UNIVERSITI PUTRA MALAYSIA

PHARMACODYNAMICS OF COLISTIN AGAINST Acinetobacter baumannii

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PHARMACODYNAMICS OF COLISTIN AGAINST *Acinetobacter baumannii*

By

RASHIZAL SAZLI BIN MOHD RASIDIN

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirements for the Degree of Master of Science

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DEDICATIONS

All praises and thanks to Allah and peace be upon Prophet Muhammad saw. My appreciation goes to my beloved wife, Zahrina binti Abdul Kadir who has always been by my side through thick and thin. My special tribute goes to my dear parents, Zaleha binti Saleh and Mohd Rasidin bin Abdul Rahman who raised me up to what I am today. All my love to other family members who have always been there for me.

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Master Science

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April 2017

Chairman : Associate Professor Syafinaz Amin Nordin, MBChB, MPath
Faculty : Medicine and Health Sciences

Nosocomial infection caused by *Acinetobacter baumannii* is endemic in hospital settings especially among immunocompromised patients. Treatment strategy is limited due to rapid emergence of extensively drug resistance and lack of novel agent in the antibiotic development pipeline. Colistin has been the last line therapy with good *in vitro* activity. However, updates in the pharmacological aspects are required to support dosing optimisation. The current study descriptively explained high proportion (89%) of extensively drug resistance among 72 multidrug resistance strains of *A. baumannii* collected from Hospital Serdang. The clinical strains were mainly isolated from elderly patients (median age 61 [IQR 25]) admitted to medical ward (35%) while common isolation site was respiratory sample (60%). Antibiogram profile showed 100% *in vitro* susceptibility of the strains to colistin with 54% of isolates exhibit MIC 1.0 µg/mL. The *in vitro* static time-kill kinetic and post-antibiotic effect experiments were conducted against two clinical isolates (colistin MIC 1.0 µg/mL and 0.75 µg/mL) as well as one reference isolate ATCC 19606 (colistin MIC 1.0 µg/mL). The development of colistin resistance was also examined after colistin exposure in time-kill study. The post-antibiotic effect was determined at colistin concentration based on the achievable plasma level after parenteral administration. Concentration-dependent killing activity was observed in time-kill experiment against all studied isolates. However, delayed bactericidal activity indicates tolerance or persistence of the strains against colistin. Development of colistin resistance after colistin exposure was not detected except for the known colistin heteroresistance ATCC 19606 strain. Meanwhile, the post-antibiotic effect was significant in a concentration-dependent manner for all studied isolates. Dosing suggestion based on observations from this study include administration of a supplemental dose 3 MIU at 12 hours after loading dose, administration of maintenance dose 9 MIU in two divided doses and the application of extended interval in dosing adjustment for patients with renal impairment. However, information of the current study is applicable for non-colistin-heteroresistance *A. baumannii* with colistin MIC ≤ 1 µg/mL. As for heteroresistance and strain with

colistin MIC > 1.0 μg/mL, combination therapy would be the more appropriate treatment strategy.

Keywords: colistin, *Acinetobacter baumannii*, pharmacodynamics

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia
sebagai memenuhi keperluan untuk ijazah Sarjana Sains

FARMAKODINAMIK KOLISTIN TERHADAP *Acinetobacter baumannii*

Oleh

RASHIZAL SAZLI BIN MOHD RASIDIN

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Jangkitan nosokomial oleh *Acinetobacter baumannii* adalah endemik di hospital terutamanya di kalangan pesakit yang lemah sistem imun. Strategi rawatan pula terhad dengan kemunculan bakteria rintang antibiotik dan kekurangan antibiotik baru. Kolistin adalah antara pilihan yang ada di mana antibiotik ini mempunyai tindakan *in vitro* yang baik. Walaubagaimanapun, maklumat terkini farmakologi diperlukan untuk penyelarasan dos klinikal. Dalam kajian ini, nisbah isolat *A. baumannii* yang dikumpulkan dari Hospital Serdang dan bersifat rintang antibiotic menyeluruh adalah tinggi (89%) di kalangan 72 isolat bersifat rintang antibiotic perlbagai, secara deskriptif. Kebanyakan isolat dikumpulkan dari pesakit berusia lanjut (median umur 61 [JIK 25]) yang dimasukkan ke Wad Perubatan (35%) manakala sampel dari sistem respirasi (60%) adalah yang paling tinggi kekerapan. Profil antibiogram menunjukkan 100% sensitiviti *in vitro* terhadap kolistin di mana 54% isolat menunjukkan MIC 1.0 µg/mL. Ujikaji kinetik masa-pembunuhan statik dan kesan pasca-antibiotik secara *in vitro* telah dilakukan terhadap dua isolat klinikal (MIC kolistin 1.0 µg/mL dan 0.75 µg/mL) dan satu isolat rujukan ATCC 19606 (MIC kolistin 1.0 µg/mL). Kerintangan terhadap antibiotik selepas pendedahan kepada kolistin dalam ujikaji masa-pembunuhan juga dianalisa. Ujikaji kesan pasca-antibiotik dilakukan dengan penggunaan kepekatan kolistin berdasarkan paras kolistin mampu capai dalam darah pesakit selepas pemberian antibiotik secara parenteral. Pembunuhan berkadar dengan kepekatan telah diperhatikan dalam ujikaji masa-pembunuhan terhadap setiap sampel bakteria yang diuji. Namun begitu, aktiviti pembunuhan yang perlahan menunjukkan toleransi atau ketahanan bakteria terhadap kolistin. Kerintangan terhadap kolistin selepas pendedahan tidak dikesan kecuali pada isolat ATCC 19606 yang telah diketahui bersifat heterorintang. Sementara itu, kesan pasca-antibiotik juga signifikan dan berkadar dengan kepekatan kolistin bagi setiap isolat yang diuji. Cadangan dos hasil dari pemerhatian-pemerhatian dalam kajian ini termasuklah pemberian dos tambahan kolistin 3 MIU pada 12 jam selepas dos muatan, pemberian dos pengekalan 4.5 MIU dua kali sehari dan perlaksanaan strategi menjarakkan kekerapan dos bagi pesakit yang mengalami kerosakan fungsi buah pinggang. Walaubagaimanapun, maklumat

