Saudi Journal of Biological Sciences (2015) xxx, xxx–xxx

King Saud University

Saudi Journal of Biological Sciences

www.ksu.edu.sa
www.sciencedirect.com

ORIGINAL ARTICLE

Menadione (vitamin K) enhances the antibiotic activity of drugs by cell membrane permeabilization mechanism

Jacqueline C. Andrade [a], Maria Flaviana B. Morais Braga [a], Gláucia Morgana M. Guedes [a], Saulo R. Tintino [a], Maria A. Freitas [a], Lucindo J. Quintans Jr. [b], Irwin R.A. Menezes [a], Henrique D.M. Coutinho [a,*]

[a] *Universidade Regional do Cariri, Crato (CE), Brazil*
[b] *Universidade Federal de Sergipe, Aracaju (SE), Brazil*

Received 25 June 2015; revised 28 August 2015; accepted 1 September 2015

KEYWORDS

Menadione;
Lipid solubility;
Microdilution;
Antibiotic-modifying;
Permeabilization

Abstract Menadione, vitamin K3, belongs to the class of lipid-soluble vitamins and lipophilic substances as menadione cause disturbances in the bacterial membrane, resulting in damage to the fundamental elements for the integrity of the membrane, thus allowing increased permeability. Accordingly, the aim of this study was to evaluate in vitro the antibiotic-modifying activity of menadione in multiresistant strains of *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Escherichia coli*, with a gradual increase in its subinhibitory concentration. In addition, menadione was compared with cholesterol and ergosterol for similarity in mechanism of drug modulatory action. Antibiotic-modifying activity and antibacterial effect were determined by the broth microdilution assay. Menadione, cholesterol and ergosterol showed modulatory activity at clinically relevant concentrations, characterizing them as modifiers of bacterial drug resistance, since they lowered the MIC of the antibiotics tested. This is the first report of the antibacterial activity of menadione and its potentiation of aminoglycosides against multiresistant bacteria.

1. Introduction

Menadione, vitamin K3, is a synthetic compound that belongs to the class of lipid-soluble vitamins, which is converted to vitamin K2 in the gut (Klack and Carvalho, 2006). Lipid-soluble vitamins are organic substances present in small amounts in foods, and they are essential to the functioning of the body as co-factors (Paixão and Stamford, 2004). Vitamin K is a biologically active substance found in functional foods, which is required particularly in the mechanism of

* Corresponding author at: Departamento de Química Biológica, Universidade Regional do Cariri – URCA, Rua Cel. Antonio Luis 1161, Pimenta, Crato (CE) 63105-000, Brazil. Tel.: +55 (88)31021212; fax: +55 (88)31021291.
E-mail address: hdmcoutinho@gmail.com (H.D.M. Coutinho).
Peer review under responsibility of King Saud University.

Production and hosting by Elsevier

http://dx.doi.org/10.1016/j.sjbs.2015.09.004
1319-562X

Please cite this article in press as: Andrade, J.C. et al., Menadione (vitamin K) enhances the antibiotic activity of drugs by cell membrane permeabilization mechanism. Saudi Journal of Biological Sciences (2015), http://dx.doi.org/10.1016/j.sjbs.2015.09.004

blood coagulation, where it is essential for the synthesis of prothrombin, besides being involved in the synthesis of proteins present in plasma, kidney and perhaps other tissues. Some studies on vitamin K2 have demonstrated growth inhibitory effects against various neoplastic cells and reduced risk of mutagenic events in rapid cell proliferation in the fetus and newborn (Klack and Carvalho, 2006). Some studies report that lipid-soluble compounds modulate plasma membrane permeability in bacteria (Pretto et al., 2004; Gibbons, 2004; Nicolson et al., 1999). Therefore, menadione with its lipid-soluble nature may cause changes in the fluidity of the bacterial membrane, making it more permeable to substances, including antibiotics.

Cholesterol is a lipid component that is necessary for normal functioning of the body, and it plays an important role in the structure and function of the plasma membrane as well as organelle membranes. Also, it is involved in the synthesis of bile acids required for the absorption of lipids and lipid-soluble vitamins from the intestine, and participates in the synthesis of steroid hormones and vitamin E (Ludke and López, 1999; Leança et al., 2010).

