Polish Journal of Veterinary Sciences Vol. 25, No. 3 (2022), 479–481

DOI 10.24425/pjvs.2022.142034

Short communication

Effect of alkaloid berberine on the susceptibility of nontuberculous mycobacteria to antibiotics

K. Puk, L. Guz

Department of Biology and Fish Diseases, Faculty of Veterinary Medicine, University of Life Sciences, Akademicka 12, 20-033 Lublin, Poland

Abstract

Nontuberculous mycobacteria (NTM) have recently emerged as important bacterial pathogens of both animals and humans. In this study, we aimed to evaluate the effect of a combination of ten antibiotics with an inhibitor of efflux pumps (EPI), i.e. berberine (BER), against 6 strains of NTM. Our results showed that the BER potentiated the anti-mycobacterial activities of the antibiotics. Overall, our findings show the importance of BER in increasing the efficacy of antibiotics in NTM.

Key words: berberine, antibiotics, mycobacterium, NTM, minimum inhibitory concentration

Introduction

The *Mycobacterium* genus includes mycobacteria causing tuberculosis, mycobacteria causing leprosy, and non-tuberculous mycobacteria (NTM), i.e. a widely diverse group of species ranging from saprophytes to pathogens of humans and animals. NTM infections are hard to treat due to the relative resistance to currently available drugs and the difficulty in tolerating prolonged treatment. Drug resistance in mycobacteria is conferred by their highly lipophilic cell wall and the various mechanisms that control the cell wall content, a low number of porins, a broad range of efflux pumps, active biotransformation by cytosolic enzymes and inducible resistance mechanisms (Van Ingen et al. 2012). Efflux systems have an essential role in NTM resistance to a variety of antibiotics by extrusion of these drugs. The upregulation of the activity of efflux pumps, induced upon antibiotic exposure, can significantly decrease the intracellular concentration of antibiotics, reducing their clinical efficacy (Menichini et al. 2020). Reversal of the function of drug efflux pumps is a promising approach to improve the efficacy of anti-mycobacterial drugs.

Berberine is an alkaloid isolated from many plant species, such as *Coptis chinensis* (Coptis, goldenthread), *Hydrastis canadensis* (goldenseal), and *Berberis vulgaris* (barberry). For centuries, berberine has been used in the traditional Chinese medicine system for the treatment of various illnesses. Berberine

Correspondence to: L. Guz, e-mail: leszek.guz@up.lublin.pl

Table 1. Effect of berberine (BER) on antibiotic susceptibility of the rapidly growing mycobacteria strains.

Fold decrease in antibiotic minimum inhibitory concentration (MIC) in presence of different concentrations of BER (μg ml $^{-1}$)	*ANT	*BER	Strain							
			MF	MP	M11	M57	M17	M22	M88	M78
	STR	64	**4**	**8**	**256**	**2048**	**2048**	**512**	**2048**	**2048**
		32	2	**4**	1	**2048**	**1024**	**512**	**1024**	**1024**
		16	1	2	1	**8**	**16**	1	2	2
	KN	64	2	**32**	**1024**	**512**	**512**	**128**	**512**	**256**
		32	1	**16**	1	**256**	**512**	**128**	**1128**	**256**
		16	1	**4**	1	2	**512**	**4**	**4**	2
	AM	64	2	**8**	**128**	**128**	**64**	**32**	**64**	**128**
		32	1	**4**	1	**64**	**64**	**32**	**32**	**64**
		16	1	2	1	2	2	1	1	2
	GEN	64	1	2	**256**	**64**	**512**	**1024**	**512**	**512**
		32	1	2	1	**64**	**512**	**1024**	**512**	**512**
		16	1	1	1	**4**	1	1	2	2
	TOB	64	1	**8**	**64**	**128**	**512**	**16**	**256**	**128**
		32	1	**8**	2	**128**	**256**	**16**	**256**	**128**
		16	1	2	1	1	2	2	2	2
	CLR	64	1	2	**256**	**128**	**2048**	**1024**	**1024**	**32**
		32	1	2	**4**	**128**	**128**	**256**	**256**	**8**
		16	1	1	2	**4**	**16**	2	16	2
	CIP	64	1	2	**8192**	**64**	**2048**	**32**	**4096**	**32**
		32	1	2	2	**64**	**2048**	**32**	**4096**	**32**
		16	1	2	1	1	1	1	1	1
	DOX	64	2	2	**8192**	**8192**	**4096**	**256**	**2048**	**4096**
		32	2	2	2	**8192**	**4096**	**256**	**2048**	**512**
		16	1	2	1	2	16	2	2	1
	RFP	64	1	1	**16384**	**4096**	**4096**	**4096**	**1024**	**4096**
		32	1	1	1	**4096**	**4096**	**4096**	**1024**	**4096**
		16	1	1	1	**8**	**16**	**32**	**4**	**8**
	SMZ	64	**4**	**8**	**4096**	**16384**	**1024**	**8192**	**1024**	**4096**
		32	2	**8**	1	**16384**	**1024**	**8192**	**1024**	**4096**
		16	1	1	1	1	1	1	1	1

MF – *M. fortuitum* ATTC 6841; MP – *M. peregrinum* ATTC700686; *BER - concentrations of BER (μg ml^{-1}); * ANT – antibiotic,
[a] Values in boldface indicate a significant synergistic effect between antibiotic and the BER

and its derivatives exhibit various pharmacological activities, such as anti-cancer, anti-microbial, anti--inflammatory, and anti-diabetes effects (Gaba et al. 2021). Berberine has been confirmed to be active against the multi-drug resistant strain of *Mycobacterium tuberculosis* (Gentry et al. 1998). The new derivatives of dihydropseudoberberine and dihydropropalmatine with various substituents at position 8 were synthesized and evaluated for their antimycobacterial activities against *M. tuberculosis* (Wang et al. 2012). Berberine is also a well-known inhibitor of efflux pumps (EPI). Synergistic and additive effects of berberine with antibiotics or antifungal drugs against pathogenic microbes have been observed (Zhou et al. 2016). However, the combined effects of berberine and antibiotics against rapidly growing mucobacteria (RGM) isolates are rarely investigated in mechanistic studies. In the present study, the interactions between berberine and ten antibiotics were examined *in vitro* on six NTM isolated from ornamental fish.