kajian ini adalah bersesuaian dengan *A. baumannii* yang tidak bersifat heterorintang terhadap kolistin ataupun strain yang nilai MIC kolistin ≤ 1 μg/mL. Bagi isolat yang heterorintang terhadap kolistin serta mempunyai nilai MIC kolistin > 1.0 μg/mL, terapi gabungan antibiotik adalah strategi yang lebih sesuai.

Kata kunci: kolistin, *Acinetobacter baumannii*, farmakodinamik

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I certify that a Thesis Examination Committee has met on 11 April 2017 to conduct the final examination of Rashizal Sazli bin Mohd Rasidin on his thesis entitled "Pharmacodynamics of Colistin Against *Acinetobacter baumannii*" in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The Committee recommends that the student be awarded the Master of Science.

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LIST OF ABBREVIATIONS

ATCC	American Type Control Culture
AUC_{24}	24 hours area under the curve
BD	*bis die* (twice a day)
CBA	Colistin Base Activity
CLSI	Clinical Laboratory Standard Institute
C_{max}	Peak concentration
CMS	Colistimethate sodium
CRE	Carbapenem Resistant Enterobacteriaceae
CRRT	Continuous renal replacement therapy
DDD	Daily Define Dose
eGFR	Estimated glomerular filtration rate (creatinine clearance)
FDA	Food and Drug Administration
HDU	High Dependency Unit
ICU	Intensive Care Unit
IQR	Interquartile range
IV	Intravenous
LPS	Lipopolysaccharide
MBLs	Metallobeta-lactamases
MDR	Multi Drug Resistance
MIC	Minimal Inhibitory Concentration
MPC	Mutant Prevention Concentration
MSW	Mutant Selection Window
NSAR	National Surveillance of Antibiotic Resistance
OD	*omne die* (once daily)
OXAs	Oxacillinases
PAE	Post Antibiotic Effect
PAPs	Population Analysis Profiles
PD	Pharmacodynamics
PK	Pharmacokinetic
QID	*quater in die* (four times a day)
T	Time
TDS	*ter die sumendum* (three times a day)
TSI	Triple Sugar Ion
VBNC	Viable but Non-Culturable
XDR	Extensive Drug Resistance

CHAPTER 1

INTRODUCTION

1.1 Background and justification of study

Nosocomial infection due to *A. baumannii* is endemic in hospital settings and commonly affects immunocompromised patients. Alarmingly, fast emergence of extensive drug resistance (XDR) strain has limited the treatment option (Peleg *et al*., 2012; Peleg, Seifert, & Paterson, 2008). Situation is worsening with non-progressive development of novel agents targeting gram negatives by pharmaceutical industry (Bassetti, Merelli, Temperoni, & Astilean, 2013; Boucher *et al*., 2013; Wright, 2012). This has prompted resurgence usage of the older antibiotic group polymyxins, particularly colistin as these antibiotics were the only agents that retain *in vitro* susceptibility against XDR gram negatives (Gales, Jones, & Sader, 2011; Landman, Georgescu, Martin, & Quale, 2008; Li, Nation, *et al*., 2006).

Excellent *in vitro* activity however, does not ensure clinical efficacy of the antibiotic. Dosing regimen of colistin recommended by the manufacturer was designed based on outdated pharmacokinetics data. These data were obtained from microbiologic assay rather than the latest chromatography and mass spectrometry method, hence prone to false higher result caused by ex-vivo hydrolysis of colistimethate to colistin during incubation, consequently leads to lower dosing recommendation (Nation *et al*., 2015). The under dosing is responsible for poor outcomes among patients (S.-K. Lim *et al*., 2011; Livermore *et al*., 2010) as well as emergence of bacterial resistance (Markou *et al*., 2008; Olofsson & Cars, 2007). Therefore, development of dosing strategy with maximum efficacy and minimum resistance development is warranted to retain the therapeutic value of colistin as salvage therapy for XDR gram negatives (L. M. Lim *et al*., 2010; Wertheim *et al*., 2013).

