

# Evaluation of biotherapics for human flu using *in vitro*, pre-clinical and clinical studies

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**Background:** Influenza viruses have been responsible for highly contagious acute respiratory illnesses with high mortality, mainly in the elderly and immune compromised patients, which encourages the development of new drugs for treatment of human flu. The biotherapics are medicines prepared from biological products, which are compounded according to homeopathic procedures<sup>1</sup> indicated for infectious diseases with known etiology.

**Aim:** In the present study, the *in vitro* and *in vivo* effects of three biotherapics were evaluated using *in vitro*, pre-clinical and clinical assays.

**Methodology:** The biotherapics were prepared according to homeopathic procedures and the ultrastructural aspects of viral particles were evaluated by transmission electron microscopy<sup>2</sup>. Cell viability was assessed by MTT method and mitochondrial respiratory function was studied by cellular oxygen consumption, lactate production, phosphofrutokinase-1 enzyme and citrate synthase enzyme activities and ATP hydrolysis. *In vivo* methodologies were previous approved by Ethical Committees (protocol numbers: 194/08; DFBCICB037; DFBCICB040). In pre-clinical methodologies the efficacy of biotherapics against the immune response of BALB/c mice was evaluated using open field, assessment of organs by weight, histometry, immunohistochemistry and flow cytometer. Additionally, the efficacy of biotherapics to prevent flu and/or respiratory diseases in Brazilian healthy children was quantified using a triple-blind, randomized, placebo-controlled clinical trial.

**Results:** The *in vitro* experiments showed alterations in important enzyme activities and in the maximum respiratory capacity when cells were treated with intact biotherapeutic 30x. The pre-clinical study indicated that the biotherapics were not able to induce pathogenetic effects. However, some important alterations in peritoneal washing fluids of mice that received intact biotherapeutic 30x were detected by flow cytometer, such as: increase in B regarding non-B cells, decrease in B2 cells and increase in B1 and CD4+ cells. These alterations indicate that biotherapeutic 30x stimulated the innate immune response when the animals were challenged with influenza hemagglutinating antigen. The clinical trial proved that the biotherapics have a prophylactic effect against flu and acute respiratory infection symptoms, when compared to placebo.

**Conclusions:** The results obtained in this study showed that *in vitro* and *in vivo* assays enable the understanding of the antiviral properties of biotherapics.

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