The antibiotic drug azithromycin acts as a motilin receptor agonist and facilitates cholinergic activity in human isolated stomach

John Broad, Cian McGuire, Daniel Sifrim, Gareth Sanger. Neurogastroenterology group, Blizard Institute, Barts & The London School of Medicine and Dentistry, Queen Mary University of London, London, UK.

The macrolide antibiotic azithromycin is licensed as a prophylactic treatment in lung transplant patients and for treatment of food poisoning. In addition, azithromycin can promote gastric emptying (Larson et al 2009) in a manner similar to erythromycin, a structurally-related antibiotic which promotes gastric motility by activating the motilin receptor. Here we show for the first time that azithromycin is also an agonist at the human motilin receptor and can facilitate cholinergically-mediated contractions of human antrum.

CHO-K1 cells expressing the human motilin receptor (Sanger et al 2009) and parental cells were seeded at 100,000 cells/well into 96 well plates in 100µl Fluo-4 Direct calcium assay buffer. 100µl Fluo-4 Direct calcium assay reagent containing 5µM probenecid was added after 10 minutes and incubated for 1 hour. Following triplicate addition of test compounds in PBS, fluorescence emission was measured at 516 nm. Human gastric antrum was obtained at surgery following informed consent. After removing the mucosa, strips were cut parallel to the circular muscle and suspended between ring electrodes in tissue baths for isometric recording (Kreb’s; 5% CO₂ in O₂; 37°C; 2g tension). Electrical field stimulation (EFS) was applied at 5Hz (0.5ms pulse width, 50V, 10s) every 1min, for sub-maximal responses. N = number of patients. All drugs were added non-cumulatively.

In the cells expressing the motilin receptor, azithromycin (0.1-100µM) caused dose dependent increases in fluorescence (Eₘₐₓ=122±22% response to 300nM motilin; pEC₅₀=5.4±0.23; n=3). No response was observed in the parental cells (n=3). In human antrum, EFS-evoked contractions were prevented by 1µM tetrodotoxin (n=3), attenuated by atropine 1µM (n=3) and facilitated by the nitric oxide synthase inhibitor L-NAME 0.3mM (by 11±7% n=14). Both azithromycin (0.1-100µM) and erythromycin (0.1-30µM) enhanced the magnitude of EFS (respectively, by 431±293% with 100µM, apparent pEC₅₀≈4.3, n=3-5, and by 161±97% with 30µM, apparent pEC₅₀=5.8±1.6, n=3-4) and increased baseline muscle tension only at the top concentrations (respectively, by 89±22% of the pre-drug contraction amplitudes to EFS, n=5, and by 23±13% EFS, n=4). In 4/5 strips (each from different patients) the response to 100µM azithromycin faded (t½=40±5 min) and the contraction amplitudes became irregular during fade in 2 strips. The response to 30µM erythromycin also faded (t½=32±4 min, n=4), but the contraction amplitudes remained regular during fade.

In the recombinant system azithromycin is a full agonist at the human motilin receptor. However, in the native human stomach there was a significant loss of efficacy (compared to the increase of 1041±592% EFS in response to 300nM motilin; Broad et al 2011). This highlights the importance of using human native tissue systems in motility research, and the potential clinical use of azithromycin as a gastric pro-kinetic.

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