Infection and Drug Resistance

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SHORT REPORT

Ascorbic Acid-Mediated Modulation of Antibiotic Susceptibility of Major Bovine Mastitis Pathogens

Zeyi Liang[1], Jiahao Shen[1], Jing Liu[1], Qinfan Li[2], Feng Yang[1], Xuezhi Ding[1]

[1]Lanzhou Institute of Husbandry and Pharmaceutical Sciences of Chinese Academy of Agricultural Science, Lanzhou, People's Republic of China; [2]College of Veterinary Medicine, Northwest A&F University, Shaanxi, People's Republic of China

Correspondence: Feng Yang; Xuezhi Ding, Lanzhou Institute of Husbandry and Pharmaceutical Sciences of Chinese Academy of Agricultural Science, No. 335 Jiangouyan, Qilihe District, Lanzhou, Gansu, People's Republic of China, Tel +86-931-2164183, Fax +86-931-2114180, Email yangfeng@caas.cn; dingxuezhi@caas.cn

Abstract: This study aimed to investigate the effects of ascorbic acid on antibiotic susceptibility of major bovine mastitis pathogens, including *Staphylococcus aureus, Streptococcus dysgalactiae, Streptococcus uberis, Streptococcus agalactiae*, and *Escherichia coli*. Minimum inhibitory concentrations (MICs) were determined by E-test method. The presence of 10 mM ascorbic acid decreased the MICs of penicillin and ampicillin while increased the MICs of erythromycin, kanamycin, streptomycin, and ciprofloxacin for all tested strains. Besides, ascorbic acid specifically reduced the MICs of tetracycline for gram-positive bacteria and chloramphenicol for gram-negative bacteria. This study highlights that ascorbic acid is a potential modulator of antibiotic activity against the major bovine mastitis pathogens.

Keywords: ascorbic acid, antibiotic susceptibility, bovine mastitis, pathogens

Introduction

Bovine mastitis is the most prevalent and costly disease of dairy industry.[1] A wide variety of microorganisms can cause this disease, but *Staphylococcus aureus, Streptococcus dysgalactiae, Escherichia coli, Streptococcus agalactiae, Streptococcus uberis* are the primary pathogens.[2,3] Antibiotics are often used to treat this disease. However, cure rates are generally poor against most mastitis pathogens due to the increasing bacterial resistance.[4,5]

The ascorbic acid is a potent water-soluble antioxidant. Its antioxidant effects had been demonstrated in many experiments in cell, animals and humans.[6,7] High ascorbic acid intake would be associated with lower risk of cardiovascular disease, stroke and cancer, and with increased longevity.[8] Recently, it was reported that ascorbic acid in combination with antibacterial agents exhibited increasing antibacterial activity against different pathogens, including *E. coli, Salmonella, Yersinia enterocolitica, Listeria monocytogenes, Staph. aureus, Helicobacter pylori, Pseudomonas aeruginosa*, and *Mycobacterium tuberculosis*.[9,10] However, the effect of ascorbic acid on antibiotic susceptibility of bovine mastitis pathogens has not been studied. The aim of the present study was to investigate the effects of ascorbic acid on antibiotic susceptibility of *Staph. aureus, Strep. dysgalactiae, Strep. agalactiae, Strep. uberis*, and *E. coli* from bovine mastitis cases.

Materials and Methods

The *Staph. aureus, Strep. dysgalactiae, Strep. agalactiae*, and *E. coli* isolates were same as those in one of our earlier studies.[11] The two *Strep. uberis* strains (LZ 2, LZ 3) were collected from subclinical bovine mastitis cases in China during 2016. Bacterial identification was performed by PCR.[12] Minimum inhibitory concentrations (MICs) of nine antibiotics, including penicillin, ampicillin, erythromycin, kanamycin, streptomycin, tetracycline, ciprofloxacin, vancomycin, and chloramphenicol, were performed by the E-test (BioMerieux, Marseille, France) on Mueller–Hinton agar plates according to the manufacturer's instructions, supplemented with 7% sheep blood for *Streptococcus* spp. Antibiotic

Received: 1 September 2022
Accepted: 1 December 2022
Published: 14 December 2022

concentration ranges were from 0.002 to 32 μg/mL for penicillin, ciprofloxacin, and chloramphenicol; 0.016 to 256 μg/mL for ampicillin, erythromycin, kanamycin, tetracycline, and vancomycin; and 0.64 to 1024 μg/mL for streptomycin.

