REVIEW

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Tyrothricin – An underrated agent for the treatment of bacterial skin infections and superficial wounds?

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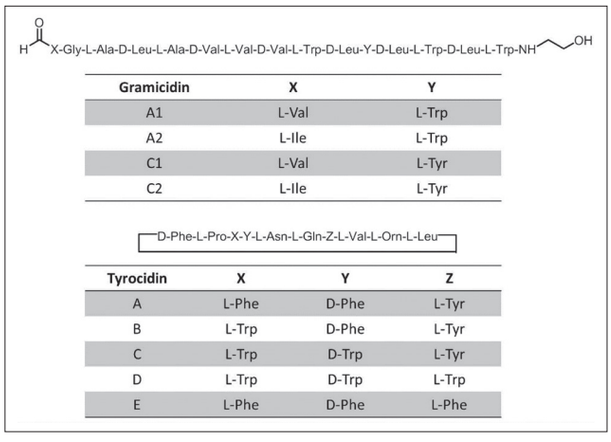
The antimicrobial agent tyrothricin is a representative of the group of antimicrobial peptides (AMP). It is produced by *Bacillus brevis* and consists of tyrocidines and gramicidins. The compound mixture shows activity against bacteria, fungi and some viruses. A very interesting feature of AMPs is the fact, that even *in vitro* it is almost impossible to induce resistances. Therefore, this class of molecules is discussed as one group that could serve as next generation antibiotics and overcome the increasing problem of bacterial resistances. In daily practice, the application of tyrothricin containing formulations is relatively limited: It is used in sore throat medications and in agents for the healing of infected superficial and small-area wounds. However, due to the broad spectrum antimicrobial activity and the low risk of resistance development it is worth to consider further fields of application.

1. Introduction

A decade after Alexander Fleming published his findings on the antibiotic effect of penicillin in 1929 (Fleming 1929), René Dubos discovered tyrothricin, a polypeptide mixture obtained from *Bacillus brevis*, which was isolated from soil. One component of tyrothricin, gramicidin, was the first clinically tested antibiotic agent and helped to revive the stalled interest in penicillin, starting the era of antibiotics (Van Epps 2006). Thereafter, further effective agents isolated from natural sources were identified and applied for the treatment of microbial infections. Today, many pathogenic bacteria show resistances to a wide range of available antimicrobial drugs (Walsh 2003; Tenover 2006; Courvalin 2016). This resulted in a warning by the world health organization (WHO) that these multiple antibiotic-resistant pathogens would very likely bring the world back to a pre-antibiotic era (Parisien et al. 2008). As a consequence alternatives to conventional antibiotics are strongly needed. In contrast to other antibacterial agents like fusidic acid, the risk of resistance formation against the topically used tyrothricin is very low (Besier et al. 2003; Stauss-Grabo et al. 2014; Chen et al. 2015). Tyrothricin belongs to the group of antimicrobial peptides (AMP, a.k.a. host defense peptides), which differ from "conventional" antibiotics in many attributes. In general, AMPs are produced by all organisms and pose their first line defense against infections. A common antimicrobial mechanism of the amphiphilic peptides, which generally consist of 6-50 amino acids, is their interaction with anionic phospholipid membranes resulting in a broad spectrum microbicidal activity against bacteria, fungi and some viruses (Korting et al. 2012). Among others, defensins and the S100-protein psoriasin comprise examples of AMPs that are produced by humans (Körber et al. 2005; Harder et al. 2010; Harder 2016; Wittersheim et al. 2013; Cordes et al. 2014). Despite the existing contact of AMPs with microorganisms over millions of years, their efficacy remains unchanged (Fjell et al. 2012). Because of their broad spectrum activity and their low risk to induce resistances, AMPs are discussed to be potential next-generation antibiotics (Mangoni et al. 2016). This review will focus on the AMP tyrothricin, which is already marketed for the treatment of superficial wounds and as a sore throat remedy since decades. Its properties might open several potential fields of application which can help to replace "conventional" antibiotics.

