K:D-Rib: cancer cell proliferation inibitor and DNAzyme folding promoter

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Abstract

We report the effects of K:D-Rib, a D-ribose and $KHCO_3$ water solution on HTB-126 human cancer cell line proliferation and the preliminary ultraviolet-visible (UV-VIS) measures of DNAzyme as biosensor of extracellular K^+ concentration. On the one hand, we demonstrate that the synergic action of $KHCO_3$ and D-ribose from one side has a cytostatic effect on human breast cancer cell line increasing by 30% the doubling population time of treated cells with respect to the control; and on the other hand we demonstrate how it seems to permit the K^+ uptake.

Introduction

A significant increase in K^+ channel expression, K^+ current and/or K^+ efflux, can be correlated with tumorigenesis and proliferation.[1] The effects of K:D-Rib, a water solution of D-ribose and $KHCO_3$, on HTB-126 cell (human breast cancer cell line) proliferation is reported. This study also shows preliminary results on DNAzyme used as biosensor to measure extracellular K^+. Cell membranes are permeable to D-ribose but, without Na^+/sugar symporter unlike glucose.[2] Under physiological conditions, the K^+ intracellular concentration is around 150 mM, while the extracellular concentration is about 5 mM. K^+ is involved in G-quadruplex folding[3] and in the apoptotic pathway.[4] G-quadruplex and hemin together form hemin-G-quadruplex nanostructures called DNAzyme[5] with a catalytic activity horseradish peroxidase-like. We employed the DNAzyme as extracellular K^+ biosensor to monitor the K^+ concentration. Following the DNAzyme formation by means of UV-VIS spectra acquisition, we measured the K^+ uptake after K:D-Rib treatment. The DNAzyme folding occurs in spectroscopy buffer containing K^+. We compared the amount of DNAzyme formed using cell growth medium containing K:D-Rib, supernatant of cells incubated with K:D-Rib, cell growth medium without treatment and supernatant of untreated cells.

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Materials and Methods

Cells and culture conditions

According to Croci *et al*.,[6] except for cell medium, Dulbecco's modified eagle medium (DMEM) without red phenol (Lonza) was used.

Drugs

Two hundred and fifty mM K:D-Rib: 0.15 g of D-ribose (Sigma Aldrich, St. Louis, MO, USA) and 0.3 g of $KHCO_3$ (BHD) mixed into 4 mL of distilled water.

Dnazyme folding

Five hundred mM of PS5.M (DNA-TE buffer stock solution frozen) was thawed at room temperature (RT), heated at 95°C for 5 min and let to cool back at RT, 500 mM of PS5.M solution are diluted up to 1.5 mM in spectroscopy buffer (50 mM MES, pH 6.2; 100 mM Tris acetate, DMSO 1% [v/v] and Triton X-100 5% [w/v]) provided with 0.5 mL of K^+ solution for 30 min at RT, to allow proper G-quadruplex folding. After 100 mM of DMSO hemin stock solution was added to G-quadruplex folded solution getting hemin concentration of 0.5 mM, for 20 min at RT.

K^+ solutions

Four K^+ solutions were prepared, one for each sample measured: supernatant of cells treated with 5 mM K:D-Rib, supernatant of control cells, cell free DMEM with 5 mM K:D-Rib and cell free DMEM. All the solutions were incubated at 37°C for 48 h.

Optical measurements

Jasco 6200 spectrometer (Jasco Inc., Oklahoma City, OK, USA) was used to acquire the UV-VIS spectra at RT.

Results

Proliferation assay

In Figure 1 the best fits of control (C) and treated (T) cell data obtained with the following equation are reported:

$$\ln_2(N) = d + \ln_2(N_0) \quad \text{(eq. 1)}$$

where N is proportional to the number of cells at t time, N_0 is proportional to the seeded cell number and d is the doubling population (DP) time. As reported in Figure 1, the linear regression of T data has a slope that is different to C data, with a consequent different DP time: d_T=59±2 h and d_C=44±1 h. The t-test has a value t=3.114>tc=2.571 (P=0.05).

