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New activity for old drug: *In vitro* activities of vitamin K_3 and menadione sodium bisulfite against methicillin-resistant *Staphylococcus aureus*

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To discover new lead compounds against methicillin-resistant *Staphylococcus aureus* (MRSA), trimethylhydroquinone, vitamins K_1, K_2, K_3 and menadione sodium bisulfite were targeted for anti-MRSA assay. Their anti-MRSA activities were evaluated by agar diffusion method, and their minimum inhibitory concentrations (MICs) were determined by broth microdilution method. The results showed that trimethylhydroquinone, vitamin K_3 and menadione sodium bisulfite presented obvious anti-MRSA activity, and their MICs against MRSA ATCC 33592 and three clinical MRSA isolates were successively 16 to 32, 8 to 16 and 16 µg/ml. Vitamins K_1 and K_2 showed no anti-MRSA activity when the test discs respectively carried 1024 µg of them. These indicated that the anti-MRSA activity would disappear when the methyl of vitamin K_3 was substituted by alkyl that contained four isopentenyl units, and vitamin K_3 probably has an ancillary effect on the treatment of MRSA infection.

Key words: Vitamin K_3, menadione sodium bisulfate, trimethylhydroquinone, methicillin-resistant *Staphylococcus aureus* (MRSA).

INTRODUCTION

Methicillin-resistant *Staphylococcus aureus* (MRSA) poses a serious threat to public health because of its resistance to multiple antibiotics (Payne, 2008; Taubes, 2008). To overcome resistance, various efforts at discovery of novel antibiotics were carried forward, such as classic screening methods, chemical modification of known antimicrobials, improving antimicrobial activity of known compounds, hybrid agents and new targets (Moellering, 2011). Moreover, it is worth noticing that discovering anti-MRSA compounds from known compounds and drugs should be an economical and effective method (Berger, 2007; Chong and Jr Sullivan, 2007).

Base on the structure-activity analysis of anti-MRSA natural products (Saleem et al., 2010; Gibbons, 2004), some phenols and quinones contained hydrophobic groups were deduced to present potential anti-MRSA activity. So, three potential anti-MRSA compounds as trimethylhydroquinone (compound 1) (Figure 1), α-tocopherol and phloroglucinol were selected for anti-MRSA assays. The results showed that compound 1 presented obvious anti-MRSA activity with daptomycin as a positive control, while α-tocopherol and phloroglucinol

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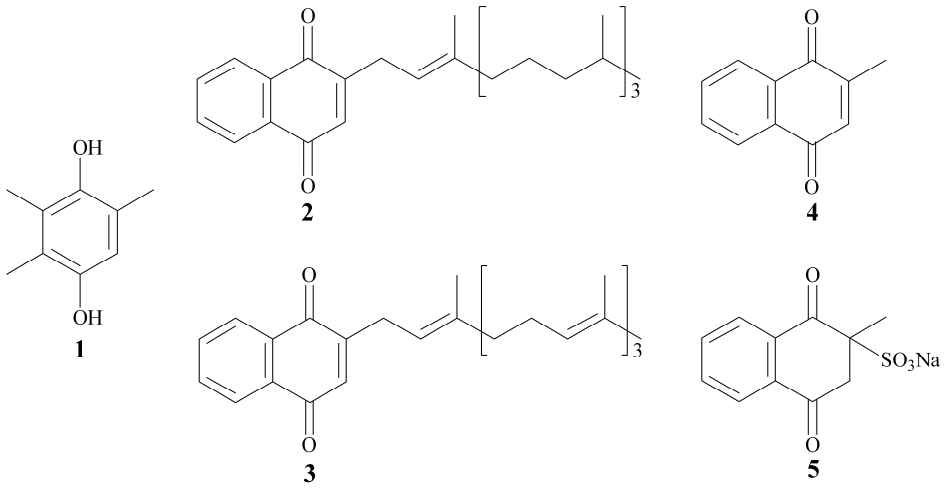

Figure 1. The structures of 1 (Trimethylhydroquinone), 2 (Vitamin K_1), 3 (Vitamin K_2, menaquinone-4), 4 (Vitamin K_3) and 5 (Menadione sodium bisulfate).

showed no activity against MRSA (Data was not reported). Since the skeleton structures of vitamins K_1 (compound 2), K_2 (compound 3), K_3 (compound 4) and menadione sodium bisulfate (compound 5) were a little similar to that of 1 (Figure 1), these four compounds may also show anti-MRSA activities. To prove this and discover new anti-MRSA activity for old drugs, compounds 2 to 5 were further targeted for anti-MRSA assay, and *in vitro* activities of them against three clinical isolates of MRSA, two reference strains as MRSA ATCC 33592 and methicillin-susceptible *S. aureus* (MSSA) ATCC 25923 were evaluated.

