Effect of 3',6-diNonylneamine, an amphiphilic aminoglycoside derivative, on *Pseudomonas aeruginosa's* shape and membrane integrity

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Molecular Life Sciences — 40TH FEBS CONGRESS — The Biochemical Basis of Life — July 4–9, 2015 • Berlin, Germany

INTRODUCTION

Amphiphilic aminoglycosides derivatives targeting the bacterial cell wall or cell membrane have emerged for the last decade aiming the discovery of potential new antibiotics [1,2]. They showed important antibacterial effect even on multi drug resistant strains. In this perspective, we previously synthesized a variety of amphiphilic neamine derivatives and studied their efficacy towards *Pseudomonas aeruginosa* strains[3,4]. 3',6-diNonylneamine (3',6-diNn) had an important antibacterial effect and a moderate toxicity on cellular models[4]. This compound interacts with LPS of the bacterial outer membrane[5] and with the negatively charged lipids of the inner membrane[6], mainly cardiolipin (CL), induces an impairment in the lateral distribution of CL and the formation of hemi fusion diaphragm (in Giant Unilamelar vesicles) [6]. Moreover, this interaction triggered local redistribution of cardiolipin in membrane models. These modifications were responsible of the membrane permeabilization and depolarization leading to bacterial lysis [5,6].

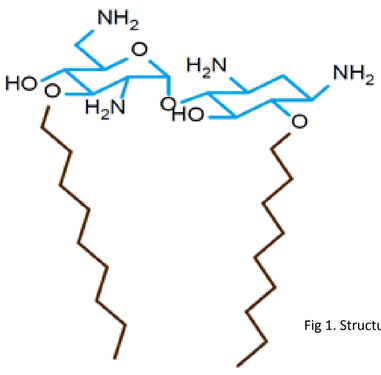

Fig 1. Structure of 3',6-diNonylneamine.

Aim

The aim of this work was to investigate more the impact of the 3',6-diNn's interaction with membrane's lipids on the bacteria's length, morphology, growth rate, and redox chain in order to elucidate its mode of action.

MATERIAL & METHODS

Bacterial strain: ATCC27853

Effect on bacterial shape: the 3',6-diNn was either added to a liquid culture of ATCC27853 in Cation Adjusted Muller Hinton Broth (CA-MHB) or to a 1 % agarose pad. Cultures were incubated at 37°C and bacteria's were observed using a Zeiss Axio Observer Z1. Cell length was measured using MicrobeTracker (version 0.937)[7]. Colistin, gentamicin, and neamine were used as controls.

Scanning electron microscopy: ATCC27853 in mid log non treated or treated with 3',6-diNn at 5 times its minimal inhibitory concentration MIC for two hours were imaged by electron scanning microscopy.

Effect on growth rate: ATCC27853 cultures in CA-MHB were incubated at 37°C in the presence of 3',6-diNn at different concentrations and the OD at 620 was followed. Data were fitted to determine the growth kinetics parameters.

Effect on redox chain using 5-cyano-2,3-ditolyl tetrazolium chloride(CTC): ATCC27853 in mid log were incubated briefly with 3',6-diNn, stained with CTC and counter stained with Syto green24. They were visualized by epifluorescence.

RESULTS

Effect on bacterial length & width

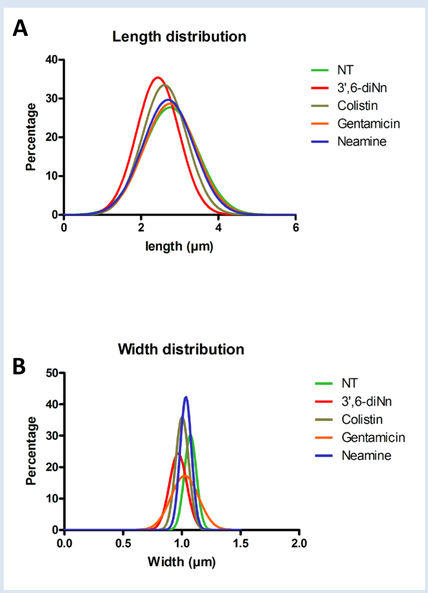

Fig 2. Overall distribution of the length (A) and the width (B) of non treated (NT) ATCC27853 or treated for 4 hours with 3',6-diNn and other antibiotics at their MICs: 3',6-diNn (4µg/mL), colistin (1µg/mL), and gentamicin (1µg/mL). Neamine, inactive against *Pseudomonas aeruginosa*, was used at the highest concentration (128 µg/mL).