2. Structure, production, properties

Tyrothricin is a mixture of polypeptides, consisting of 50 % - 70 % tyrocidines and 25 % to 50 % gramicidins (Ph.Eur. 8th edition 2014). The group of tyrocidines are basic, cyclic peptides, whereas the fraction of gramicidins are neutral, linear peptides. Both groups each are composed of structurally related compounds, that differ in particular amino acids in certain positions of the molecule (Voigt and Ehlers 1989, Ph.Eur. 8th edition 2014): The tyrocidine fraction includes the decapeptides tyrocidine A, B, C, D, and E in which Phe and Tyr residues are gradually replaced by Trp, depending on the relative concentrations of these amino acids in the growth medium (Mootz and Marahiel 1997; Ph.Eur. 8th edition 2014). The gramicidin fraction mainly includes the pentadecapeptides gramicidin A1, A2, C1 and C2, which contain solely amino acids with hydrophobic residues and have blocked C- (ethanolamine) and N- (formylation) termini, rendering them unable to adopt a net charge or form a zwitterion at any pH (Fig. 1) Apart from the above mentioned tyrocidines and gramicidins, other related compounds may be present in smaller amounts (Kessler et al. 2004; Ph.Eur. 8th edition 2014). Besides physiological and essential L-amino acids, the components of tyrothricin also contain D-amino acids (Voigt and Ehlers 1989; Vogt et al. 2003). Tyrocidines contain two



Gramicidin	X	Y
A1	L-Val	L-Trp
A2	L-Ile	L-Trp
C1	L-Val	L-Tyr
C2	L-Ile	L-Tyr

D-Phe-L-Pro-X-Y-L-Asn-L-Gln-Z-L-Val-L-Orn-L-Leu

Tyrocidin	X	Y	Z
A	L-Phe	D-Phe	L-Tyr
B	L-Trp	D-Phe	L-Tyr
C	L-Trp	D-Trp	L-Tyr
D	L-Trp	D-Trp	L-Trp
E	L-Phe	D-Phe	L-Phe

Fig. 1: Components of tyrothricin according to Ph. Eur.

amino acids in D configuration and the nonproteinogenic amino acid L-ornithine (Mootz and Marahiel 1997). Gramicidins have an alternating L- and D-amino acid composition except for the non-chiral amino acid glycine in position 2. This results in the formation of a β-helix by the gramicidins with all hydrophobic residues pointing outwards (Kessler et al. 2004).

Tyrothricin is produced by the gram-positive aerobic spore-forming bacterium *Bacillus brevis* (Strain ATCC 8185) during its sporulation phase via the nonribosomal pathway (Dubos and Hotchkiss 1941; Stokes and Woodward 1943; Vogt et al. 2003; Vosloo et al. 2013). In general, the Bacillus species is industrially important e.g. due to its excellent safety record, its short fermentation cycles and its high capacity for protein secretion into the extracellular medium (Sumi et al. 2015). Tyrocidines are formed by an enzyme complex comprising the three peptide synthetases TycA, TycB and TycC (tyrocidine synthetases 1,2, and 3) (Mootz and Marahiel 1997). Gramicidins are assembled by the four multimodular peptide synthetases LgrA, LgrB, LgrC, and LgrD (Kessler et al. 2004). Besides their well-known antimicrobial properties, it was shown that tyrocidines and gramicidins play a regulatory role in the initiation process of sporulation by interacting with RNA and DNA of the *Bacillus brevis* (Chakraborty et al. 1978; Paulus et al. 1979; Sarkar et al. 1979; Bohg and Ristow 1986; Bohg and Ristow 1987).

Tyrothricin is a white or almost white powder with a potency of 180 to 280 international units/mg with regard to the dried substance. The compound is practically insoluble in water, whereas it is soluble in ethanol (96 %) and in methanol (Brayfield 2014; Ph.Eur. 8th edition 2014).