MATERIALS AND METHODS

Reagents

Compounds 1, 2, 4 and 5 were purchased form Aladdin Industrial Corporation (Shanghai, China), and compound 3 and daptomycin (Purity of 90%) were purchased form Sigma-Aldrich (St. Louis, MO, USA). These compounds avoided light, and were stored at 4°C. Mueller-Hinton Agar (MHA) and Mueller-Hinton broth (MHB) used for anti-MRSA assay were purchased from Qingdao Hope Bio-Technology Co., Ltd. (Qingdao, China) and were used for the measurement of anti-MRSA activities. The water used was freshly distilled, deionized and purified with Milli-Q plus equipment (Millipore, Bedford, MA). All other solvents and reagents used were of analytical grade. A top pipette 20 to 200 µl and an eight channels pipette 50 to 300 µl were purchased from Dragon Laboratory Instruments Limited (Beijing, China). Ninety six-well plates were purchased from Sangon Biotech (Shanghai) Co., Ltd. (Shanghai, China).

Strains

Two reference strains as MRSA ATCC 33592 (gentamycin and methicillin-resistant) and MSSA ATCC 25923 were purchased from ATCC (American Type Culture Collection, USA). Three clinical MRSA isolates as MRSA 01, MRSA 02 and MRSA 03 were obtained from Clinical Laboratory of the Second Affiliated Hospital, Sun Yat-sen University, Guangzhou, China (Yuan et al., 2012). These three MRSA isolates were identified by PBP2a Latex Agglutination Kit test, and were respectively resistant to erythromycin and methicillin (MRSA 01), methicillin (MRSA 02) and gentamycin and methicillin (MRSA 03). All these MRSA and MSSA strains were sub-cultured with MHB at 35°C for 24 h prior to anti-MRSA assay.

Inhibition zones

The inhibition zone of compounds 1 to 5 against all MRSA strains were performed using agar diffusion method described as Clinical and Laboratory Standards Institute standard with a little modification (CLSI, 2012). Compounds 2 to 4 were respectively dissolved in ethyl acetate to obtain sample solutions with the concentrations of 20.48, 20.48 and 5.12 mg/ml, and compounds 1, 5 and daptomycin were respectively dissolved in purified water to obtain sample solutions with the concentrations of 2.56 mg/ml, 5.12 mg/ml and 64 µg/ml. Daptomycin solution and ethyl acetate were respectively used as a positive control and a solvent control. Diluted inoculums (100 µl, 0.5 of McFarland standard) of MRSA ATCC 33592 and MSSA ATCC 25923 were respectively spread on MHA plates using sterile cotton swab. Next, each sample solution was circularly dropped on a 10 mm diameter disc with pipette and dried in the clean bench until 50 µl of it was carried. After this, each disc was placed on the surface of MHA plates, and then incubated at 35°C for 18 h. All tests were repeated twice, and the test plates stood in dark to avoid the degradation of compounds 1 to 5 in the culture process. The results were described as the diameters (in mm) of inhibition zones, and expressed as mean value ± standard deviation (SD).

Minimum inhibitory concentrations (MICs)

The MICs of compounds 1, 4 and 5 against all MRSA strains were determined by broth microdilution method described as CLSI standard with a little modification (CLSI, 2012). The tests were

Table 1. Diameters of inhibition zones of 1 to 5 against two reference strains (*n* = 3).

Reference strain	Diameters of inhibition zones (mm)*					
	1	2	3	4	5	Daptomycin
MRSA ATCC 33592	20.3 ± 1.2	-**	-	30.3 ± 1.5	29.0 ± 1.5	21.0 ± 1.7
MSSA ATCC 25923	21.3 ± 1.5	-	-	30.0 ± 2.0	30.0 ± 1.7	21.7 ± 1.5

*The diameter of all discs was 10 mm, and the amount of **1**, **2**, **3**, **4**, **5** and daptomycin were respectively 128, 1024, 1024, 256, 256 and 32 µg. **Indicated that there was no inhibition zone.