Bacteria do not have cholesterol as part of their cytoplasmic membrane, nor ergosterol, a cholesterol derivative and lipid component of fungal membranes. Both sterols were used as complex lipid substances (Santos and Carvalho, 2001; Thevissen et al., 2003; Loguercio-Leite et al., 2006) that can act on the fluid mosaic of the bacterial membrane, modifying its fluidity, for comparison with menadione with regard to effect on the bacterial plasma membrane.

Bacteria are simple organisms found in most natural environments, where the bacterial cell has several structures, some present only in certain species. An essential structure is the cytoplasmic membrane, which is responsible for numerous functions including DNA replication, enzyme secretion, biosynthesis of components, solute transport and energy production (Schaechter et al., 2002). The cell wall is a structure that gives rigidity to many bacteria, and according to its constitution, bacteria are divided into two classes, Gram-positive and Gram-negative bacteria, the difference being mainly due to their permeability properties and surface components (Tortora et al., 2008; Schaechter et al., 2002; Pretto et al., 2005).

Bacterial infections are currently the focus of public health, mainly due to the significant growth of bacterial resistance. Infections caused by *Staphylococcus aureus* are the most common, showing a greater difficulty in treatment due to its resistance to various antibiotics (Tortora et al., 2008). The species *Pseudomonas aeruginosa* is the leading cause of nosocomial infections, attacking the skin, urinary tract, ear, and eye (Murray et al., 2004). *Escherichia coli* are the most common species of the genus *Escherichia*, associated with severe urinary tract infections, meningitis and gastroenteritis (Murray et al., 2004; Tortora et al., 2008).

The aim of this study was to evaluate in vitro the antibiotic-modifying activity of menadione in multiresistant strains of *S. aureus*, *P. aeruginosa* and *E. coli*, with gradual increase in its subinhibitory concentration. Also, menadione was compared to cholesterol and ergosterol with regard to mechanism of modulating action (see Figs. 1 and 2).

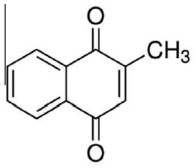

Figure 1 Structural formulae of menadione.