3. Mechanism of action

3.1. Tyrocidines

Due to the amphiphilic character of the basic and cyclic tyrocidines, consisting of a hydrophobic and hydrophilic face, they can easily insert into bacterial membranes. It appears that the hydrophilic residues of the peptide interact with the negatively charged phosphate groups abundant in the bacterial cell membrane, while the hydrophobic groups form a nonselective porous channel (Marques et al. 2007; Pálffy et al. 2009). This causes a leakage of nitrogen- and phosphor-containing substances from the bacterial cell membrane. In consequence the bacterial cell loses essential molecules such as amino acids, phosphates, purines, pyrimidines, etc. to the surrounding medium resulting in a dilution of these essential metabolites causing the bactericidal effect. This action is independent of the bacterial growth (Hotchkiss 1944; Gale 1963; Voigt and Ehlers 1989). Recently a high resolution crystal structure of tyrocidine A was published revealing an amphipathic dimer, which is discussed to be the physiological agent of the interaction with bacterial target membranes (Loll et al. 2014) (Fig. 2). Further actions of tyrocidines described in the literature are the reversible inhibition of the soluble form of acetylcholinesterase and the inhibition of β-galactosidase from *E. coli* (Changeux et al. 1969).

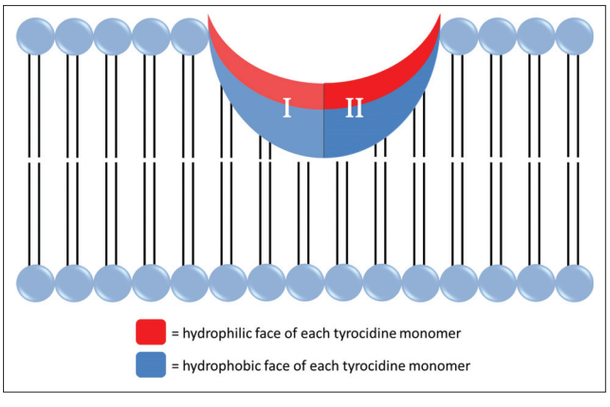


Fig. 2: Potential physiological interaction of tyrocidine with bacterial target membranes as an amphipathic dimer. **I** and **II** indicate the respective monomers

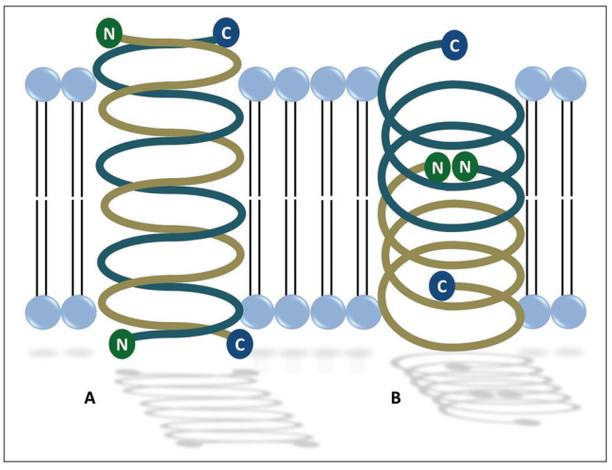


Fig. 3: Structures of channels formed by gramicidin according to (Wallace 1998): **A** Double helix (pore) **B** Helical dimer (channel)

3.2. Gramicidins

The structural feature of gramicidins, having an alternating D- and L- amino acid sequence leads to the formation of β-helix-like channels with all hydrophobic residues pointing outwards and the relatively hydrophilic polypeptide backbone pointing inwards (Kessler et al. 2004). Having this property, the molecules partition strongly into the hydrophobic core of phospholipid membranes forming dimeric channels (helical dimer) or pores (double helix) for monovalent cations (Busath and Szabo 1981; Wallace 1998, 2000) (Fig. 3). Although the formation of these channels in phospholipid membranes and a loss of monovalent cations (mainly K^+) from cells caused by gramicidin was shown (Harold and Baarda 1967), it is controversially discussed, whether the primary antibiotic activity of the peptide is related to this fact (Paulus et al. 1979; Wallace 1998; Novartis 1999; Sorochkina et al. 2012). The main mechanism of antibiotic action seems to be the uncoupling of the oxidative phosphorylation by gramicidin (Hotchkiss 1944; Gale 1963; Voigt and Ehlers 1989). In detail, findings suggest a direct interaction of gramicidin with the H^+-ATPase: The direct proton transfer from redox pumps to H^+-ATPase appears to be short-circuited by gramicidin (Rottenberg and Koeppe 1989; Rottenberg 1990).