Table 2. Minimum inhibitory concentrations of 1, 4 and 5 against MRSA strains.

MRSA strain	Minimum inhibitory concentrations (µg/ml)			
	1	4	5	Daptomycin
MRSA ATCC 33592	16	8	16	1
MRSA 01	32	16	16	1
MRSA 02	16	8	16	0.5
MRSA 03	16	8	16	1

performed on 96-well plates in duplicate, and daptomycin was used as a positive control. The sample solution of compound 4 was prepared with dimethyl sulphoxide (DMSO) and MHB, and the initial well contained 5% DMSO in the 96-well plate. So, 5% DMSO aqueous solution was used as a solvent control. Briefly, 100 µl MHB was added into each well. 100 µl each compound solution (256 µg/ml) was respectively added into column 1, and was mixed with pipette. Then starting with the concentration of 128 µg/ml for each compound, two-fold dilution was followed. Subsequently, 100 µl diluted inoculum (0.5 of McFarland standard) was added to each well. Finally, the test plates were incubated at 35°C for 24 h, and also stood in dark to avoid the degradation of compounds 1, 4 and 5 in the culture process. The microbial growth was observed by adding 20 µl of 2.0 mg/ml 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT). When the microbial growth in the well of solvent and growth controls was good (Color change from yellow to blue purple), the MICs of samples were determined as the lowest concentration where bacterial growth was visibly inhibited (no obvious color change from yellow).

RESULTS

As the most important member of vitamin K_2 homologs, compound 3 (menaquinone-4, MK-4) was used as vitamin K_2 in this research. The results (Table 1) indicated that compounds 1, 4 and 5 presented obvious activity against MRSA ATCC 33592 and MSSA ATCC 25923 with respective inhibition zones of 20.3 to 21.3, 30.0 to 30.3 and 29.0 to 30.0 mm, and those for daptomycin were 21.0 to 21.7 mm (the reference for the inhibition zones of 30 µg daptomycin against MSSA ATCC 25923 were 18 to 23 mm). While compounds 2 and 3 showed no anti-MRSA activity. Further, the minimum inhibitory concentrations (MICs) of compounds 1, 4 and 5 against all four MRSA strains were respectively 16 to 32, 8 to 16 and 16 µg/ml, and those of daptomycin were 0.5 to 1 µg/ml (the reference for MICs of daptomycin against MRSA ATCC 33592 was less than 1 µg/ml) (Table 2).

DISCUSSION

Vitamin K_1 (compound 2) known as phylloquinone, is biosynthesized by plants. Menaquinone-4 (compound 3, MK-4) was the most important member of vitamin K_2 homologs called menaquinones, has several subtypes, and is biosynthesized by animals and bacteria. While compound 4 is a synthetic form of vitamin K. Considering that compound 4 is insoluble in water, compound 5 is usually its application form. Meanwhile, vitamin K_3 can be converted to vitamin K_2 *in vivo* (Shearer and Newman, 2008). After the anti-MRSA activity of compound 1 was discovered, their similar skeleton structures to compound 1 deduced that compounds 2 to 5 may also show anti-MRSA activity. Based on these facts, a hypothesis that anti-MRSA compounds would be found everywhere (in plant, animal, human and bacteria) was put forward if compounds 2 to 4 were proved to be active against MRSA. So, the anti-MRSA assays of compounds 2 to 5 were further evaluated with daptomycin used as a positive control. The results showed that the anti-MRSA activity of compound 4 was basically equal to that of compound 5 by comparing their molar concentrations calculated from their MICs, while it was about twofold stronger than that of compound 1. Many new bioactivities of vitamin K_3 (compound 4 or 5) and its derivatives were discovered recently, such as anti-cancer (Matzno et al., 2008; Tomasetti et al., 2012; Tanahashi et al., 2011), anti-Alzheimer's disease (Huy, et al., 2013), anti-allergic and anti-inflammatory effects (Kohli et al., 2011; Chinzei

et al., 2011). Here, the obvious antibacterial activity especially anti-MRSA activity of vitamin K_3 (compound 4 or 5) was first discovered by us, which showed that vitamin K_3 probably have an ancillary effect on the treatment of MRSA infection except for being used as vitamin K supplement and potential cancer treatment.