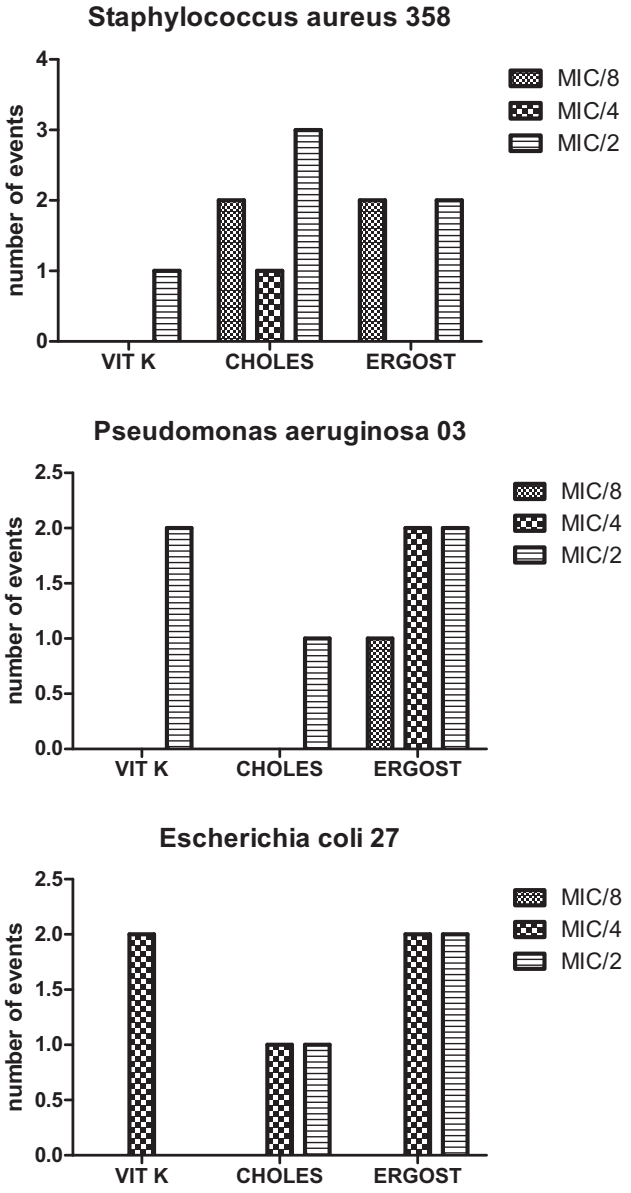

Figure 2 Comparison of the number of events modulator concentrations subinhibitory MIC/8, MIC/4, MIC/2 between the solutions of menadione, cholesterol and ergosterol. *MIC: minimum inhibitory concentration, VIT K: vitamin K.

Table 1 Bacterial resistance profile against antibiotics.

Bacterial strain	Source	Resistance profile
Staphylococcus aureus SA 358	Surgical wound	Oxa, Gen, Tob, Ami, Can, Neo, Para, But, Sis, Net
Staphylococcus aureus ATCC 25923	–	–
Escherichia coli EC27	Surgical wound	Ast, Ax, Amp, Ami, Amox, Ca, Cfc, Cf, Caz, Cip, Clo, Im, Can, Szt, Tet, Tob
Escherichia coli ATCC 10536	–	–
Pseudomonas aeruginosa PA03	Catheter tip	Cpm, Ctz, Im, Cip, Ptz, Lev, Mer, Ami
Pseudomonas aeruginosa ATCC 15442	–	–

Ast – Aztreonam; Ax – Amoxacillin; Amp – Ampicillin; Ami – Amikacin; Amox – Amoxicillin; Ca – Cefadroxil; Cfc – Cefaclor; Cf – Cephalothin; Caz – Ceftazidime; Cip – Ciprofloxacin; Clo – Chloramphenicol; Im – Imipenem; Can – Kanamycin; Szt – Sulphametrophim, Tet – Tetracycline; Tob – Tobramycin; Oxa – Oxacillin; Gen – Gentamicin; Neo – Neomycin; Para – Paramomicin; But – Butirosin; Sis – Sisomicin; Net – Netilmicin; (–) absence of resistance or non-significant resistance.

Table 2 Minimum inhibitory concentration (MIC) of menadione (vitamin K3) against bacterial strains.

Bactérias	MIC (μg/mL)	
	Menadiona	DMSO
S. aureus	128	128
P. aeruginosa	64	256
Klebsiella pneumoniae	128	128
E. coli	128	128

2. Experimental

2.1. Bacterial material

Bacteria used in the minimal inhibitory concentration (MIC) test were the standard strains of *S. aureus* ATCC 25923, *P. aeruginosa* ATCC 15442 and *E. coli* ATCC 10536. To evaluate the modulatory activity of the extract, the following multi-resistant bacterial strains were used, isolated from clinical environments: *P. aeruginosa* 03, *E. coli* 27 and *S. aureus* 358, with the resistance profile demonstrated in Table 1 in the MIC test. All strains were obtained from the Laboratory of Clinical Mycology – UFPB. All strains were maintained on heart infusion agar slants (HIA, Difco Laboratories. Lawrence, USA) and prior to assay, the cells were grown overnight at 37 °C in brain heart infusion (BHI, Difco Laboratories, Lawrence, USA).

2.2. Drugs

2.2.1. Liposoluble vitamins

The vitamin K3 (Menadione) was obtained from Sigma Chemical Co., St. Louis, USA. Stock solutions were prepared in 1 mL of dimethylsulfoxide (DMSO), at a concentration of 100 mg/mL, after which they were diluted to a concentration of 1024 μg/mL in distilled water, except menadione which was diluted in DMSO. Thus, a DMSO control was included to determine any possible interference with the results.

2.2.2. Sterols

Cholesterol and ergosterol were obtained from Sigma Chemical Co., St. Louis, USA. Stock solutions were prepared in 2 mL of DMSO/Tween 80 at a concentration of 200 mg/mL, after which they were diluted to 1024 μg/mL in distilled water.

