Q - Membrane Permeation: Channels and Transporters

Q.01 - Expression profile of zinc channels in human Renal Cell Carcinoma after Temsirolimus treatment

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INTRODUCTION: Zinc is an essential trace element for cell proliferation and growth. Cellular Zn is regulated of by ZnT and ZIP family channels but its mechanism still not completely understood. Renal cell carcinoma (RCC) is one of the most malignant renal tumors. The RCC clear cell pathological subtype is associated with the VHL gene mutation, that is responsible for its aggressiveness. Temsirolimus (TEM), an antineoplastic drug used in the treatment of RCC, is a selective inhibitor of mTOR. OBJECTIVES: To evaluate the expression of zinc channels in clear cell renal carcinoma cell line with and without TEM treatment. MATERIALS AND METHODS: The MTS (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium-bromide) assay was used define IC50. The expression of ZnT1, ZnT4, ZIP11 and ZIP14 channels from the HK-2, 786-0 and 786-0/TEM cells was evaluated by real-time PCR and Western blot analysis. DISCUSSION AND RESULTS: The IC50 dose was 10µM. Gene expression analysis comparing Hk-2 and 786-0 cell lines revealed decreased levels of ZnT1 of 79.20±3.58% ($P< 0.0001$ vs HK-2) and an increase for the ZIP 11 of 243.3±62.84% ($P< 0.01$ vs HK-2). The comparison between 786-0 with and without TEM treatment showed decreased levels of ZnT1 of 34.03±20.45% ($P< 0.05$ vs 786-0), ZnT4 of 92.82±0.72% ($P< 0.0001$vs 786-0), ZIP14 of 11.24% ($P< 0.01$ vs 786-0) and ZIP11 of 95.96±0.54% ($P< 0.0001$ vs 786-0). Western blot data corroborated the real time results. CONCLUSION: There is a difference in the Zn channel expression profiles between HK-2 and 786-0. The treatment with TEM modulates the expression of these channels

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Q.02 - Effects of $CoCl_2$ on posterior gill K^+-phosphatase activity in the swimming crab *Callinectes danae*

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INTRODUCTION: The aquatic biome is extremely impacted by heavy metals such as cobalt. The swimming crab *Callinects danae* is one of many species affected. Co^{2+} may have an effect on the posterior gill K^+-phosphatase, an enzyme associated with the (Na^+-,K^+-)-ATPase, a transmembrane protein important in crustacean osmoregulation. OBJECTIVES: This study aimed to better understand the effects of Co^{2+} on gill K^+-phosphatase activity. MATERIALS AND METHODS: The kinetic assays were performed continuously at 25 ºC and 410 nm. Total K^+-phosphatase activity was measured in 50 mM Hepes buffer under variable *p*NPP and $CoCl_2$ concentrations, and saturating $MgCl_2$ (7 mM) and KCl (15 mM) concentrations in a final volume of 1 mL. K^+-phosphatase activity is the difference between the total and ouabain (7 mM) insensitive *p*NPPase activities. DISCUSSION AND RESULTS: K^+-phosphatase was inhibited by 72% (K_I = 3 mM) under increasing $CoCl_2$ concentrations (10^{-5} to 2 x 10^{-2} mM) and saturating $MgCl_2$ and *p*NPP (10 mM) concentrations; V_M decreased from 157.5 to 44.3 *p*PNP^- min^{-1} mg^{-1} protein). Interestingly, a K^+-phosphatase stimulated monophasic curve (V_M = 122.5 *p*PNP^- min^{-1} mg^{-1} protein) was seen in the absence of $MgCl_2$. $CoCl_2$ concentrations greater than 10^{-2} mM increasingly inhibits K^+-phosphatase activity, similarly to $MgCl^2$. K^+-phosphatase was stimulated by a single curve (V_M = 128.2 *p*PNP^- min^{-1} mg^{-1} protein) with increasing *p*NPP concentrations, with both saturating $CoCl_2$ (3 mM) and KCl concentrations. Under the same conditions and Mg^{2+}, V_M was 63.1 *p*PNP^- min^{-1} mg^{-1} protein. CONCLUSION: Therefore, it is possible to say that under these conditions Mg^{2+} is able to dislocate up to 2 mM Co^{2+} from its binding site.

Keywords: K^+-phosphatase, $CoCl_2$, Callinectes danae
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