

Biochemical responses induced by Biotherapics prepared from intact influenza A (H3N2) and inactivated influenza A (H3N2) virus at 12x and 30x in MDCK cells and RAW-264-7 macrophages

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ABSTRACT

Background: "Roberto Costa's Biotherapics" are homeopathic remedies prepared from intact microorganisms which have been proposed for treatment of diseases like influenza.

Aim: This study aimed to compare the biochemical effects, in MDCK cells and RAW-264-7 macrophages, of biotherapics prepared from intact influenza virus diluted in water as well as from a sample of the same virus inactivated by ethanol 70% (v / v), both in the homeopathic potencies of 12x and 30x. Water 30x, non-dynamized water and cells without treatment (control cells) were used as control.

Methodology: Treatments were performed by incubating MDCK cells with DMEM medium added in a 1:10 ratio for 6 times (3 different aliquots per day) or 18 times (up to 4 aliquots per day) in each experimental situation. Each aliquot was added with an interval of at least 2 hours. After that, the mitochondrial activity of MDCK cells was analyzed by MTT assay. The effects of treatments with intact biotherapics on MDCK cells respiratory parameters were studied using high resolution respirometry (Oroboros Oxygraph-O2K). RAW-264-7 macrophages were treated with intact and inactivated biotherapeutic 30x (4 treatments, 24 hours) to verify the nitric oxide production. These macrophages were also submitted to MTT assay.

Results: Both biotherapeutic preparations 1x (intact and inactivated virus sample) were analyzed by transmission electronic microscopy. The presence of virus particles was detected when water was used as solvent. The use of ethanol as biotherapeutic solvent induced complete virus lysis. There was no alteration in cell osmolarity revealed by neutral red assay, when 10% of each test solution was used. Cellular viability analyzed by MTT method increased when MDCK cells were treated with 18 stimuli of inactivated biotherapeutic 30x when compared to intact biotherapeutic 30x ($p < 0.05$). However, no statistically significant differences ($p > 0.05$) were detected when these cells were compared to control cells.

The maximum respiratory capacity of MDCK cells increased after treatment with 18 stimuli of intact biotherapeutic 30x when compared to control cells. However, no statistically significant differences ($p>0.05$) induced by biotherapeutics in macrophage cells were observed by MTT and nitric oxide assays. Moreover, a reduction in nitric oxide was observed in macrophages treated with dynamized water when compared to control cells.

Conclusions: These results indicate that the method of biotherapeutic compounding (intact or inactivated virus as starting point) can modify the cellular parameters with the tendency to increase cellular response with longer treatments and higher potencies.

Keywords: Biotherapeutic, influenza, cell alteration.



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