Antibiotics and Antioxidants: Friends or Foes During Therapy?

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Abstract

Different types of biochemical and physiological factors (including commonly used cellular and dietary antioxidants) affect the activity of therapeutic antibiotics. The findings from our lab have shown that the presence of glutathione, N-acetylcysteine or ascorbic acid in the growth medium decreases the bacterial susceptibility towards fluoroquinolone and aminoglycoside antibiotics. However the mechanism behind the antioxidant mediated protection against these two groups of antibiotics could be different. In addition, presence of glutathione increased antibacterial activity of β-lactam antibiotics showing that glutathione could act as a differential antibiotic susceptibility modulator for bacteria. Our data therefore demonstrate that therapeutic effectiveness of antibiotic treatment could be modulated by the dietary intake and cellular level of these antioxidants.

Introduction

Antibiotics are natural or synthetic compounds with selective bactericidal or bacteriostatic effects that eliminate pathogens or slow their growth such that host defense mechanisms can clear the infection. The mechanisms of antibiotics actions are well studied, particularly in relation to their targets interactions. Majority of the commonly used antibiotics fall into following groups: DNA damage-causing agents, inhibitors of protein synthesis, inhibitors of cell wall biosynthesis and metabolic inhibitors. Antibiotics are weapons of choice in fight against infectious bacterial diseases, however, their overuse and misuse has contributed significantly to the growing problem of antibiotic resistance and emergence of superbugs such as Methicillin Resistant *Staphylococcus aureus* (MRSA) and New Delhi Metallo-beta-lactamase-1 (NDM-1) isolates. Therefore knowledge about molecular mechanism of antibiotic action, related bacterial response and factors modulating antibiotic activity could be of used for development of improved antibacterial substances and therapeutic regimen, which could help us in keeping pace with remarkable adaptability of bacteria.

In our lab, we are studying the effect of dietary and cellular antioxidants on antibacterial effect of commonly used antibiotics. Dietary supplements such as vitamin C (Ascorbic acid) and E (α-tocopherol), having antioxidant properties, are prescribed many a times by the physicians along with antibiotics during the course of treatment of an infection. Besides antioxidants such as N-acetylcysteine (NAC) are used as auxiliary medication in certain pathological conditions along with the antibiotic therapy (NAC is used as a mucolytic agent in combination with clinically relevant antibiotics for treatment of lower respiratory tract infection). Therefore it is important to understand the effects of antioxidants on the antibacterial action of commonly used antibiotics. We are actively working to understand how different antioxidants affect the action of diverse antibiotics under *in-vitro* and *in-vivo* conditions and what are the mechanisms operating behind the observed effect. The overall aim of our studies was to understand the effect of commonly used antioxidants on antibacterial efficacy of therapeutically relevant antibiotics. It was followed up by understanding the role of reactive oxygen species (ROS) in the antibacterial action of antibiotics which displayed

reduced effectivenessin presence of the antioxidants.

We have used wild type *E. coli* K-12 strain MG1655 as the model test organisms for our study. During the course of our studies we have examined the effect of a number of antioxidants *viz.* glutathione, ascorbic acid, histidine, mannitol, sodium pyruvate and α-ketoglutarate on susceptibility of MG1655 against antibiotics such as streptomycin, chloramphenicol, tetracycline, ampicillin, penicillin and ciprofloxacin *etc.* After initial rounds of standardization 10 mM of antioxidant concentration was chosen for further studies (depending on their maximum achievable intra-cellular concentration and toxic side effects). The major findings of our studies are abridged under following sub-headings:

Glutathione and Ascorbic Acid Antagonize ROS Mediated Anti-bacterial Action of Ciprofloxacin