4. Antibacterial efficacy

In order to set the following MIC values obtained from *in vitro* experiments into a clinical context, it has to be mentioned that formulations for the treatment of superficial wounds marketed in Germany contain tyrothricin concentrations of 1 mg/g. The efficacy spectrum of tyrothricin mainly covers gram-positive but also several gram-negative bacteria. Ruckdeschel et al. (1983) conducted extensive investigations to identify germs of clinical origin, that are susceptible to tyrothricin: Between concentrations of 16 and 64 µg/ml the AMP inhibited 400 of 401 tested Streptococcus strains. Corynebacteria and staphylococci showed a broader range of tyrothricin concentrations for inhibition (2 – 256 µg/ml). In contrast to the gramicidin fraction alone, tyrothricin was able to inhibit all Staphylococcus strains at a maximum concentration of 128 µg/ml. Furthermore, the production of penicillinase had no influence on the susceptibility of either staphylococci or gonococci against tyrothricin. Among the gram-negative organisms, gonococci and meningococci were the most sensitive germs (96 µg/ml); also 4 of 5 strains of *Branhamella catarrhalis* were susceptible. Reduced activities were found against other strains of Neisseria and *Haemophilus influenzae* (Table 1). Other gram-

Table 1: Cumulative percent of isolates susceptible to indicated tyrothricin concentrations according to Ruckdeschel et al. (1983). All values were obtained by agar dilution tests

		Cumulative % of isolates susceptible to indicated concentrations [µg/ml]																	
Organism	**n**	**≤0.03**	**0.06**	**0.125**	**0.25**	**0.5**	**1**	**2**	**4**	**8**	**16**	**24**	**32**	**48**	**64**	**96**	**128**	**192**	**256**	**>256**
Staphylococcus aureus (penicillin sensitive)	**15**										0	7	13	13	67	93	100			
Staphylococcus aureus (penicillin resistant)	**34**										0	3	21	26	76	97	100			
Staphylococcus aureus MRSA values obtained from (Chen et al 2014)	**223**						1.3	3.1	40.4	100										
Streptococcus pyogenes	**44**		0	5	7	25	48	61	61	75	100									
Streptococcus agalactiae	**80**				0	1	14	55	65	75	96	96	100							
Streptococcus group C	**29**	3	3	3	14	59	97	97	100											
Streptococcus group F	**39**		0	3	5	13	41	77	97	100										
Streptococcus group G	**37**						0	19	68	89	95	95	100							
Streptococcus pneumoniae	**54**	4	24	24	28	43	56	70	83	89	93	93	96	96	98	100				
Streptococcus mitis (incl. S. mutans, S. salivarius)	**59**		0	7	19	32	39	56	68	80	85	85	90	90	100					
Streptococcus faecalis	**35**						0	3	3	9	31	43	91	94	100					
Streptococcus faecium	**24**				0	13	38	46	50	63	67	67	88	92	100					
Neisseria meningitidis	**30**						0	3	3	13	13	23	46	69	86	100				
Neisseria gonorrhoeae (penicillin sensitive)	**29**							0	3	3	17	45	93	97	100					
Neisseria gonorrhoeae (penicillin resistant)	**11**							0	9	9	9	36	91	100						
Neisseria sicca (incl. N. mucosa, N. flava, Branhamella catarrhalis)	**60**								0	5	7	7	17	17	20	20	73	78	93	100
Corynebacteria (C. xerosis, C. haemolyticum, C. pseudodiphteriticum, C. ulcerans	**19**								0	11	63	63	89	89	89	89	89	89	100	
Haemophilus influenzae (Ampicillin sensitive)	**53**												0	9	9	38	51	68	72	100
Haemophilus influenzae (Ampicillin resistant)	**16**															0	6	81	88	100