The effects of ascorbic acid (Sigma-Aldrich, Lyon, France) on antibiotic susceptibility of the major bovine mastitis pathogens were evaluated by their MICs in the presence and absence of 10 mM ascorbic acid in the medium, respectively. Antibiotic susceptibility of two *Staph. aureus* strains, two *Strep. dysgalactiae* strains, two *Strep. agalactiae* strains, two *Strep. uberis* strains, and two *E. coli* strains were tested for this purpose. The presence of 10 mM ascorbic acid did not affect growth of these bacteria. The experiments were performed more than twice and the representative results are mentioned here.

Results

The effects of ascorbic acid on antibiotic susceptibility of *Staph. aureus* are shown in Table 1, *Strep. dysgalactiae* and *Strep. agalactiae* in Table 2, and *Strep. uberis* and *E. coli* are shown in Table 3. In the case of β-lactam antibiotics, the presence of ascorbic acid decreased the MICs of both penicillin and ampicillin for all tested strains. Conversely, ascorbic acid increased the MICs of erythromycin, ciprofloxacin, and aminoglycosides including kanamycin and streptomycin

Table 1 Effect of Ascorbic Acid[a] (AA) on Antibiotic Susceptibility of *Staphylococcus aureus*, as Measured by MIC (μg/mL)

Antibiotics	*Staph. aureus*			
	LZ 0215		LZ 84184	
	Control	+AA	Control	+AA
Penicillin	1.5	0.5	0.5	0.19
Ampicillin	2	0.5	0.75	0.38
Erythromycin	0.094	3	0.064	3
Kanamycin	3	8	1.5	8
Streptomycin	192	>1024	128	>1024
Tetracycline	0.75	0.38	0.5	0.125
Ciprofloxacin	0.19	0.38	1	1.5
Vancomycin	1.5	1.5	1.5	1.5
Chloramphenicol	3	3	4	4

Note: [a]In each case, the final ascorbic acid concentration was 10 mM.

Table 2 Effect of Ascorbic Acid[a] (Vitamin C) on Antibiotic Susceptibility of *Streptococcus dysgalactiae* and *Streptococcus agalactiae*, as Measured by MIC (μg/mL)

Antibiotics	*Strep. dysgalactiae*				*Strep. agalactiae*			
	LZ 717		LZ 211		LZ 17		LZ 21	
	Control	+AA	Control	+AA	Control	+AA	Control	+AA
Penicillin	1.5	0.75	0.016	<0.016	0.047	0.016	0.032	0.012
Ampicillin	0.75	0.25	0.032	0.016	0.094	<0.016	0.064	0.016
Erythromycin	0.75	4	0.032	0.125	0.032	0.125	0.047	0.25
Kanamycin	32	> 256	128	>256	>256	>256	>256	>256
Streptomycin	>1024	>1024	>1024	>1024	6	1024	24	512
Tetracycline	> 256	256	0.25	0.125	48	32	96	64
Ciprofloxacin	0.5	4	0.75	2	0.5	2	0.5	3
Vancomycin	2	2	0.75	0.75	1.5	1.5	1.0	1.0
Chloramphenicol	4	4	3	3	2	2	2	2

Note: [a]In each case, the final ascorbic acid concentration was 10 mM.

Table 3 Effect of Ascorbic Acid[a] (Vitamin C) on Antibiotic Susceptibility of *Streptococcus uberis* and *Escherichia coli*, as Measured by MIC (μg/mL)

Antibiotics	*Strep. uberis*				*E. coli*			
	LZ 2		LZ 3		LZ 2552		LZ 282	
	Control	+AA	Control	+AA	Control	+AA	Control	+AA
Penicillin	3	1.5	2	1.5	>32	32	>32	>32
Ampicillin	0.75	0.5	0.5	0.38	2	1.5	3	2
Erythromycin	3	16	3	24	16	> 32	48	>256
Kanamycin	48	96	32	96	0.75	1.5	3	32
Streptomycin	32	192	48	192	1.5	24	1.5	24
Tetracycline	0.38	0.25	0.38	0.25	3	3	3	3
Ciprofloxacin	0.5	2	0.5	2	0.004	0.016	0.012	0.023
Vancomycin	0.38	0.38	0.38	0.38	>256	>256	>256	>256
Chloramphenicol	3	3	3	3	2	1.5	3	1.0