➔Only 3',6-diNn decreased significantly the bacterial length

➔This effect on length is concentration dependent.

➔ 3',6-diNn affects slightly bacteria's width only at its MIC. At concentrations higher than 5 MIC, it induced bacteria's blebbing and membrane deformation(data not shown)

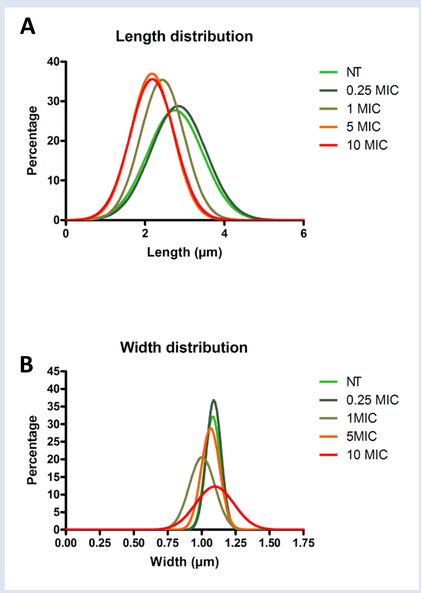

Fig 3. Overall distribution of the length (A) and the width (B) of non treated (NT) ATCC27853 or treated for 4 hours with 3',6-diNn at concentrations ranging from 0,25 to 10 times MIC.

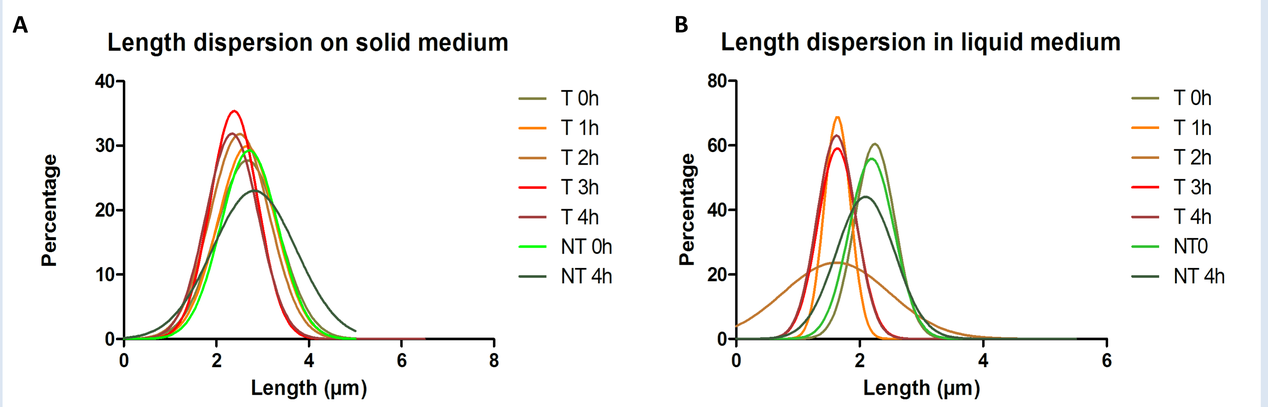

Fig 4. Overall distribution of the length of non treated (NT) ATCC27853) or treated (T) followed for 4 hours with 3',6-diNn at its MIC either on agarose pad (A) or in liquid medium (B). 0, 1, 2, 3, and 4 represent the duration of incubation in hour.

➔The effect of 3',6-diNn was more important in liquid medium than on solid medium where after 2 hours of incubation time a maximum decrease was observed.