negative bacilli, including *E. coli*, *Klebsiella pneumoniae*, *Serratia marcescens*, *Proteus mirabilis*, *Proteus vulgaris*, *Proteus morganii* and *Pseudomonas aeruginosa* were not inhibited by tyrothricin in concentrations up to 256 µg/ml. Kretschmar et al. (1995) demonstrated the fast bactericidal effect of the AMP: Clinical isolates and reference strains of streptococci, staphylococci and enterococci were investigated for their minimum inhibitory concentration (MIC) revealing values of 0.048-1.562 µg/ml, 1.562-3.125 µg/ml and 0.195-1.562 µg/ml, respectively (Table 2). Interestingly, multi-resistant germs (MRSA) did not differ in their MIC compared to their respective sensitive strains. The fast bactericidal effect is exemplified by the reduction of the colony forming units (cfu) of MRSA from $5x10^5$ cfu/ml to 10^3 cfu/ml within the first 20 min when using 25 µg/ml tyrothricin (8-fold MIC). After further 40 min, no cfu´s were detectable any more. The use of concentrations as low as 6.25 µg/ml also resulted in a fast bactericidal effect within 60 min. The same group was able to show that tyrothricin is even effective against MRSA that show reduced susceptibility to mupirocin, which is a common agent for the treatment of mucosal colonization with MRSA (Kretschmar et al. 1996a). This was confirmed by a recent publication which emphasizes the use of tyrothricin as a potential alternative for MRSA decolonization (Chen et al. 2014). Furthermore, the activity against other microorganisms e.g. plasmodiidae (Rautenbach et al. 2007), trichomonadidae (Voigt and Ehlers 1989) and mycoplasmae (Ruckdeschel et al. 1983) is mentioned in the literature.

Table 2: MICs of tyrothricin. Values according to reference 1: Micro dilution assay (Kretschmar et al. 1996b) 2: Micro dilution assay (Stauss-Grabo et al. 2014) 3: Agar dilution test (Kretschmar et al. 1995)

Organism (n)	Ref.	Origin	MIC [µg/ml]
Candida albicans	1	DSM 1386	6.2
Candida albicans (5)	1	Clinical isolates	4.8-6.7
Candida albicans (20)	2	Clinical isolates	4-16
Candida glabrata (5)	1	Clinical isolates	6.4-9.1
Candida glabrata	1	DSM 70614	8.6
Candida parapsilosis (20)	2	Clinical isolates	4-32
Candida tropicalis	1	DSM 1346	7.8
Candida tropicalis (5)	1	Clinical isolates	5.3-7.6
Corynebact. spec. (20)	2	Clinical isolates	0.5-2
Enterococcus faecalis	3	ATCC 29212	1.562
Enterococcus faecalis (6)	3	Clinical isolates	0.195-1.562
Enterococcus faecalis (20)	2	Clinical isolates	1-8
Enterococcus faecium	3	DSM 2146	0.390
Staphylococcus aureus	3	DSM 799	1.562
Staphylococcus aureus (12)	3	Clinical isolates	1.562-3.125
Staphylococcus aureus MRSA (10)	3	Clinical isolates	1.562-3.125
Staphylococcus aureus MRSA (20)	2	Clinical isolates	1-8
Staphylococcus aureus MSSA (20)	2	Clinical isolates	1-8
Staphylococcus epidermidis	3	DSM 706	1.562
Staphylococcus epidermidis (12)	3	Clinical isolates	1.562-3.125
Staphylococcus haemolyticus (20)	2	Clinical isolates	1-4
Streptococcus agalactiae (B) (6)	3	Clinical isolates	0.048-1.562
Streptococcus mutans	3	DSM 20523	1.562
Streptococcus oralis	3	DSM20627	1.562
Streptococcus pneumoniae (5)	3	Clinical isolates	0.048-0.195
Streptococcus pyogenes	3	DSM 20565	0.390
Streptococcus pyogenes (A) (6)	3	Clinical isolates	0.048-0.390
Streptococcus pyogenes (20)	2	Clinical isolates	0.25-1
Streptococcus sanguis	3	DSM 20565	0.390

5. Antifungal efficacy

Tyrothricin exerts fungicidal effects on a variety of *Candida* species. The determination of the MIC revealed a good activity of tyrothricin against *Candida albicans*, *Candida tropicalis* and *Candida glabrata* (Table 2). The fungicidal effect of the AMP underlies a quick onset: Four times the MIC of tyrothricin reduced the inoculum (5×10^5 cfu/ml) below the detection limit of 10^2 CFU/ml after 1 h of incubation (Kretschmar et al. 1996b). Furthermore, it was shown, that the species *Candida parapsilosis* is also susceptible to tyrothricin.

