

POSTER PRESENTATION

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# Effect of ouabain on NF $\kappa$ B and p-38 activation in macrophages: a new biotechnological application

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## Background

Inflammation is a response to external challenge or cellular injury that leads to the release of several inflammatory mediators and restoration of tissue structure and function. On the other hand, inflammation deregulation can cause tissue damage and is related to many diseases. During inflammation, a complex program of intracellular signal transduction and transcription events, driven by multiple pro-inflammatory mediators and cytokines, is activated. Ouabain, a potent inhibitor of the Na<sup>+</sup>, K<sup>+</sup>-ATPase, was identified as an endogenous substance of human plasma, able to affect various immunological processes, such as lymphocyte proliferation, apoptosis and monocyte function. These effects are particularly attractive for anti-inflammatory molecules bioprospection. We have also demonstrated the ability of ouabain to modulate inflammation, but little is known about the mechanisms involved.

## Aim

The aim of this work was to evaluate, *in vivo*, the role of ouabain on NF $\kappa$ B e p-38 activity. Additionally, we tested ouabain effect (1, 10 and 100 nM) on FITC-dextran endocytosis ability of peritoneal cells.

## Methods

In all experiments, 0.56mg/kg ouabain or phosphate buffered saline (PBS) was given intraperitoneally (i.p.) for three consecutive days. After 1h of the last day of ouabain treatment, mice were euthanized and macrophages and neutrophils from peritoneal cavity were collected. Cells were seeded in 24-well plates and incubated for 1h. After the incubation period, macrophages were tested using antibodies anti-P-NF $\kappa$ B e anti-Pp38 by flow cytometry.

Besides that, ouabain was tested on FITC-dextran endocytosis ability of peritoneal cells. Peritoneal cells from unstimulated animals, including macrophages and neutrophils, were placed in sterile 96-well plates and incubated with different concentrations of ouabain (1, 10, 100nM) for 1h. This was followed by the addition of 0.5 mg/ml of 40 kDa FITC-dextran. After incubation, cells were washed with PBS and analyzed by flow cytometry. The FITC-dextran uptake was determined as the mean fluorescence relative to the background staining of the respective sample incubated with FITC-dextran at 4°C.

## Results

Ouabain treatment decreased basal levels of P-p38 and P-NF $\kappa$ B expression compared to the PBS group. Ouabain led to a 65% reduction of p38 activation and 60% reduction of P-NF $\kappa$ B. Besides that, ouabain was also capable of reducing by 84% the expression of both protein NF $\kappa$ B and p-38 in the double positive cell subpopulation. Finally, after one hour of incubation, approximately 58% of cells endocytosed FITC-dextran particles, and this process was inhibited at 4°C. Moreover, we also observed that ouabain did not affect the endocytosis of dextran particles.

## Conclusion

In agreement with the immunosuppressive effects that have been previously observed by us, ouabain pretreatment reduced the basal activation of proteins NF $\kappa$ B and p-38, but did not alter *in vitro* phagocytosis of dextran particles by peritoneal cells, suggesting a new mode of action of this substance. The present work reveals ouabain anti-inflammatory potential with biotechnological applications related to the immune system. However, further studies are necessary to elucidate the mechanisms involved.

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