DNAzyme like a K+ biosensor

In Figure 2 the spectra UV-VIS of DNAzyme are reported. The DNAzyme UV-VIS absorbance peaks are at 404, 503 and 629 nm.[7]

DNAzyme spectra in presence of cell free DMEM treated with 5 mM K:D-Rib has the highest absorbance value at 404 nm with respect to all the other sample spectra. The DNAzyme spectrum in presence of treated HTB-126 cell supernatant has a significantly higher absorption at 404 nm with respect to both spectra in presence of cell free DMEM and control HTB-126 cell supernatant.

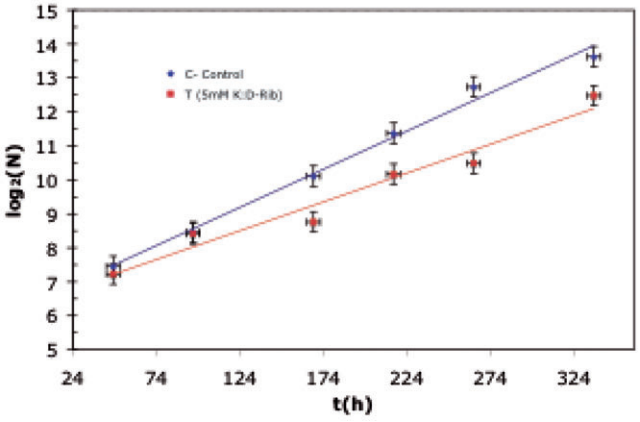

Figure 1. Best fits of cell number against incubation time of control (blue diamond) and treated cells (red square). The linear regressions have a different slope.

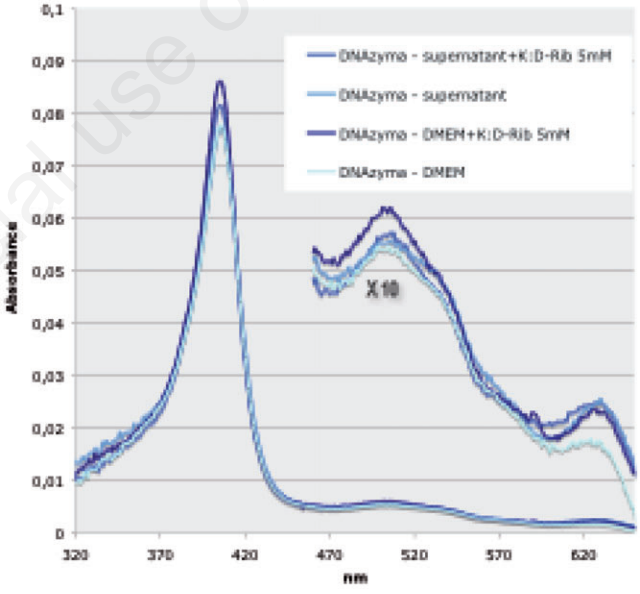

Figure 2. UV-VIS spectra of DNAzyme. On the right part of the figure, under the legend, the ten fold VIS spectra of DNAzyme are reported.

Discussion

The results reported in Figure 1 prove that K:D-Rib, water solution of D-ribose and $KHCO_3$, at the concentration of 5 mM decreases the HTB-126 cell line proliferation as shown by the significative duplication time increase. The preliminary results (Figure 1) seem to indicate that the sample, prepared with supernatant of the HTB-126 cell line treated with 5 mM K:D-Rib up to 48 h has a concentration of DNAzyme lower compared to the one prepared with cell free DMEM incubated with 5 mM K:D-Rib.

Conclusions

Since DNAZyme folding occurs in presence of K+, these evidences demonstrate that K+ enters into the cells and that the DNAzyme can be used as a biosensor for measuring the K+ concentration.

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