6. Antiviral efficacy

In vitro suspension experiments showed an anti-infectious activity of tyrothricin against parainfluenza virus (type Sendai). The authors discuss a virostatic mode of action by the AMP. Furthermore, an inhibiting effect on viral infectivity by tyrothricin could also be shown in other viruses such as influenza-, mumps- and herpes viruses. The authors postulate a connection of the activity of the AMP with the lipid content of the viral envelope (Grossgebauer and Hartmann 1978; Voigt and Ehlers 1989). The same authors transferred these experiments to animal models using herpes-simplex-virus (HSV) type 1. They were able to show that a pre-incubation of the virus suspension with tyrothricin could significantly decrease the lethality in mice. The effect could only be shown after a direct contact between tyrothricin and the virus (Hartmann and Grossgebauer 1979).

7. Resistance

The very low probability of resistance formation is one important feature of AMPs. The same fact applies to tyrothricin, which was recently confirmed by the comparison of current MICs with MICs from the past, showing an unchanged susceptibility of the investigated germs (Stauss-Grabo et al. 2014). In addition, the 2-3 times daily application of a tyrothricin containing toothpaste to 99 children over a period of 2 years did not result in a significant difference of the sensitivity of the germs as compared to 107 other children that did not obtain a tyrothricin containing toothpaste (Lind and Swanton 1954; Florey 1960). The difficulty of a resistance development is also depicted in the fact that compared to conventional antibiotics a resistance formation *in vitro* by the repeated use of concentrations lower than the MIC is extremely challenging (unpublished data). Several reasons for the low probability of resistance formation against AMPs and tyrothricin are discussed in the literature: *a.* Tyrothricin exerts a fast bactericidal mechanism, aggravating the possibility for bacteria to adapt to the situation and to develop a defense strategy to protect against the antimicrobial agent (Kretschmar et al. 1995; Vosloo et al. 2013). Furthermore, the antimicrobial effect is not limited to growing or dividing bacteria (Voigt and Ehlers 1989). *b.* The target of tyrothricin are membrane structures of the bacteria. Therefore, pathogens would have to alter their membrane composition and organization to achieve resistance. This is a much more complicated mechanism compared to e.g. mutations of an enzyme binding site (Marques et al. 2007; Sumi et al. 2015). *c.* As previously noted, tyrothricin is a mixture of tyrocidines and gramicidins, both underlying different mechanisms of action. In case the pathogen would be able to develop a resistance against one of the components, it is still confronted with the remaining compound class. *d.* The components of tyrothricin partly consist of D-amino acids. These peptides with "unnatural" amino acids often show resistance to enzymatic hydrolysis, which aggravates the formation of enzymes by bacteria which are able to destroy the AMP. Furthermore, the high content of hydrophobic side chains is discussed to influence the stability of tyrothricin in the presence of enzymes (Hotchkiss 1944). In addition, cross resistances with tyrothricin are not observed due to the lacking resorption of the compound and the mechanism of action that is different to systemically applied antibiotics (Ruckdeschel et al. 1983; Voigt and Ehlers 1989).

8. Selectivity towards bacteria/toxicity

As tyrothricin contains membrane active substances, the question about selectivity towards bacterial membranes arises: In contrast to the zwitterionic phospholipids found in eukaryotes, bacterial surfaces consist of negatively charged phosphate groups which are discussed to mediate a selective attraction of the positively charged tyrocidines. Furthermore, eukaryotic membranes contain cholesterol which helps to stabilize and protect the cells against an attack of the cyclopeptides (Prenner et al. 2001; Qin et al. 2003; Marques et al. 2007). Nevertheless disruption of the integrity of eukaryotic membranes is observed at higher tyrothricin concentrations *in vitro* (Kretschmar et al. 1995; Rautenbach et al. 2007). This effect is exemplified as hemolytic activity of tyrothricin in *in vitro* studies and when applied to animals i.v. (Hotchkiss 1944; Florey 1960; Voigt and Ehlers 1989). When applied to skin or mucosa, tyrothricin does not cause tissue damage, but rather shows a very good tolerability besides its antibacterial efficacy (Voigt and Ehlers 1989). This observation was confirmed in a recent study, comparing the healing of standardized wounds using a tyrothricin containing gel or the respective vehicle. An impairment of wound healing by tyrothricin was not observed (Wigger-Alberti et al. 2013). In contrast to an intravenous ($LD_{50\ mouse}$: 3,7 mg/kg) and an intraperitoneal ($LD_{50\ mouse}$: 20-45 mg/kg) application the oral application is very well tolerated, as tyrothricin is destroyed in the gastro-intestinal tract. Furthermore, due to its poor solubility and its relatively high molecular weight, there is no detectable resorption when applied locally on skin or mucosa (Voigt and Ehlers 1989). Although the application on mucosa is generally safe, the nasal application should be omitted, as the loss of the olfactory sense was observed in 7 case reports after treatment with tyrothricin containing nasal spray or drops in a concentration of 1:5000. The condition persisted for 4 to 8 months (Seydell and McKnight 1948).