The effects of antioxidants on the antibiotic susceptibility of MG1655 were first analyzed by the antibiotic disk diffusion method, in which zone of inhibition around the antibiotic disk placed on the bacterial cell lawn reveals antibiotic susceptibility of the bacteria under study. Reduction in the zone of inhibition around the ciprofloxacin disks indicated that the presence of 10 mM glutathione (GSH) or ascorbic acid (ASC) in the growth medium leads to reduced ciprofloxacin susceptibility of MG1655 cells (Fig. 1). The action of antibiotics such as chloramphenicol and tetracycline was not visibly affected due to presence of these antioxidants. Other antioxidants, such as histidine, mannitol (scavengers specific for singlet oxygen and hydroxyl radicals respectively), sodium pyruvate and α-ketoglutarate did not alter MG1655 susceptibility towards any of the antibiotics (data not shown) suggesting that only nonspecific antioxidants (capable of neutralizing different types of ROS) having low redox potential could provide protection against ciprofloxacin.

Quantitative estimates of the protection offered by GSH and ASC were made by measuring minimum inhibitory concentration (MIC) of ciprofloxacin for

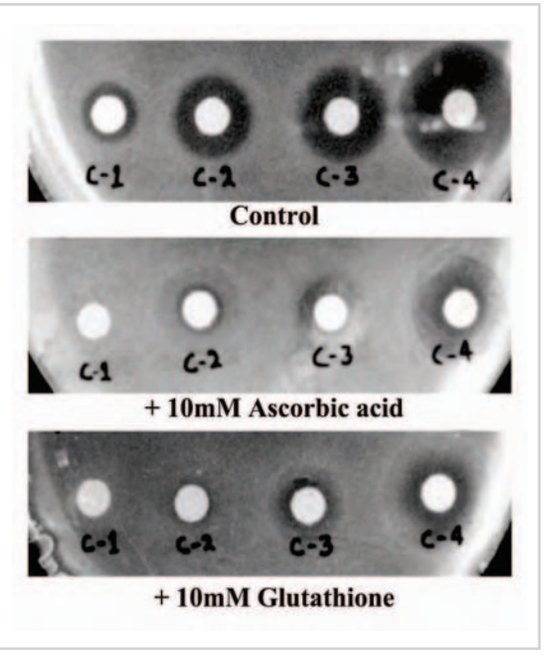

Fig. 1: Decreased susceptibility of MG1655 towards ciprofloxacin in the presence of 10 mM glutathione (GSH) or Ascorbic acid (ASC). C-1, C-2, C-3 and C-4 correspond to 40, 200, 400 and 2000 ng of ciprofloxacin spotted on the Whatman disk.

MG1655 in LB-agar in presence and absence of either antioxidant as per the clinical laborarory standard institute (CLSI) guidelines and our results showed that the protective effect against ciprofloxacin is more pronounced with GSH than for ASC. The MIC of ciprofloxacin increased 3 fold in the presence of ASC and >10 fold in the presence of GSH as compared to the control (Table 1). Ciprofloxacin is a representative member of fluoroquinolone group of antibiotics, which act by inhibiting DNA topoisomerase II and DNA topoisomerase IV activities. It was further found that GSH gives protection against other fluoroquinolones as well such as ofloxacin, norfloxacin and gatifloxacin (please see table 1 and reference 3 for details).

Antioxidant mediated protection against ciprofloxacin could be through scavenging of ROS generated in the presence of antibiotic. Consequently effect of mutations in oxidative stress defense genes *viz.* superoxide dismutases (*sodA, sodB* & *sodC*), catalases (*katE* & *katG*) and alkyl hydro peroxide reductase (*ahpCF*) on ciprofloxacin

Table 1: Susceptibility of MG1655 towards different antibiotics in the presence and absence of 10 mM GSH or ASC.

