds (Rice-Evans et al. 1995; Pietta 1998; Oktyabrsky et al. 2009; Tang and Halliwell 2010). Antibiotics and medicinal herbs may be used simultaneously under therapy of infectious diseases. However, influence of plant extracts on sensitivity of bacteria to antibiotics has not been well studied. Recent data show that medicinal plant extracts and polyphenols may protect bacterial cells against ciprofloxacin toxicity (Smirnova et al. 2012; Marathe et al. 2013).

The aim of the present work was to study antioxidant activity and influence of the medicinal herbs on antibiotic susceptibility of *E. coli* cultures. Considering that the quantification of antioxidants through *in vitro* chemical assays may not reflect the full complexity of action of antioxidants in cellular systems, antioxidant activity of extracts under peroxide stress in *E. coli* cells was also studied.

2. Materials and methods

2.1. Materials

All reagents for determination of β-galactosidase activity, Amplex Red, DPPH (2,2-diphenyl-1-picrylhydrazyl), ferrozine (3-(2-pyridyl)-5,6-diphenyl-1,2,4-triazine-4′,4″-disulfonic acid sodium salt), trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylicacid), 2,2′-dipyridyl, deferoxamine mesylate, thiamine, gallic acid, Folin–Ciocalteu's phenol reagent were from Sigma Chemical Co. (St. Louis, MO, USA). All other reagents were of analytical grade (Reachim, Russia).

Water extracts of medicinal herbs and teas were prepared from commercial pharmaceutical preparations (OAO Krasnogorskleksredstva and ZAO Ivan-Chai, Russia) and commercial samples of green and black tea (Greenfield "Golden Ceylon", Greenfield tea Ltd., London, UK). Dry herbs (1 g) were boiled in 30 ml of distilled water in a water-bath during 30 min, cooled and sequentially filtrated through paper and membrane (0.45 μm pore size) filters. Fresh extracts were used in all experiments.

2.2. Microbial strains and culture conditions

In this work we used strains of bacteria *Escherichia coli* BW25113 (wt, Keio collection), BN407 containing plasmid ColV-K30 with fusion *iucC::lacZ* (Lorenzo et al. 1987) received from Dr. J. Imlay (Maringanti and Imlay 1999), and NM3021, which was constructed by introduction of plasmid pKT1033 with gene fusion *katG::lacZ* (Tao et al. 1989, 1991) into strain BW25113. Bacteria were grown in M9 minimal glucose ($2\,g\,l^{-1}$) medium ($Na_2HPO_4{\cdot}12H_2O$ – $15.13\,g\,l^{-1}$, KH_2PO_4 – $3\,g\,l^{-1}$, NH_4Cl – $1\,g\,l^{-1}$, NaCl – $0.5\,g\,l^{-1}$, $MgSO_4{\cdot}7H_2O$ – $0.246\,g\,l^{-1}$, $CaCl_2$ – $0.011\,g\,l^{-1}$) supplemented with 0.2% casamino acids and thiamine (10 μg/ml) (Miller 1972).

2.3. Determination of bacterial susceptibility to antibiotics

In order to evaluate the influence of plant extracts and chelators on bacterial susceptibility to ciprofloxacin (3 μg/ml), ampicillin (10 μg/ml) and kanamycin (30 μg/ml), duration of lysis delay and colony-forming ability were studied. After centrifugation cells grown overnight were resuspended in 50 ml of fresh medium to initial OD_{600} = 0.1 and then were grown aerobically at 37 °C in 250-ml flasks with shaking at 150 rpm. In mid-log phase (OD_{600} = 0.5) extracts (400 μl) and antibiotics were added and bacteria were cultivated for 160 min. Specific growth rate was calculated according to the equation:

$$\mu = \ln(N/N_0)/t$$

where μ is the specific growth rate and N_0 and N are the optical densities at time zero and t, respectively. Time point when μ dropped below zero was taken as a start of lysis.

For colony-forming studies, samples of control culture and cultures treated with compounds and antibiotics were removed at intervals, washed and diluted in 0.9% NaCl and then mixed with molten soft LB-agar (0.8%) at 42 °C and poured onto agar plates containing solid LB-agar (1.5%) without antibiotics and extracts. Colonies were counted in 24 h after incubation at 37 °C.

2.4. Assessment of antioxidant activity using microbial test system

E. coli cells from mid-log phase (100 ml culture with OD_{600} = 0.5) were centrifuged and resuspended in 8 ml of M9 medium. These cells were further used in plate experiments. Each well of plate contained 5 μl of the extracts or chelators (0.1 mM), 5 μl of concentrated cells (up to OD_{600} = 0.1) and M9 medium to the final volume of 200 μl. OD_{600} was measured before and after addition of the cells to subtract the color of the extracts. Plates were incubated for 20 min at 37 °C with shaking at 140 rpm, OD_{600} was measured and then H_2O_2 was added to final concentration of 4 mM. After 40 min incubation OD_{600} was measured. The obtained values of OD_{600} were used to determine the specific growth rate before and after oxidative stress. Index of antioxidant activity (AOA) was expressed as the ratio of specific growth rate in cultures pretreated with the tested compounds to the untreated control after H_2O_2 addition.

2.5. Determination of β-galactosidase activity

β-Galactosidase activity was determined as described by Miller (1972), modified for measurements using plate reader xMark Bio-Rad (Smirnova et al. 2012). Cultures of *E. coli* NM3021 and BN407 were used for study the *katG* and *iucC* genes expression, respectively.

2.6. Measurement of total phenol content

Total polyphenols content was measured using a modified Folin–Ciocalteu method (Wu et al. 2006). Plant extracts (12.5 μl) were mixed with 40 μl of Folin–Ciocalteu reagent for 15 s and incubated at room temperature for 3 min. About 125 μl of 7% sodium carbonate solution was then added to the test tubes, and the mixture was diluted to 3 ml with de-ionized water. Total polyphenols were determined after 90 min of incubation at room temperature, and absorbance was measured at 760 nm using Shimadzu UV–vis spectrophotometer. The measurement was compared to a standard curve of gallic acid solutions and the results were expressed as gallic acid equivalents (GAE) in mg per 1 g of dry weight.

2.7. Determination of DPPH• free radical-scavenging capacity

Antiradical activity of extracts was assessed on the basis of the free radical-scavenging effect of the stable DPPH• radical. The reaction mixture contained 3 ml of 0.3 mmol l^{-1} of DPPH–ethanol