2.2.3. Antibiotics

Drugs used in the tests were the aminoglycosides amikacin, neomycin and gentamicin (Sigma Co., St. Louis, USA). All drugs were diluted in sterile water, to a concentration of 5000 μg/mL.

2.3. Antibacterial and modulatory activity

The minimal inhibitory concentration (MIC) was determined by the broth microdilution assay. MIC is defined as the lowest concentration at which no microbial growth is observed. MIC was determined using a bacterial suspension of 10^5 CFU/mL in 10% brain heart infusion (BHI) broth. In 96-well microdilution plates, 100 mL of inoculum were added to each well followed by 100 mL of a serially diluted solution of menadione, cholesterol or ergosterol starting at a concentration of 1024 μg/mL. The final concentrations varied from 1024 to 8 μg/mL. The plates were incubated for 24 h at 35 °C (Javadpour et al., 1996). The potential of the vitamin, cholesterol and ergosterol as modifiers of antibacterial resistance was determined as proposed by Coutinho et al. (2008) with modifications. The solution of vitamin was tested at three subinhibitory concentrations (MIC/8, MIC/4 and MIC/2). In a microdilution plate, 100 mL of BHI with bacterial inoculum and liposoluble vitamins were added to each well, and 100 mL of antimicrobial drugs were then serially diluted 1:2 with concentrations varying 1024–0.5 μg/mL. The plates were incubated for 24 h at 37 °C.

3. Results and discussion

This is the first report of antibacterial activity and potentiation of menadione by the aminoglycosides, against multiresistant bacteria. Until now, there had been no report on the use of menadione as a modulator of antibiotics.

In view of the development of bacterial resistance to antibiotics, which is responsible for the lack of efficacy in the treatment of many existing infections, the pharmaceutical industry, in recent years, has shown increased interest in substances isolated from natural products that possess antimicrobial properties (Köhler et al., 1999; Lee et al., 2003; Taleb-Contini et al., 2003).

Please cite this article in press as: Andrade, J.C. et al., Menadione (vitamin K) enhances the antibiotic activity of drugs by cell membrane permeabilization mechanism. Saudi Journal of Biological Sciences (2015), http://dx.doi.org/10.1016/j.sjbs.2015.09.004

Table 3 Antibiotic modulatory activity of menadione alone or associated with aminoglycosides in sub-inhibitory concentrations (MIC/8, MIC/4 and MIC/2).

Menadione (vitamin K) (μg/mL)									
	Antibiotic + MIC/8	MIC DMSO	Antibiotic alone	Antibiotic + MIC/4	MIC DMSO	Antibiotic alone	Antibiotic + MIC/2	MIC DMSO	Antibiotic alone
Staphylococcus aureus 358									
Amikacin	78.1	39.1	39.1	9.7	19.5	78.1	2.4	9.7	156.2
Gentamicin	9.7	78.1	4.8	2.4	2.4	2.4	2.4	2.4	312.5
Neomycin	156.2	156.2	156.2	2.4	4.8	156.2	2.4	2.4	156.2
Pseudomonas aeruginosa 03									
Amikacin	156.2	156.2	156.2	39.1	39.1	78.1	2.4	9.7	312.5
Gentamicin	39.1	39.1	39.1	9.7	19.5	19.5	2.4	2.4	625
Neomycin	156.2	78.1	78.1	78.1	78.1	78.1	2.4	78.1	78.1
Escherichia coli 27									
Amikacin	78.1	156.2	156.2	9.7	78.1	78.1	2.4	4.8	156.2
Gentamicin	19.5	19.5	39.1	2.4	19.5	9.7	2.4	2.4	625
Neomycin	78.1	2.4	312.5	78.1	78.1	312.5	2.4	4.8	312.5

Table 4 Antibiotic modulatory activity of cholesterol alone or associated with aminoglycosides in sub-inhibitory concentrations (MIC/8, MIC/4 and MIC/2).