Antibiotic	MIC in μg/ml		
	Control	+ GSH	ASC
Ciprofloxacin	0.03	> 0.3	0.09
Ofloxacin	0.05	> 1.0	N.D.
Streptomycin	8	> 250	20
Kanamycin	6	> 250	> 10
Gentamycin	2	> 200	N.D.
Spectinomycin	20	> 300	N.D.
Tetracycline	4	4	4
Chloramphenicol	8	4	8
Ampicillin	8	4	8
Penicillin	64	48	64

susceptibility of MG1655 was studied. These genes encode enzymatic defense system against ROS, regulating their intracellular steady-state level. Besides effect of multi-copy *sod* genes on ciprofloxacin susceptibility of MG1655 was also examined. Among different single and multiple mutants of *katE, katG* & *ahpCF* studied, *katG ahpCF* double mutant (JI374) and the *katE katG ahpCF* triple mutant (JI377) strains having severely compromised H_2O_2 scavenging functions (7) exhibited significantly increased ciprofloxacin susceptibility in comparison to MG1655 (Fig. 2). Similarly *E. coli* cells carrying *sodC* mutation also

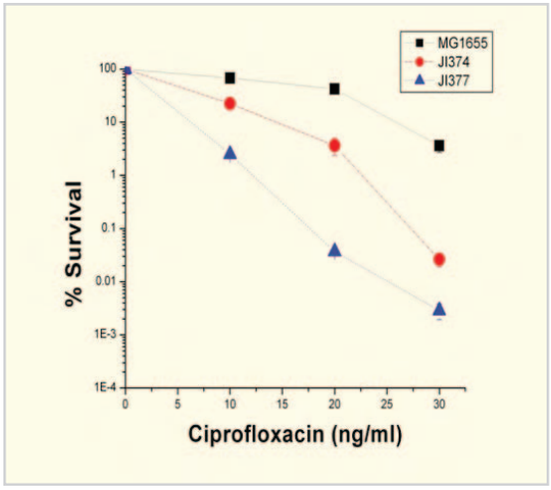

Fig. 2: Graph showing increased ciprofloxacin susceptibility in *katG ahpCF* double mutant (JI374) and the *katE katG ahpCF* triple mutant (JI377) strains in comparison to the wild type parent strain MG1655.

exhibited slightly increased ciprofloxacin susceptibility (3). Furthermore *E. coli* cells having any of the multi-copy *sod* genes showed better survival in comparison to wild type parent strain at low ciprofloxacin concentration (3). Our genetic data therefore suggested that the antibacterial action of ciprofloxacin involves ROS, such as superoxide anions and hydrogen peroxide and antioxidant mediated protection against ciprofloxacin could be due to scavenging of ROS.

No Role of ROS Scavenging in Antioxidant Mediated Reduced Bacterial Streptomycin Susceptibility

Like in case of ciprofloxacin, GSH and ASC were found to protect *E. coli* cells against streptomycin as well. Streptomycin belongs to aminoglycoside group of antibiotics, which act by interfering with bacterial protein synthesis machinery. GSH was found more effective as compared to ASC, since MIC of streptomycin increased by 2.5 fold in the presence of ASC and more than 30 fold in presence of GSH as compared to the control (Table 1). Both GSH and ASC were found to inhibit the antibacterial action of other aminoglycosides (such as kanamycin, gentamycin and spectinomycin, please see table #1 for details). During the course of our studies we also investigated whether this antioxidant-mediated protection against streptomycin is specific to MG1655 or it can be seen across diverse *E. coli* strains such as W3110, Xl-1 blue and DH5á. Our results showed that GSH and ASC were effective against streptomycin in the above mentioned strains as well. It implies that, irrespective of the genetic background, GSH and ASC interfere with a step that is crucial for antibacterial action of streptomycin in *E. coli*.

Our genetic data suggested that unlike ciprofloxacin, mitigated streptomycin susceptibility of *E. coli* cells is not due to antioxidant mediated scavenging of ROS. None of the single and multiple mutants of *katE, katG* and *ahpCF* exhibited increased streptomycin susceptibility. Similarly mutated or multi-copy *sod* genes also failed to alter the bacterial

streptomycin susceptibility levels. Our genetic data was further corroborated by the NBT reduction values which demonstrated that streptomycin treatment does not lead to induction of oxidative stress in *E. coli*, whereas ciprofloxacin indeed increases the ROS levels in bacterial cells (4). It implies that the mechanism by which antioxidant mediated protection is brought about against these two classes of antibiotics is not same. Our data therefore also emphasized that other than ROS scavenging process, additional and alternate mechanisms operate behind antioxidant-mediated protection against various antibiotics.

