Assessment of inhibitory potency of antibiotics by measurement of apparent T_2 in bacterial cultures

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Introduction: The emergence of resistance to antibacterials requires the search for new drugs. In the recent years, several *in vivo* studies applied magnetic resonance spectroscopy (MRS) methods in addition to routine magnetic resonance imaging (MRI) in the diagnosis of bacterial cerebral infections and in monitoring antibiotic treatment [1]. In *in vitro* experiments characteristic spectral patterns of cell wall constituents and bacterial metabolites permitted identification of different bacterial groups. Parallelization of this method, to allow for high throughput screening, is in principle possible with chemical shift imaging (CSI) [2]. However, CSI implies very long measurement times if high spatial resolutions are required for separation of a large number of samples. Prompted by the development in therapy of bacterial infections and the recent biological applications of MR, we have established a novel cytotoxicity assay on the species *Streptococcus vestibularis* by correlating cell growth with the chemical-exchange dependent T_2 relaxation [3], measured by MRI.

Experiments: MR images were obtained on a Bruker Avance 750 MHz wide bore spectrometer (Rheinstetten, Germany) equipped with a 1 T/m gradient system and a 20 mm birdcage coil. The determination of the transverse relaxation time T_2 was carried out using a multi spin echo (CPMG) sequence with the following parameters: matrix size, 64x64; field of view, 20x20 mm^2; TE, 10 ms; TR, 5 s. 64 echoes were acquired following one excitation.
NMR spectroscopy was performed on a 400 MHz Bruker Avance NMR spectrometer (Rheinstetten, Germany) with BBO probe equipped with z-gradient. 1D proton spectra were acquired using watergate W5 water suppression.
Bacterial samples in a concentration of 10^6 cells/ml were prepared in 5 mm NMR tubes. Seven of these rudiments were packed in a 20 mm tube and measured together simultaneously at a temperature of 30 °C during 12 h.

Results: Measured T_2, which is sensitive to the variation of medium the bacteria are suspended in, changed with bacterial growth. Bacterial metabolism, observed in ^{1}H spectra, changes the composition, the pH of the medium, and consequently the rate of chemical exchange and T_2 relaxation. ^{1}H NMR of bacterial cultures were correlated with the extracellular parameters T_2, OD_{600} (optical density at 600 nm) and pH. The time course of T_2, measured on samples in presence of different vancomycin concentrations showed a typical threshold behaviour and allowed for distinction between growing cultures and cultures where growth was suppressed. From three dilution series the minimum inhibitory concentration of vancomycin was calculated to be 0.33 µM.

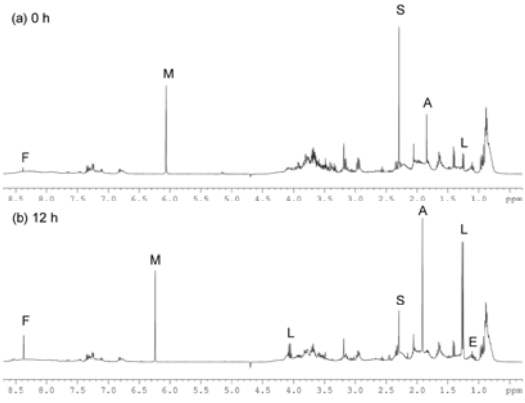

Figure 1: *^{1}H spectra of the cell suspension of S. vestibularis at (a) t=0 h and (b) t=12 h of the growth curve. (M = maleic acid, internal standard; L= lactate; S = succinate; A = acetate; F = formiate; E = ethanol)*

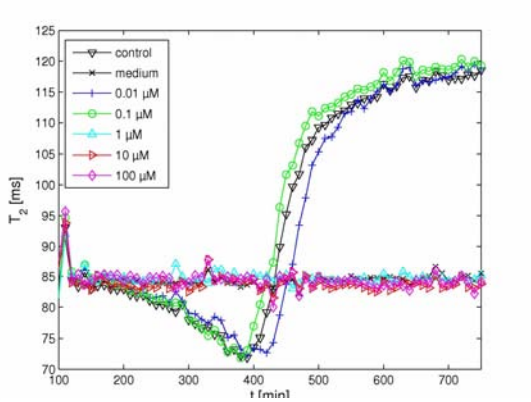

Figure 2: *T_2 growth curves of S. vestibularis at different concentrations of vancomycin*

Conclusion: We have shown that MRI is applicable to assess the antibacterial potency of drugs. T_2 measurements can be performed on a large number of cultures simultaneously and avoid the problems of resonance identification and extensive scan times required by CSI methods.

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References:

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