Cholesterol (μg/mL)						
	Antibiotic + MIC/8	Antibiotic alone	Antibiotic MIC/4	Antibiotic alone	Antibiotic + MIC/2	Antibiotic alone
Staphylococcus aureus 358						
Amikacin	78.1	39.1	39.1	19.5	19.5	156.2
Gentamicin	4.8	19.5	2.4	2.4	2.4	9.7
Neomycin	39.1	156.2	4.8	78.1	9.7	156.2
Pseudomonas aeruginosa 03						
Amikacin	78.1	39.1	156.2	156.2	39.1	78.1
Gentamicin	19.5	39.1	39.1	39.1	4.8	19.5
Neomycin	156.2	312.5	156.2	312.5	156.2	156.2
Escherichia coli 27						
Amikacin	39.1	39.1	19.5	78.1	39.1	156.2
Gentamicin	9.7	9.7	2.4	4.8	4.8	9.7
Neomycin	156.2	156.2	78.1	156.2	78.1	78.1

Menadione demonstrated antibacterial activity against *P. aeruginosa* 03, with a MIC of 64 μg/mL, while DMSO, used as a negative control, showed a much higher MIC of 256 μg/mL against the strain tested. Menadione showed no significant antibacterial activity against the other bacterial strains analyzed, where MIC was the same as the DMSO control (Table 2). Dimethylsulfoxide is versatile and has several pharmacological and therapeutic properties, including antimicrobial action, showing in vitro bactericidal or bacteriostatic activity at concentrations of 5–50% against various pathogenic bacteria, including *Staphylococcus* spp., *E. coli*, *Mycobacterium tuberculosis*, *Streptococcus* spp., *Salmonella* spp. and *Proteus* spp. (Brayton, 1986; Stone, 1993; Mangia, 2008).

Lipophilic substances and menadione cause disturbances in the bacterial membrane, resulting in damage of the fundamental elements needed for membrane integrity, such as reduced membrane potential and loss of ions, cytochrome C, proteins and radicals, followed by the collapse of the proton pump and ATP depletion (Sikkema et al., 1994; Turina et al., 2006; Hirayama et al., 2006). Permeabilization of the outer and inner membrane can subsequently occur and facilitate the entry of antibiotics, causing cell lysis and death (Knowles et al., 2005).

When combined with the aminoglycoside class of antibiotics, menadione decreased the antibiotic MIC with increasing subinhibitory concentration of vitamin K3, MIC/8 to MIC/2, indicating a synergistic relationship (Table 3).

Menadione at the subinhibitory concentration of MIC/8 did not show antibiotic-modifying potential in any of the bacterial strains used. Subinhibitory concentration MIC/4 showed a significant synergistic interaction in *S. aureus* 358 with amikacin and neomycin and *E. coli* 27 with all aminoglycosides. Subinhibitory concentration MIC/2 was synergistic with all antibiotics and strains tested, including *P. aeruginosa* 03.

Especially notable is that menadione was significantly effective against *S. aureus* 358 and mainly *E. coli* 27, microorganisms with different morphologies. Gram-positive *S. aureus* has a thick and rigid cell wall formed by layers of peptidoglycan. The cell walls of Gram-negative bacteria such as *E. coli*

Please cite this article in press as: Andrade, J.C. et al., Menadione (vitamin K) enhances the antibiotic activity of drugs by cell membrane permeabilization mechanism. Saudi Journal of Biological Sciences (2015), http://dx.doi.org/10.1016/j.sjbs.2015.09.004

Table 5 Antibiotic modulatory activity of ergosterol alone or associated with aminoglycosides in sub-inhibitory concentrations (MIC/8, MIC/4 and MIC/2).

Ergosterol (μg/mL)						
	Antibiotic + MIC/8	Antibiotic alone	Antibiotic + MIC/4	Antibiotic alone	Antibiotic + MIC/2	Antibiotic alone
Staphylococcus aureus 358						
Amikacin	78.1	39.1	9.7	19.5	19.5	156.2
Gentamicin	4.8	19.5	2.4	2.4	2.4	9.7
Neomycin	19.5	156.2	39.1	78.1	78.1	156.2
Pseudomonas aeruginosa 03						
Amikacin	78.1	39.1	39.1	156.2	19.5	78.1
Gentamicin	4.8	39.1	9.7	39.1	4.8	19.5
Neomycin	312.5	312.5	625	312.5	156.2	156.2
Escherichia coli 27						
Amikacin	39.1	39.1	9.7	78.1	9.7	156.2
Gentamicin	9.7	9.7	2.4	4.8	2.4	9.7
Neomycin	156.2	156.2	39.1	156.2	39.1	78.1

contain only a thin peptidoglycan layer (Tortora et al., 2008), which suggests that menadione, due to its lipid-soluble nature has a lipophilic action on the bacterial cell envelope, causing a disruption of the fluid mosaic membrane (Nostro et al., 2004), affecting *E. coli* more so than *S. aureus*.

