Monitoring New Delhi Metallo-β-lactamase activity in live bacterial cells using NMR spectroscopy: new methods for antibacterials drug discovery

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Introduction

In the perpetual 'arms race' between pathogenic bacteria and their human and animal hosts, the microbes are once again gaining the upper hand, after close to three quarters of a century in retreat. A combination of factors that includes over-prescription of marketed antibiotics and a dearth of novel agents being brought into clinical use has contributed to the emergence of resistant strains, against which we currently have no effective therapies. Antibacterial drug discovery has been challenging, not least because inhibitors effective against isolated bacterial enzymes are often ineffective against whole bacterial cells; hence, new methods that permit measurement of enzyme activity in living bacteria are required. The β-lactam antibiotics that include the penicillins and cephalosporins have been among our most effective weapons against bacterial infection. Yet, their efficacy is waning as a result of the evolution of resistance mechanisms in the form of β-lactamases that disarm β-lactams through opening of the cyclic amide ring. New Delhi Metallo-β-lactamase (NDM-1) is a particularly alarming and rapidly disseminating resistance gene capable of cleaving even carbapenems, until recently considered the β-lactams of last resort because of their resistance to most β-lactamases. Development of new drug candidates that can inhibit the activity of these zinc-dependent β-lactamases, hence restoring the effectiveness of carbapenem antibiotics, is urgently needed.

Results

In work performed with AstraZeneca, we have demonstrated that NMR spectroscopy can be used to monitor the effect of known inhibitors of NDM-1 on its β-lactamase activity in live bacterial cells. To demonstrate our ability to measure the enzymatic activity of NDM-1 in real time using

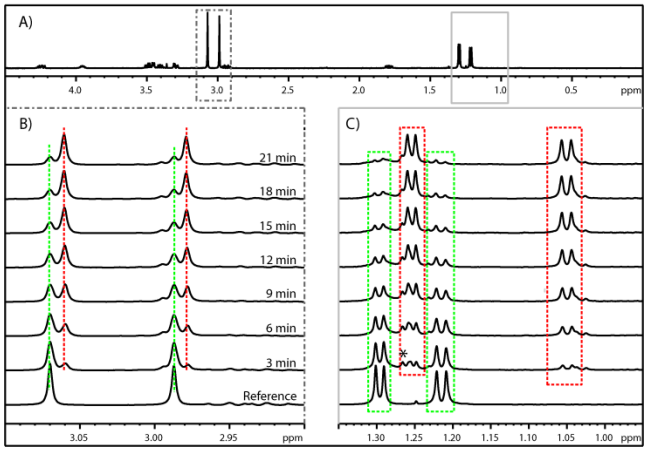

Figure 1: ^{1}H NMR spectra (600 MHz) of meropenem hydrolysis in the presence of NDM-1 *E. coli* cells. A) The full ^{1}H NMR spectrum of 100 μM meropenem in 50 mM sodium phosphate at pH 7.0. The hydrolysis of meropenem incubated with NDM-1 *E. coli* cells (OD_{600} = 2.5) at different time points was monitored via the ^{1}H NMR signals from B) the nitrogen-attached methyl groups and C) the carbon-attached ones. The green and red dotted lines/boxes represent the signals of substrate and product, respectively. The signals labelled with asterisks are from residual buffer contaminants.

NMR spectroscopy, we used a strain of *E. coli* that expresses the NDM-1 resistance gene. When we incubated the carbapenem antibiotic meropenem with NDM-1-expressing *E. coli* cells suspended in buffer in an NMR tube, we could clearly follow, in real time, the hydrolysis of the lactam ring using ^{1}H NMR (Figure 1). Controls showed that this hydrolysis was not seen with non-NDM-1-expressing *E. coli* cells, nor was it due to cell lysis and release of NDM-1 into the medium, since hydrolysis was not observed in supernatants following centrifugation.

We next went on to examine whether we could observe inhibition of NDM-1 activity in the bacterial cell suspension using ^{1}H NMR. There are very few documented inhibitors of Zn-dependent metallo-β-lactamases (hence the need for additional research in this area); however, although not suitable as an antibacterial drug, the marketed ACE inhibitor, L-captopril, has moderate inhibitory potency against a range of metallo-β-lactamases. Addition of captopril and another NDM-1 inhibitor, EDTA, to suspensions of NDM-1 *E. coli* cells in the presence of meropenem effectively inhibited the hydrolysis of the antibiotic in a dose-dependent fashion, as measured by the intensity of the ^{1}H NMR resonances monitored in Figure 1. We were able to calculate half-maximal inhibition (IC_{50}) values of 175.0 μM and 1.6 μM, respectively, for L-captopril and EDTA (Figure 2), which are similar to literature values reported using isolated enzyme preparations and conventional enzyme inhibition assays.

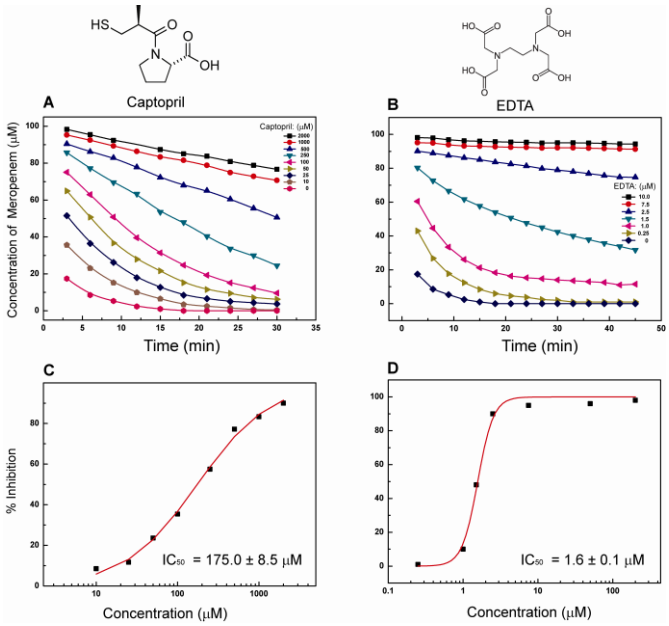

Figure 2: Inhibition of meropenem hydrolysis in the presence of NDM-1 *E. coli* cells by A) L-captopril and B) EDTA at various concentrations, and IC_{50} measurements for L-captopril C) and EDTA D). For each experiment, the NDM-1 *E. coli* cells (OD_{600} = 5.0) were first incubated with the inhibitor for 10 minutes before addition of 100 μM meropenem.

Our demonstration that ^{1}H NMR can be used to monitor enzymatic activity in whole bacterial cell suspensions may open up new possibilities in drug discovery by bridging the gap between activity measured *in vitro* using isolated enzymes, and the desired activity against living cells – a gap that is often hard to cross in conventional antibacterials discovery approaches. We have subsequently gone on to show that we can use our NMR-based assay to screen compound collections against *E. coli* cells carrying the NDM-1 gene – an approach we are calling target-based whole cell NMR screening (Ma et al., *Angew. Chem. Int. Ed.*, in press).

Publications

Ma, J., Mcleod, S., Maccormack, K., Sriram, S., Gao, N., Breeze, A. & Hu, J. (2014) Real-time monitoring of New Delhi metallo-β-lactamase activity in living bacterial cells by H^{1} NMR spectroscopy. *Angew. Chem. Int. Ed. Engl.* **53**: 2130-2133.

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Collaborators

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