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Formulation of carbapenems loaded gold nanoparticles to combat multi-antibiotic bacterial resistance: *In vitro* antibacterial study

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ABSTRACT

Despite the fact that carbapenems (powerful β-lactams antibiotics) were able to fight serious infectious diseases, nowadays the spread of carbapenems-resistant bacteria is considered the main challenge in antibacterial therapy. In this study, we focused on evaluating the surface conjugation of carbapenems (imipenem and meropenem) with gold nanoparticles as a delivering strategy to specifically and safely maximize their therapeutic efficacy while destroying the developing resistance of the pathogens. Different particle size formulae (35, 70 and 200 nm) were prepared by citrate reduction method. The prepared nanoparticles were functionalized with imipenem (Ipm) or meropenem (Mem) and physico-chemically characterized for loading efficiency, particle size, morphology, and *in-vitro* release. The antibacterial efficacy was also evaluated against carbapenems resistant Gram-negative bacteria isolated from infected human, through measuring the minimum inhibitory concentration and antibiotic kill test. All the obtained gold nanoparticles showed a distinct nano-size with loading efficiency up to 72% and 74% for Ipm and Mem, respectively. The conjugation and physico-chemical stability of the formulated carbapenems were confirmed by FTIR and X-RPD. Diffusion driven release behavior was observed for both Ipm and Mem from all of the loaded gold nanoparticles. For both Ipm and Mem, formula with 35 nm diameter showed the most significant enhancement in antibacterial activity against all the selected isolates including *Klebsiella pneumoniae*, *Proteus mirabilis* and *Acinteobacter baumanii*. Ipm loaded Gold nanoparticles demonstrated decrease in the MIC of Ipm down to four folds, whereas, Mem loaded gold nanoparticles showed decrease in the MIC of Mem down to three folds on the tested bacterial isolates. Based on these results, the formulation of carbapenems-loaded gold nanoparticles demonstrated to be a promising nano-size delivery vehicle for improving the therapeutic activity and destroying the bacterial resistance for carbapenems.

1. Introduction

Carbapenems are the broadest spectrum class of β-lactam antibiotics and clinically showed the greatest potency against multi-antibiotic resistant Gram-positive/negative aerobic as well as anaerobic bacteria (Sotgiu et al., 2016). They are mainly indicated for systemic administration via multiple intravenous injection regimens for the empirical treatment of hospitalized patients with life-threatening bacterial diseases such as complicated urinary tract infections (Drulis-Kawa et al., 2006; Sun et al., 2014), blood stream infections (Sotgiu et al., 2016; Yang et al., 2016), as well as nosocomial infections of unidentified origin (Baroud et al., 2013; Drulis-Kawa et al., 2006; Sun et al., 2014; Yang et al., 2016). Carbapenems therapy has always been considered the drug for last resort, however, the recent outgrowth of resistant bacteria started to diminish this clinical relevance (Baroud et al., 2013). This bacterial resistance is due to the occurrence of various avenues, including the irreversible adhesion of bacteria through extracellular polymeric film 'biofilm' formation with a high growth rate and more resistance to conventional carbapenems therapy (Baroud et al., 2013; Holmes et al., 2016). Also, developed resistance may be due to the bacterial production of the carbapenems' degradation enzyme carbapenemases (Holmes

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et al., 2016), as well as, the active efflux or the anticipated membrane impermeability as a result of bacterial membrane mutations or alterations in membrane channels (Baroud et al., 2013; Brooks and Brooks, 2014; Holmes et al., 2016). Consequently, this developing bacterial resistance enforces a main research concern on the clinical future of carbapenems all over the world and regrettably, the discovery of new non-resistant derivatives/generations are extremely beaten by the pharmaceutical production of existing members (Brooks and Brooks, 2014).

In consequence with this bacterial challenge, different research strategies have been explored to address and smash that developed resistance to prolong the effective life of carbapenems (Tang et al., 2016). The first strategy is the use of combination therapy between two or more antibiotics of the same/different mechanism of action, in the hope that they will act synergistically (Miliani et al., 2011; Sun et al., 2014; Yang et al., 2016). Combination therapy would ensure delay in carbapenem resistance if utilized rationally, nonetheless, the biggest challenges in employing combination therapy are the reported adverse effects due to potential drug–drug interactions and patient compliance due to multiple routes of administration or multiple dosing that may be required. The second strategy is the protection of carbapenem itself from the perceived *in vivo* hydrolysis, which is anticipated to be adequate cause of diminishing its bactericidal action, by incorporating into the structures of complexing agent such as cyclodextrins (Paczkowska et al., 2016). Complex formation would increase the chemical stability; however, such studies are required to clinically examine its ability to fit in the pharmaceutical production pipeline. The third strategy focused on the development of intelligent drug delivery systems capable of achieving sustained