According to Nicolson et al. (1999) Gram-positive and Gram-negative bacteria are more sensitive to low-polarity compounds due to the presence of polysaccharide chains in the bacterial membrane that act as barriers to hydrophobic substances.

Ergosterol and cholesterol are major components of biological membranes of eukaryotes, where they control permeability, but they are absent in the membrane of bacteria. The cell can control its fluidity by regulating the level of cholesterol, or the saturation of phospholipid hydrocarbon chains (Frézard and Schettini, 2005). Due to their lipophilic structure, analogous to menadione, both were used as controls to check and compare the mechanism of action of menadione in the bacterial membrane.

Cholesterol and ergosterol had no antimicrobial activity, showing MIC $\geqslant$1024 μg/mL, which was already expected. However, the combination of either with antibiotics, against multiresistant bacteria, reduced the antibiotic MIC significantly as the subinhibitory concentration of the sterols was increased (Tables 4 and 5). There is no previous report on the use of cholesterol and ergosterol as modifiers of the action of antibiotics or any other drug, so this is the first study in this area. Thus, it was possible to determine the similarity between menadione between cholesterol, and ergosterol with regard to antibiotic-modifying activity.

4. Conclusion

Menadione demonstrated clinically relevant results in the modulation of aminoglycosides against multiresistant bacteria. This represents an interesting alternative to increasing bacterial resistance, since menadione is present in the diet, and is not toxic to humans. Pre-clinical and clinical studies are warranted to determine bioavailability and mechanisms of action involved in this interaction.

References

Brayton, C.F., 1986. Dimethyl sulfoxide (DMSO): a review. Cornell Vet. 76, 61–90.

Coutinho, H.D.M., Costa, J.G., Lima, E.O., Falcão-Silva, V.S., Siqueira-Júnior, J.P., 2008. Enhancement of the antibiotic activity against a multiresistant *Escherichia coli* by *Mentha arvensis* L. and chlorpromazine. Chemotherapy 54, 328–330.

Frézard, F., Schettini, D.A., 2005. Lipossomas: propriedades físico-químicas e farmacológicas, aplicações na Quimioterapia à base de antimônio. Quím. Nova 28, 511–518.

Gibbons, S., 2004. Anti-staphylococcal plant natural products. Nat. Prod. Rep. 126, 263–277.

Hirayama, Karin B., Speridião, Patrícia G.L., Fagundes Neto, U., 2006. Ácidos graxos poliinsaturados de cadeia longa. Electron. J. Pediatr. Gastroenterol. Nutr. Liver Dis. 10.

Javadpour, M.M., Juban, M.M., Lo, W.C., Bishop, S.M., Alberty, J. B., Cowell, S.M., Becker, C.L., Mclaug, M.L., 1996. De novo antimicrobial peptides with low mammalian cell toxicity. J. Med. Chem. 39, 3107–3113.

Klack, K., Carvalho, J.F., 2006. Vitamina K: metabolismo, fontes e interação com o anticoagulante varfarina. Rev. Bras. Reumatol. 46, 398–406.

Knowles, J.R., Roller, S., Murray, D.B., Naidu, A.S., 2005. Antimicrobial action of carvacrol at different stages of dual-species biofilm development by *Staphylococcus aureus* and *Salmonella enterica* Serovar typhimurium. Appl. Environ. Microbiol., 797–803

Köhler, T., Pechère, J.C., Plésiat, P., 1999. Bacterial antibiotic efflux systems of medical importance. Cell. Mol. Life Sci. 56, 771–778.