Theoretically, antibiotic pharmacodynamics involves the study of antibiotic activity against targeted bacteria. The integration between pharmacokinetics and pharmacodynamics (PK/PD) provides information which is required for dosing optimisation. This is important as to design standard regimen with good efficacy in a particular group of patient. The present pharmacodynamics study is aimed to evaluate the *in vitro* activity of colistin against *A. baumannii* by static time-kill kinetics and post antibiotic effect experiments. Static time-kill kinetics determines the *in vitro* bacterial killing activity at different (multiples MIC) but constant drug concentration based on the MIC of studied bacteria. Meanwhile, post antibiotic effect (PAE) reflects the antibiotic ability to retain inhibition or killing effect after concentration had decreased below MIC. Longer PAE of the antibiotic would therefore allow prolong dosing interval without reduced efficacy giving the advantage of minimizing adverse effects (Burgess, 1999).

1.2 Problem statements

Colistin is an old antibiotic entering clinical practice in 1960s. However, this antibiotic was abandoned by medical practitioner during 1970s because of its nephrotoxicity side effect in addition to the availability of potent and less toxic antibiotics. As such, research and update on the pharmacological aspects of colistin are lacking. Unfortunately, increase prevalence of multi drug resistance (MDR) gram negatives in the following decades has led to the resurgence use of this antibiotic (Balaji, Jeremiah, & Baliga, 2011; Falagas, Kasiakou, & Saravolatz, 2005).

The National Surveillance of Antibiotic Resistance (NSAR) reported that around 55% to 58% of *A. baumannii* isolated from Malaysian hospitals were resistance to carbapenem in two consecutive years 2013 and 2014 (MOH, 2014b). Meanwhile, the National Antibiotic Guideline stated that colistin usage by mean DDD/1000 patient days in Malaysian hospitals is higher than polymyxin B and the usage has been gradually increased from 2011 to 2013 (MOH, 2014a). These reports indicate the revival of colistin in Malaysian hospitals parallel to the increase incidence of XDR *A. baumannii.* Therefore, update on the pharmacological properties of colistin is urgently warranted among local population to support dosing standardisation.

Several international studies have been conducted on the population pharmacokinetics-pharmacodynamics (PK/PD) of colistin, and the application of loading dose is recommended (Kift, Maartens, & Bamford, 2014; Mohamed, Cars, & Friberg, 2014; Mohamed *et al*., 2012). The Malaysian National antibiotic guideline has recently adhered to the recommendation for treatment against MDR *A. baumannii*. However, the administration of maintenance dose in either two or three divided doses is left to clinician discretion. Moreover, detail on renal dose adjustment is not outlined although the adjustment is stated as required (MOH, 2014a). The vagueness in dosing regimen is due to lack of local data on colistin safety and efficacy in relation to patient infected with *A. baumannii*. Recent studies evaluating colistin dosing regimen based on PK/PD modelling was conducted in Sweden and the targeted organism was *Pseudomonas aeruginosa* (Mohamed *et al*., 2014; Mohamed *et al*., 2012). Therefore, the Malaysian population PK/PD research of colistin among critically ill patients with MDR gram negative bacterial infections is currently underway. And, the present study aims to provide pharmacodynamics data of colistin against *A. baumannii* from local isolation. The information obtained from this study would be beneficial for development of dosing strategy with enhanced efficacy and minimal resistance development related to infection caused by *A. baumannii*.

1.3 Research questions

The current study main goal is to answer research questions in relation to colistin pharmacodynamics activity against *A. baumannii*.

1. What is the killing profile of colistin against Malaysian strain of *A. baumannii*?
2. Does colistin effectively eradicate the strain in an *in vitro* pharmacodynamics experiment and what is the concentration associated with efficient killing activity?
3. Does *A. baumannii* develop resistance against colistin after exposure to the antibiotic in time-kill experiment?
4. What is the *in vitro* post antibiotic effect of colistin at suboptimal and optimal concentration against *A. baumannii*?

1.4 General objectives

The present study aims to determine the time-kill and post antibiotic effect of colistin at different static concentration in *in vitro A. baumannii* system. Resistance development after exposure to the antibiotic is also observed in the time-kill experiment.

1.5 Specific objectives

In particular, the following are the specific objectives of this study:

1. To examine *in vitro* static time killing profile of colistin at multiple MIC and the concentration of colistin with effective killing activity against ATCC strain and randomly selected clinical isolates.
2. To observe resistance development by the studied isolates after exposure to sub-inhibitory and inhibitory concentration of colistin throughout 24 hours period of time-kill experiment.
3. To determine colistin post antibiotic effect against the studied isolates at sub-optimal and optimal concentration of the antibiotic.

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