Glutathione can act as bacterial antibiotic susceptibility modulator

An important observation made during the course of our study was that GSH not only reduces the antibacterial action of above mentioned antibiotics but it also augments the efficacy of β-lactams such as penicillin and ampicillin (1, 5), since *E. coli* cells were found to be more susceptible towards them in presence of GSH (Table 1). However, we found that this effect was specific to GSH, as the presence of ASC did not make any difference to the antibiotic susceptibility of MG1655. Therefore our studies demonstrate that GSH can act as an important modulator of antibiotic susceptibility for bacteria (Fig. 3).

Effect of Glutathione Precursors on Bacterial Antibiotic Susceptibility

GSH is a naturally occurring tri-peptide (glutamic acid-cysteine-glycine) and its biosynthesis is limited by the amino acid l-cysteine. N-acetylcysteine (NAC) is acetylated derivative of the amino acid cysteine. It is used as an important antioxidant and precursor of GSH in higher organisms. Since we have demonstrated that GSH is an important antibiotic susceptibility modulator, therefore it is of interest to study the effect of GSH precursors on antibiotic susceptibility of bacteria. Consequently the effect of cysteine and NAC supplementation on bacterial antibiotic susceptibility was determined. Like GSH, both cysteine and NAC exhibited protection against antibacterial action of fluoroquinolones and aminoglycosides (please see reference 2 for details). The extent of protection was found to be higher with NAC in comparison to cysteine. Apart from *E. coli*, NAC had profound effect on other bacteria such as *Klebsiella aerogens, Klebsiella pneumoniae,* and *Pseudomonas aeruginosa* in terms of their survival and antibiotic susceptibility modulation. Besides, NAC also augmented the action of β-lactam antibiotics. Our studies therefore suggest that administration of aerosolized NAC as a mucolytic agent during the course of antibiotic therapy for respiratory tract infection could modulate the outcome of therapeutic process depending on the target bacterial pathogen and antibiotic being used for the therapy. Taken together our data further indicates that GSH and its precursors in general act as crucial determinant of antibiotic activity against bacterial species.

Concluding Remarks

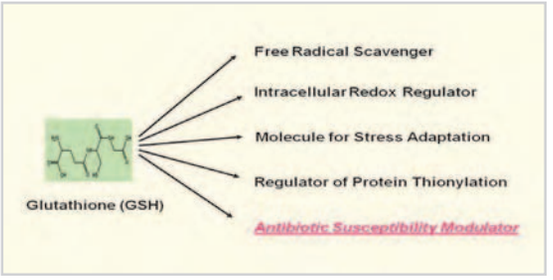

Fig. 3: Biochemical functions associated with glutathione inside bacterial cells (according to Masip *et al.*, 2006). The function indicated in red color is assigned by us on the basis of results from our lab (1,5).

On the basis of our studies it can be concluded that antibacterial action of therapeutically relevant antibiotics could be either diminished or augmented by the presence of antioxidants like GSH, NAC and ASC. Our study adds to the knowledge that apart from existence of other antibiotic activity modulation factors, dietary and cellular antioxidants also play a critical role in determination of bacterial antibiotic susceptibility. Although detailed mechanism(s) of antioxidant mediated modulation of antibiotic activity are yet to be fully understood, these findings

are of immense value. Dietary supplements such as vitamin C (ASC), cystiene rich foodstuffs and auxiliary medication like NAC, which have antioxidant properties, are prescribed sometimes along with antibiotics during the course of treatment of an infection. The therapeutic effectiveness of antibiotic mediated treatment under such conditions might be altered due to increased dietary intake and cellular levels of these antioxidants. Hence, further investigations surrounding the intake of antioxidants on antibacterial effect of different antibiotics for treatment of various infections are warranted in future. Currently, we are working in this direction to understand the real time effects of antioxidant supplementation on antibiotic mediated clearance of bacterial infection in an animal model of study.

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