Leança, C.C., Passarelli, M., Nakandakare, E.R., Quintão, E.C.R., 2010. HDL: the yin-yang of cardiovascular disease. Arq. Bras. Endocrinol. Metabol., 54–59

Lee, E.W., Chen, J., Huda, M.N., Kuroda, T., Mizushima, T., Tsuchiya, T., 2003. Functional cloning and expression of *emeA*, and characterization of EmeA, a multidrug efflux pump from *Enterococcus faecalis*. Biol. Pharm. Bull. 26, 266–270.

Loguercio-Leite, C., Groposo, C., Dreschler-Santos, E.R., Figueiredo, N.F., Godinho, P.S., Abrão, R.L., 2006. A particularidade de ser um fungo – I. Constituintes celulares. Biotemas 19, 17–27.

Ludke, M.C.M.M., López, J., 1999. Colesterol e composição dos ácidos graxos nas dietas para humanos e na carcaça suína. Ciênc. Rural 29, 181–187.

Mangia, S.H., 2008. Tratamento experimental de cães naturalmente infectados com o vírus da cinomose na fase neurológica com o uso

de ribavirina e dimetil-sulfóxido (DMSO) (MSC thesis). 186f. Universidade Estadual Paulista, Botucatu – SP.

Murray, P.R., Rosenthal, K.S., Kobayashi, G.S., Pfaller, M.A., 2004. Microbiologia Médica, fourth ed. Guanabara Koogan, Rio de Janeiro.

Nicolson, K., Evans, G., Otoole, P.W., 1999. Potentiation of methicillin. Activity against methicillin-resistant *Staphylococcus aureus* by diterpenes. FEMS Microbiol. Lett. 179, 233–239.

Nostro, A., Blanco, A.R., Cannatelli, M.A., Enea, V., Flamini, G., Morelli, I., Roccaro, A.S., Alonzo, V., 2004. Susceptibility of methicillin-resistant staphylococci to oregano essential oil, carvacrol and thymol. FEMS Microbiol. Lett. 230, 191–195.

Paixão, J.A., Stamford, T.L.M., 2004. Vitaminas lipossolúveis em alimentos – uma abordagem analítica. Quím. Nova 27, 96–105.

Pretto, J.B., Cechinel Filho, V., Noldin, V.F., Sartori, M.R.K., Isaias, D.E.B., Cruz, A.Z., 2004. Antimicrobial activity of fractions and compounds from *Calophyllum brasiliense* (clusiaceae/guttiferae). Naturforsch 59c, 657–662.

Santos, A.R., Carvalho, H.F., 2001. Biomembranas. In: Carvalho, H. F.E., Recco-Pimentel, S.M. (Eds.), A Célula 2001. Manole, Barueri.

Schaechter, M., Engleberg, N.C., Eisenstein, B.I., Medoff, G., 2002. Microbiologia: Macanismo das doenças infecciosas, third ed. Guanabara Koogan, Rio de Janeiro.

Sikkema, J., Bont, J.A.M., Poolman, B., 1994. Interaction of cyclic hydrocarbons with biological membranes. J. Biol. Chem. 269, 8022–8028.

Stone, R.W., 1993. Clinical updates on the use of dimethyl sulfoxide. Canine Pract. 18, 16–19.

Taleb-Contini, S.H., Salvador, M.J., Watanabe, E., Ito, I.Y., Dionéia, C.R.O., 2003. Atividade antimicrobiana dos flavonóides e esteróides isolados de duas espécies de *Chromolaena*. Rev. Bras. Ciênc. Farm. 30, 403–408.

Thevissen, K., Kathelijne, K.A., Ferket, I., François, E.J.A., Cammue, B.P.A., 2003. Interactions of antifungal plant defensins with fungal membrane components. Peptides 24, 1705–1712.

Tortora, G.J., Funke, B.R., Case, C.L., 2008. Microbiologia, eighth ed. ARTMED, Porto Alegre.

Turina, A.V., Nolan, M.V., Zygadlo, J.A., Perillo, M.A., 2006. Natural terpenes: self-assembly and membrane partitioning. Biophys. Chem. 122, 101–113.

Please cite this article in press as: Andrade, J.C. et al., Menadione (vitamin K) enhances the antibiotic activity of drugs by cell membrane permeabilization mechanism. Saudi Journal of Biological Sciences (2015), http://dx.doi.org/10.1016/j.sjbs.2015.09.004