Effect on bacterial morphology

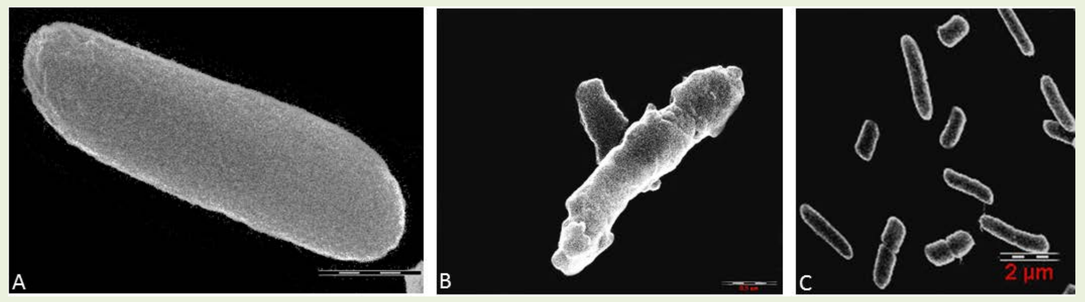

Fig 5. Scanning electron microscopy of non-treated ATCC27853 (A) or treated for 2 hours with 3',6-diNn at 5xMIC (B,C) in liquid medium. Scale bars in A, and B correspond to 5 µm.

➔3',6-diNn induced a loss of the membrane smoothness, membrane blebbing, and an heterogeneity in bacterial length. Doubling bacteria's were unable to establish scission

Effect on bacterial growth rate & redox chain

Growth kinetics parameters of ATCC27853 non treated (NT) or in the presence of different concentrations of 3',6-diNn

	3',6-diNn					NT
	0.1 MIC	0.25 MIC	0.5 MIC	0.75 MIC	1 MIC	
Growth rate µx (min^{-1})	0.0105	0.0103*	0.01**	0.0095***	0.00535***	0.012
Standard deviation	0.0059	0.0059	0.0057	0.0006	0.00027	0.0065
Doubling time g (min)	66	67	69	73	133	58
% Growth rate reduction	12.5	14.17	16.67	20.83	57.02	

Growth rate values were compared using one way ANOVA test and Bonferroni's post test. Data labelled with * represent significant difference compared to NT

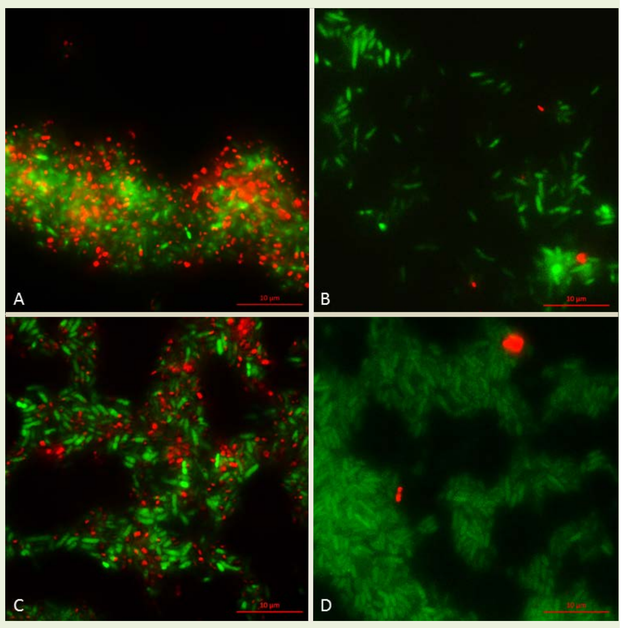

Fig 6. CTC stained non treated ATCC27853(A) , treated with 20% ethanol (B, negative control), and with 3',6-diNn at its MIC (C) or 5x MIC (D).

➔3',6-diNn inhibited the growth kinetics, and the redox chain in a concentration depending manner

CONCLUSION

3',6-diNn has a major effect on bacterial shape and morphology. It also seems to affect bacterial membrane proteins leading to a growth rate reduction at sub inhibitory concentrations, and an inhibition of the bacterial redox chain.

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ACKNOWLEDGEMENT

We thank Dr. Géraldine Laloux for her support in initiating time lapse studies. This work was financed by the F.R.S.-FNRS and the Université catholique de Louvain.

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