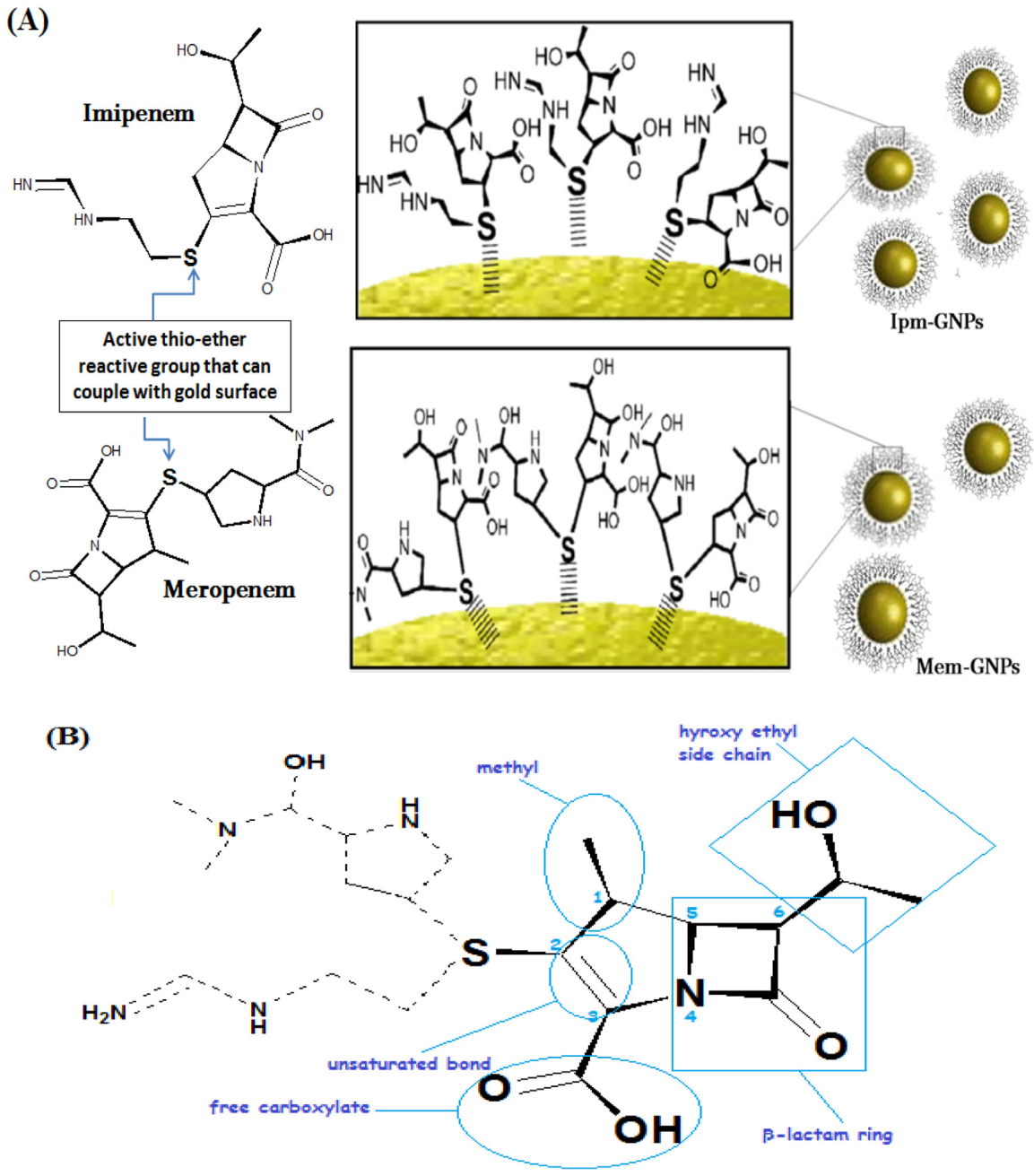

Fig. 1. **(A)** Schematic representation for the conjugation of imipenem and meropenem with citrate-capped gold nanoparticles **(B)** Structural analysis of the carbapenems mapped to pharmacophore model.