

Impact of per os antibiotic treatments on the gastrointestinal colonization with a multiresistant *Klebsiella pneumoniae* strain

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Colonization resistance refers to the ability of microbiota to prevent expansion and persistence of exogenously acquired bacterial species. It is a pivotal defense mechanism that can be impaired by antibiotic treatment. Previous research shows that changes in microbiota composition, mainly the elimination of specific groups of anaerobic bacteria lead to multi-drug resistant bacteria domination of the gastrointestinal tract in antibiotic-treated mice.

In this study our goal was to determine the effect of different per os antibiotic treatments on colonization rate and resistance gene copy numbers in intestinally carried *K. pneumoniae* (KP5825) in mice. This strain expressed a PMQR gene (*aac(6')-Ib-cr*) an extended-spectrum β -lactamase enzyme (CTX-M-15) as well as a carbapenemase enzyme.

All experiments were carried out using 6–8 weeks-old C57BL/6 male mice. For colonization, 5×10^6 CFU of KP5825 was administered orally on the 14th and 15th days of ampicillin pretreatment. After the colonization mice were divided into 6 groups. These groups received the following antibiotic treatments for 15 days: 0.5g/L ampicillin (AMP), 0.1g/L ceftazidime (CAZ), 0.5g/L ceftazidime, 0.1g/L ciprofloxacin (CIP) and 0.5g/L ciprofloxacin was administered in the drinking water (6th group: control). Fresh fecal pellets were collected from the animals every 3 days throughout the 15 days period. From the fecal samples microbial DNA was extracted.

In the second phase of our experiments the V3-V4 region of the 16S rRNA gene was amplified and sequenced with the Illumina MiSeq platform to determine the microbiota composition in the intestine of colonized mice. We used qPCR technique to determine the KP5825 DNA quantities and assess the resistance gene copy numbers.

Our results show a significant perturbation in the intestinal microbiota. We could also observe that after 15 days KP5825 decreased significantly ($p < 0,004$) in the control group and in the CIP treatment groups ($p < 0,0001$, $p < 0,002$). In the AMP and CAZ treated groups the amount of KP5825 DNA did not change.

Furthermore, our qPCR results show that higher dose CAZ treatment lead to 4-fold increase in quantities of plasmids with CTX-M. Thus, the CAZ had higher power on the dissemination of resistance genes than CIP. This raises the possibility of in vivo plasmid transfer, which we intend to investigate in our future experiments.

Former publication(s): None.

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Inhibition of phosphoinositide 3-Kinase/akt signaling hampers the vasopressin-dependent stimulation of myogenic differentiation

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Background: Vasopressin (AVP) promotes muscle differentiation, hypertrophy and regeneration in in vitro and in vivo experimental models. The effect of AVP is mediated by the cooperation of the calcineurin and CaMK pathways leading to b-HLH MRFs and MEF2 transcriptional activation, which results in the formation of multifactorial complexes on the promoters of muscle-specific genes. The PI3K/Akt/mTOR signaling pathway plays a major role in regulating the muscle mass, controlling protein synthesis and protein degradation.

Methods: To determine the role of the PI3K/Akt/mTOR signaling in the AVP-dependent control of myogenic differentiation, we treated L6 myogenic cells with the PI3K inhibitor LY294002. We evaluated the effect of LY294002 on L6 cells differentiation by: morphological analysis, Creatine Kinase assay, RT-PCR, Immunofluorescences and Western Blotting of key regulators of myogenic differentiation and protein degradation. By luciferase assay, we demonstrated the role of PI3K/Akt/mTOR pathway in the AVP-dependent activation of different fragments of the myogenin promoter. Statistical analysis was performed using the Student's t-test.

Results: LY294002 treatment dramatically inhibited the AVP-dependent myogenic differentiation modulating the expression of MEF2 and myogenin and preventing the nuclear translocation of MEF2. LY294002 also repressed the AVP-dependent nuclear export of HDAC4 and, consequently, the binding of multifactorial complexes, which include NFATc3 and MEF2, on the promoter region of muscle-specific genes, thus impinging on the whole differentiation program.

Conclusions: These results demonstrate that the activation of the PI3K pathway is essential for the fully effect of AVP on myogenic differentiation.

Former publication(s): Scicchitano B.M., Spath L., Musarò A., Molinaro M., Adamo S. and Nervi C. (2002) AVP induces myogenesis through the transcriptional activation of the myocyte enhancer factor 2. *Mol Endocrinol.* 16(6),1407-16.

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