9. Indications of medicinal products containing tyrothricin and clinical studies

The two main fields in which tyrothricin containing medicinal products are marketed are lozenges as sore throat medications and topical formulations for the treatment of infected wounds. As inflammatory processes in the laryngopharyngeal area are in most cases caused by viruses, the application of tyrothricin (often combined with an local-anaesthetic and/or antiseptic) is controversely discussed because the primary efficacy spectrum consists of gram-positive bacteria. However, as *in vitro* experiments also showed an efficacy of the AMP against some viruses, the application might be reasonable in some cases. Studies using tyrothricin for the treatment of throat infections are of older date and were extensively reviewed by Ehlers and Voigt (1989). Thus, studies regarding this indication are not further discussed in this review.

The more relevant indication for tyrothricin is the topical treatment of infected wounds. Since the summary of clinical applications by Ehlers and Voigt (1989), several studies about the application of tyrothricin on the human skin were published. These will be described hereafter:

The efficacy and tolerability of a tyrothricin containing wound powder was investigated in a prospective, randomized, placebo-controlled, double-blind multicenter trial in 131 patients (age: 18 – 85 years) with posttraumatic and surgical cutanious lesions (wound area ≥ 200 mm^2) (Bayerl and Völp 2004). The wounds of 62 patients were treated two times daily with tyrothricin containing powder (0.1 g per 100 g vehicle), 69 patients received a placebo formulation. Both treatments were conducted over a period of 9 days. The primary aim was to evaluate the average daily reduction of the radius of the lesion area between the start and the end of the treatment. Here, an overall superiority of the verum treatment could be shown (reduction: 0.55±0.31 mm/day vs. 0.47±0.30 mm/day, p=0.016; one-sided). Also in the secondary parameter, the reduction of the wound-index (range 0-15; calculated from the assessment of rubor, crusting, exudation, pain and functional impairment), an advantage was detected for the verum therapy (reduction: 4.2±1.7 vs. 3.3±1.9, p=0.0048, one-sided). In every single symptom of the wound index there was a higher improvement in the verum group. The authors conclude that the advantages might also be attributable to tyrothricin's effect of promoting granulation and epithelialisation during the proliferation phase and the repair phase of wound healing. The very good tolerability of the verum treatment is depicted in the fact that no adverse drug reaction (ADR) could be observed in the 62 patients of the tyrothricin group. Furthermore, the treatment tolerability was rated as "very good" by 70 % of the patients and in no case the tolerability was rated worse than "good".

In another double-blind, randomized, intraindividual study, the influence of the wound-healing by a tyrothricin containing hydrogel (concentration 0.1 %) was compared with the respective vehicle or untreated wounds (Wigger-Alberti et al. 2013). Therefore, 33 healthy volunteers (age: 23 – 58 years) underwent three standardized, superficial abrasions on their forearm skin each (10 mm in diameter). One of the three lesions remained untreated, the other two were either treated with the vehicle or the verum (approx. 100 µl) once daily under semiocclusive conditions over a period of 12 days. The wound healing was assessed by a trained investigator based on the level of reepithelialization (6 point scale: 0 = no healing to 5 = complete healing) on days 2, 5, 8 and 12. A significant improvement of the area under the curve (AUC) of wound healing scores and the mean reepithialization scores could be observed in the verum and the vehicle treated group as compared to the untreated lesion. As moist wound treatment accelerates wound healing and only the direct wound healing beyond antimicrobial activity was investigated in this study, no difference between verum and vehicle was expected and observed. Moreover it was established that the verum has significant advantages over untreated conditions and that the AMP is compatible with the wound tissue, as no hindering effects on improved wound healing were observed compared to the vehicle. The very good tolerability is depicted in the low number of adverse events (n=5, all non serious) which were all considered not to be related to the study medication.