Moreover, it is unfortunate to find that compounds 2 and 3 have no anti-MRSA activity when the test discs, respectively carried 1024 µg of them, which showed that the anti-MRSA activity would disappear when the C_2 methyl of compound 4 was substituted by alkyl that contained four isopentenyl units. This raises the following questions: (1) What is the reason? and (2) how do we interpret another obvious and thoughtful fact that vitamin K_3 has obvious toxicity and aforementioned bioactivities, while vitamin K_2 has no toxicity and bioactivities except as a supplement of vitamin K? It maybe very worthy to further research the molecular mechanism and the structure-activity and structure-toxicity relationships involved the alkyl side-chain substituted at C_2 position of vitamin K_3.

Conclusions

Conclusively, *in vitro* activities of vitamin K_3, menadione sodium bisulfate and trimethylhydroquinone against MRSA were determined. The results indicated that these three compounds presented obvious anti-MRSA activity, and showed that vitamin K_3 or menadione sodium bisulfite probably has an ancillary effect on the treatment of anti-MRSA infection.

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ABBREVIATIONS

MRSA, Methicillin-resistant *Staphylococcus aureus*; **MIC,** minimum inhibitory concentration.

REFERENCES

Berger PS (2007). Old laws stop drugs being used in valuable new ways. Nature 449:972.

Chinzei R, Masuda A, Nishiumi S, Nishida M, Onoyama M, Sanuki T, Fujita T, Moritoh S, Itoh T, Kutsumi H, Mizuno S, Azuma T, Yoshida M (2011). Vitamin K_3 attenuates cerulein-induced acute pancreatitis through inhibition of the autophagic pathway. Pancreas 40:84-94.

Chong C, Jr Sullivan DJ (2007). New uses for old drugs. Nature 448:645-646.

CLSI (2012). Clinical and Laboratory Standards Institute (2012). Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically; approved standard, 9rd ed.; Wayne, PA, USA, M07-A9.

Gibbons S (2004). Anti-staphylococcal plant natural products. Nat. Prod. Rep. 21:263-277.

Huy PDQ, Yu Y, Ngo ST, Van Thao T, Chen C, Li M, Chen Y (2013). *In silico* and *in vitro* characterization of anti-amyloidogenic activity of vitamin K_3 analogues for Alzheimer's disease. Biochim. Biophy. Acta. 1830:2960-2969.

Kohli V, Sharma D, Sandur SK, Suryavanshi S, Sainis KB (2011). Immune responses to novel allergens and modulation of inflammation by vitamin K_3 analogue: A ROS dependent mechanism. Int. Immunopharmacol. 11:233-243.

Matzno S, Yamaguchi Y, Akiyoshi T, Nakabayashi T, Matsuyama K (2008). An attempt to evaluate the effect of vitamin K_3 using as an enhancer of anticancer agents. Biol. Pharm. Bull. 31:1270-1273.

Moellering Jr RC (2011). Discovering new antimicrobial agents. Int. J. Antimicrob. Agents. 37:2-9.

Payne GJ (2008). Desperately seeking new antibiotics. Science 321:1644-1645.

Saleem M, Nazir M, Ali MS, Hussain H, Lee YS, Riaz N, Jabbar A (2010). Antimicrobial natural products: an update on future antibiotic drug candidates. Nat. Prod. Rep. 27:238-254.

Shearer MJ, Newman P (2008). Metabolism and cell biology of vitamin K. Thromb. Haemost. 100:530-547.

Tanahashi T, Osada S, Imai H, Sasaki Y, Takahashi T, Yamaguchi K, Yoshida K (2011). Signal transduction of vitamin K_3 for pancreas cancer therapy. Oncol. Rev. 5:57-60.

Taubes G (2008). The bacteria fight back. Science 321:356-361.

Tomasetti M, Nocchi L, Neuzil J, Goodwin J, Nguyen M, Dong L, Manzella N, Staffolani S, Milanese C, Garrone B, Alleva R, Borghi B, Santarelli L, Guerrieri R (2012). Alpha-tocopheryl succinate inhibits autophagic survival of prostate cancer cells induced by vitamin K_3 and ascorbate to trigger cell death. Plos One 12:1-12.

Yuan G, Li P, Yang H, Wu X, Tu G, Wei S (2012). Chemical screening of sixty-one actinomycetes strains and anti-methicillin-resistant *Staphylococcus aureus* assays of target strains. Chin. J. Nat. Med. 10:155-160.