P1688
Poster Session VI
PK/PD of antifungals and miscellaneous antibacterials

AZITHROMYCIN SUPPRESSES EFFECTOR FUNCTIONS OF CD4+ T-CELLS VIA INHIBITION OF MTOR SIGNALING

K. Schmetterer[1], F. Ratzinger[1], G. Hoermann[1], H. Haslacher[1], W. Poeppl[2], H. Burgmann[2]

[1]Department of Laboratory Medicine, Medical University of Vienna, Vienna, Austria ; [2]Department of Internal Medicine I, Medical University of Vienna, Vienna, Austria

Background

Azithromycin (AZM) is an antimicrobial agent with immunomodulatory properties, showing beneficial clinical effects in inflammatory pulmonary diseases including chronic obstructive pulmonary disorder, cystic fibrosis or bronchiolitis obliterans. Obviously, these effects also occur in patients without any need of immunomodulation. Consequently, characterization of the modulatory effects on immune cell subsets is of importance for its prudent use. The purpose of this study was to assess effects of AZM in therapeutically relevant concentrations on purified $CD4^+$ T-cell.

Material and Methods

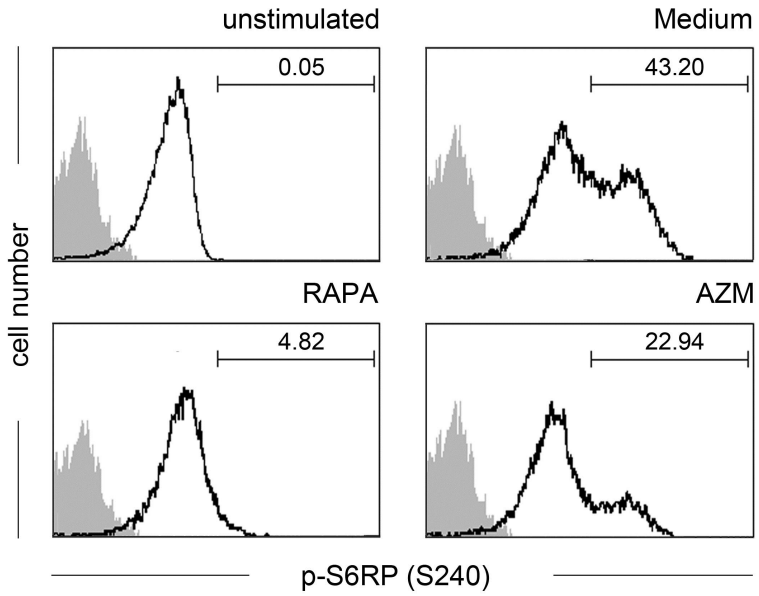
In the presence (0.6-40 mg/L) or absence of AZM, isolated $CD4^+$ T-cells from peripheral blood mononuclear cells of 14 healthy volunteers were stimulated with agonistic anti-CD3/anti-CD28 monoclonal antibodies. Cell proliferation was measured using thymidine incorporation assays and fluorescent tracers. Cytokine secretion of IL-2 (24 hours after stimulation) and IL-4, IL-10, IL-13, IL-17 and IFN-gamma (72 hours after stimulation) was assessed in corresponding supernatants using multiplex analysis. Cell viability was quantified by annexin V/propidium iodide staining. Intracellular signaling was evaluated using flow cytometry and immunoblotting to identify the mechanism of action.

Results

A dose dependent inhibition of the cell-proliferation rate, starting at 2.5 mg/L AZM was observed (normalized median: 0.74, IQR: 0.72-0.78, p=0.001), when compared to T-cells in antibiotic-free medium. Concomitantly, significant suppression of IL-2, IL-10, IL17 and IFN-gamma could be detected at 10 mg/L (p-value: <0.001 to 0.013), which is reached in leukocytes of patients receiving standard therapy. At these concentrations, no significant reduction of cell viability was observed median: (0.85, IQR: 0.69-0.90, p=0.139). Flow cytometric analysis of various intracellular signaling pathways revealed that treatment with AZM reduced the phosphorylation of the S6 ribosomal protein, a downstream target of the mammalian target of rapamycin (mTOR). While rapamycin (RAPA) nearly completely abrogated this signaling response, AZM also significantly reduced the S6RP phosphorylation (p<0.001). This finding was confirmed by a time course using immunoblotting, which has a higher sensitivity for protein phosphorylation than flow cytometry (p<0.001).

Conclusion

These data show for the first time the immunosuppressive activity of AZM on CD4+ T-cells, mediated by the inhibition of mTOR signaling. Since AZM and RAPA share structural similarities and in the line of clinically proven immunomodulatory effects of AZM, this finding is comprehensible and should give impetus to conduct further basic and clinical studies.



A) Flow cytometry results using phosflow S6RP antibody; left upper panel: unstimulated cells; right upper panel: stimulated cells in antibiotic-free medium; left lower panel: stimulated cells with RAPA (100 µM); right lower panel: stimulated cells with AZM (10 mg/L). Black line depicts specific staining; grey shaded histograms depict staining with non-specific isotype-matched control antibody