As the antimicrobial activity of tyrothricin against *Propionibacterium acnes* was demonstrated *in vitro*, the efficacy and tolerability of a tyrothricin containing gel (concentration 0.1 %) was evaluated in the treatment of Acne papulopustulosa in a randomized, active comparator-controlled, observer-blind study (Richter et al. 2015). Therefore, 24 patients (age 18-25 years) with mild to severe Acne papulopustulosa were included. Over a period of 25 days, one side of the face of all patients was treated with a tyrothricin containing gel. The respective other side was treated either with clindamycin + benzoyl peroxide (BPO) (n=12) or BPO 5 % (n=12) alone. The counts of inflammatory lesions, non inflammatory lesions and total lesions served for the assessment of the treatment efficacy. There was an overall statistically significant decline in the number of inflammatory and total lesions in all three treatments. The treatment effect of clindamycin + BPO was shown to be best. Tyrothricin was able to reduce inflammatory (mean difference: -7.7) and non-inflammatory lesions (mean difference: -6.5). With respect to inflammatory lesions there was no statistically significant difference in the efficacy of the tyrothricin- and BPO 5 %-treatment, but in non-inflammatory and total lesions there was a higher efficacy on the BPO 5% side. The results of this exploratory study indicate that tyrothricin might be a candidate for treating acne and seems to be better tolerable than both comparators. As the probability of resistance formation against tyrothricin is very low it could serve as an alternative to conventional antibiotics in the treatment of acne.

10. Conclusion/summary

Tyrothricin is a compound mixture which is produced by *Bacillus brevis* containing tyrocidines and gramicidins. It belongs to the group of AMPs and shows antimicrobial efficacy against bacteria, fungi and viruses. The activity relies in the properties of its components: Tyrocidines cause a leakage in the bacterial membrane, resulting in a loss of essential molecules like amino acids, purines,

pyrimidines and phosphates (Voigt and Ehlers 1989). Gramicidins mediate the uncoupling of oxidatve phosphorylation (Rottenberg 1990) and are able to form channels for monovalent cations causing their efflux from bacteria (Harold and Baarda 1967). Due to the fast bactericidal activity and the simultaneous attack of the two compound groups with different mechanisms, a resistance formation is hardly possible, which is a characteristic for AMPs in general (Stauss-Grabo et al. 2014). The most promising field of application of tyrothricin, which is restricted to topical use, poses the treatment of superficial wounds: Due to the microorgansims of the skin flora or the source of the injury, every wound is potentially colonized / contaminated (Percival et al. 2012). This colonization can develop into a clinically relevant infection with signs of inflammation or in severe cases even to a generalised infection like bacteremia or sepsis. In case of an infection exceeding the critical number of 10^5 germs/g tissue, the probability of a successful wound healing decreases dramatically from 94 % to 19 %. (Probst and Vasel-Biergans 2010). Therefore the use of modern antimicrobial agents in the treatment of wounds seems reasonable in many cases. The benefits of tyrothricin in the treatment of these wounds are evident: The compound shows a very good antimicrobial activity including the important germs that are generally responsible for wound infections (Ruckdeschel et al. 1983; Kretschmar et al. 1996a, 1996b; Dissemond 2014), the compound is tissue compatible (Wigger-Alberti et al. 2013), the development of resistances is very unlikely (Stauss-Grabo et al. 2014) and the compound is very well tolerated when applied topically. The use of a hydrogel as a base for tyrothricin containing medications has further advantages, as hydrogels support the ideal moist wound treatment (Probst and Vasel-Biergans 2010). The activity against bacteria, fungi and viruses and e.g. the first probatory application in patients with acne shows, that the topical use of tyrothricin does not necessarily have to be limited to small, superficial, infected wounds. Investigations of the efficacy e.g. in chronic, large area wounds, scratch wounds in patients with atopic dermatitis or other superficial skin diseases caused by bacteria, fungi or viruses appear as very interesting.

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Conflict of interest: CL and CS are employees of Engelhard Arzneimittel GmbH & Co. KG. A few products in the company´s portfolio contain tyrothricin as active ingredient.

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