Materials and Methods

The study involved 6 NTM strains originally isolated from diseased ornamental fish between January 2015 and December 2016 in the Department of Biology and Fish Diseases, Faculty of Veterinary Medicine,

University of Life Sciences in Lublin, Poland. The following atypical mycobacteria were studied: *M. abscessus* (M11), *M. chelonae* (M57), *M. fortuitum* (M17, M22, M88), and *M. peregrinum* (M78). The mycobacterial strains were identified on the basis of molecular characteristics as described previously (Puk and Guz 2020). *M. fortuitum* ATTC 6841 and *M. peregrinum* ATTC 700686 were used as reference strains.

Lyophilisates of eight antimicrobial agents, i.e. streptomycin (STR), amikacin (AMK), gentamycin (GEN), ciprofloxacin (CIP), clarithromycin (CLR), doxycycline (DOX), rifampicin (RMP), sulfamethoxazole (SMX), and tobramycin (TOB), were purchased from the Sigma-Aldrich Company (St Louis, MO, USA), while kanamycin (KAN) was purchased from A&A Biotechnology (Gdynia, Poland). Cation-adjusted Mueller-Hinton broth (CA-MHB) and albumin, dextrose, and catalase (ADC) supplement were supplied by Difco (Detroit, MI). Resazurin and berberine (BER) were ordered from Sigma-Aldrich (St. Louis, MO, USA).

The minimum inhibitory concentration (MIC) values of the antibiotics in the presence and absence of BER for each isolate were determined with the use of the resazurin microtiter assay (REMA) as previously described by Guz and Puk (2022). The final concentrations of BER were 64 μg ml $^{-1}$, 32 μg ml $^{-1}$, and 16 μg ml $^{-1}$. A growth control containing BER without antibiotics and a sterility control without inoculum were included in each plate. A fourfold or higher reduction in MIC levels was considered as an indication of a significant synergistic effect between the antibiotic and BER.

Results and Discussion

Given the importance of drug resistance as well as the increasing incidence of NTM infections, novel therapeutic strategies against NTM are needed. It has recently been reported that EPIs may help to increase the efficacy of antimycobacterial drugs (Song et al. 2016, Menichini et al. 2020). The ability of EPIs to counteract the efflux-mediated resistance to various classes of antibiotics is highly important. Thus, combination therapy based on the association of antibiotics and EPIs seems a promising approach to treat NTM infections. In our study, we aimed to determine whether the combination of antibiotics and EPI (BER) act synergistically against a panel of NTM isolates. We have shown for the first time that BER has moderate activity against NTM alone (MIC = 128 μg ml^{-1} – 512 μg ml^{-1}, data not shown) but effectively reduces the MIC of antibiotics in isolates of NTM isolated from fish (M11, M57, M17, M22, M88, M78). BER applied at the dose of 64 μg ml^{-1} decreased the MIC of all the tested antibiotics against all the NTM strains isolated from fish, always with at least a fourfold reduction of the MIC value. The effects of BER on MICs for the ten antibiotics against the NTM strains are summarised in Table 1.

References

Gaba S, Saini A, Singh G, Monga V (**2021**) An insight into the medicinal attributes of berberine derivatives: a review. Bioorg Med Chem 38: 116143.

Gentry EJ, Jampani HB, Keshavarz-Shokri A, Morton MD, Velde DV, Telikepalli H, Mitscher LA, Shawar R, Humble D, Baker W (**1998**) Antitubercular natural products: berberine from the roots of commercial *hydrastis canadensis* powder. Isolation of inactive 8-oxotetrahydrothalifendine, canadine, beta- hydrastine, and two new quinic acid esters, hycandinic acid esters-1 and -2. J Nat Prod 61: 1187-1193.

Guz L, Puk K (**2022**) Antibiotic susceptibility of mycobacteria isolated from ornamental fish. J Vet Res 66: 69-76.

Menichini M, Lari N, Rindi L (**2020**) Effect of efflux pump inhibitors on the susceptibility of *Mycobacterium avium* complex to clarithromycin. J Antibiot 73: 128-132.

Puk K, Guz L (**2020**) Occurrence of *Mycobacterium* spp. in ornamental fish. Ann Agric Environ Med 27: 535-539.

Song L, Wu X (**2016**) Development of efflux pump inhibitors in antituberculosis therapy. Int J Antimicrob Agents 47: 421-429.

Van Ingen J, Boeree MJ, Van Soolingen D, Mouton JW (**2012**) Resistance mechanisms and drug susceptibility testing of nontuberculous mycobacteria. Drug Resist Updat 15: 149-161.

Wang Y, Fu H, Li Y, Jiang J, Song D (**2012**) Synthesis and biological evaluation of 8-substituted berberine derivatives as novel anti-mycobacterial agents. Acta Pharm Sin B 2: 581-587.

Zhou XY, Ye XG, He LT, Zhang SR, Wang RL, Zhou J, He ZS (**2016**) *In vitro* characterization and inhibition of the interaction between ciprofloxacin and berberine against multidrug-resistant *Klebsiella pneumoniae*. J Antibiot 69: 741-746.