Note: [a]In each case, the final ascorbic acid concentration was 10 mM.

against all the strains. Interestingly, the MICs of tetracycline decreased for all of the strains except that of *E. coli* in the presence of ascorbic acid, while the MICs of chloramphenicol reduced for *E. coli* alone. In addition, the presence of ascorbic acid did not alter the MIC of vancomycin against any of the strains.

Discussion

In the presence of ascorbic acid, two β-lactam antibiotics (ampicillin and penicillin) reduce MIC of bacteria, whereas kanamycin and streptomycin, both aminoglycosides, increase MIC. This may be due to the different ways and targets in which different types of drugs inhibit bacteria.[13] In our study, there is a clear variation related to the effect of ascorbic acid against gram-positive and gram-negative bacteria with tetracycline and chloramphenicol. This illustrates that the influence of ascorbic acid on antibacterial activity of the two antibiotics are dramatically associated with bacterial cell wall.[14] In addition, results of the present study showed that the presence of ascorbic acid can either enhance the antibacterial activity of β-lactams antibiotics or give protective effect against erythromycin, kanamycin, streptomycin, and ciprofloxacin for all tested pathogens. Amábile-Cuevas et al[15] reported the similar data that ascorbic acid increased the efficacy of β-lactams against *Staph. aureus*, which was explained by losing penicillinase plasmids after being treated with ascorbic acid. Besides, the enhanced antibacterial activity of antibiotics in combination with ascorbic acid was previously observed in *Staph. aureus* and *H. pylori*.[9] It was claimed that ascorbic acid, as a prodrug for hydrogen peroxide formation, can help the antibiotics to eradicate bacteria. Moreover, it was also suggested that the effects of ascorbic acid on antibacterial activity of ciprofloxacin was related to induction of reactive oxygen species (ROS). However, Goswami et al[16] revealed that the effects of ascorbic acid on streptomycin sensitivity of *E. coli* was not attributed to the antioxidant-mediated scavenging of ROS. Streptomycin acts on the redox potential of the bacterial translational machinery, and ascorbate affects this site.[16] We therefore conclude that the effects of ascorbic acid on bacterial antibiotic susceptibility are dramatically correlated with the target bacterial pathogen and antibiotic being used. The specific molecular mechanisms are the subjects of going investigation in our lab. Ascorbic acid can be used as an adjunctive treatment for mastitis in dairy cows. Studies have shown that Ampicillin sodium 75 mg and cloxacillin sodium 200 mg/kg infusion of ascorbic acid for 25 mg/kg results in faster recovery of affected cows.[17] However, in clinical application of ascorbic acid, the pathogen of infection and the drug used should be considered. When erythromycin, kanamycin, streptomycin, and ciprofloxacin are used, ascorbic acid should be avoided.

Conclusions

In conclusion, the present study highlights that ascorbic acid is a potential modulator of antibiotic activity against the major bovine mastitis pathogens. The combination of ascorbate with streptomycin, erythromycin, kanamycin and

ciprofloxacin may lead to increased drug resistance in pathogens. Meanwhile, our results provide new perspectives for use of ascorbic acid combined with β-lactams antibiotics against the major bovine mastitis pathogens.

Ethics Approval and Consent to Participate

Compliance with ethical standards: This study was approved by Ethics Committee of Lanzhou Institute of Husbandry and Veterinary Medicine, Chinese Academy of Agricultural Sciences (SYXK-2019-0012) and was conducted in compliance with ethical, legal, and regulatory norms. The animal owners were informed about the purpose of the study, and consent of each animal owner was obtained before the physical examination of cows for clinical mastitis and the collection of milk samples.

Acknowledgments

This study was supported by the Key Research and Development Program of Gansu Province (No. 21YF5NA141) and the National Natural Science Foundation of China (No. 31860724).

Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

Disclosure

The authors report no conflicts of interest in this work.

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