Abstract
Background: External and internal changes are factors that can disrupt the balance between neuroendocrine and immune systems; however, currently studies that evaluate the modulation of the immune system by neuroendocrine factors in fish are scarce, and for some aspects are null. Objective: To evaluate the effects of sub-basal increase of GABA and acetylcholine (ACh) in pro and anti-inflammatory cytokines (IL-1β, TNF-α, and TGF-β) levels in the immune system organs and serum of Nile tilapia. Experimental procedures: Basal value of GABA was quantified in the serum by liquid chromatography using 2-hydroxynaphthaldehyde as derivatizing reagent. ACh was estimated by Hestrin method with modifications. Fish were intravenously dosed into the caudal vein at day 0, 3 and 6 with three doses below tenth of the basal levels of ACh and one hundred of GABA. Based on genome assemblies of *O. Niloticus* (BioProject: PRJNA354796), recombinant IL-1β, TNF-α and TGF-β were obtained by Gateway® method, and polyclonal antibodies against the recombinants were induced in mice. On the eighth day, specimens were sedated by freezing on ice for sampling the blood and after, the fish were euthanized by rapid freezing (-80°C). Levels of IL-1β, TNF-α and TGF-β were evaluated by indirect ELISAs. Results: By effect of sub-basal increases of neurotransmitters, a dose-dependent response of pro and anti-inflammatory cytokines synthesis was detected. In pronefros and thymus of ACh-treated fish, an increase was found ($p \leq 0.001$), as in the spleen ($p \leq 0.05$). Similarly in fish treated with GABA; however, a noticeable enhancement of IL-1β and TNF-α synthesis was noted in pronefros and spleen ($p \leq 0.001$). In contrast, the great induction of TGF-β was estimated in thymus. Nevertheless, high basal values of these cytokines as well as a clear dose-dependent response were quantified in serum. Discussion: Contrasting with this study, an inhibitory effect of GABAergic agents in macrophages and APC from a mouse multiple sclerosis model evaluated as IL-1β and IL-6 levels was detected. In macrophages, ACh suppresses the release of TNF-α, IL-1β and IL-18. Contrasting, in splenectomized mice dosed with an α7nAChR agonist (nicotine), an increase of serum TNF-α and IL-1β was observed. Despite there are not preceding reports about the role of dosed ACh or GABA on TGF-β level in serum or immune system organs, TGF-β and ACh present in the tumoral microenvironment of colon adenocarcinoma switch-on in agreement with tumor development, as facilitating IL-1β production. Conclusion: Results of this study suggest that GABA and ACh function as paracrine or autocrine factors in the immune system organs of the Nile tilapia.
keywords: Autocrine and paracrine factors; IL-1β; TNF-α; TGF-β.

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Alpinone exhibit immunostimulant effects in Atlantic salmon kidney

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Abstract
Viral diseases are very harmful in aquaculture since they cause high mortalities in fish that have great economic losses. At present, vaccines to prevent viral diseases in fish are limited and their efficiency is not satisfactory. Therefore, it is of great importance for aquaculture to find new antiviral agents that can modulate the innate antiviral immune response in salmonids and this way can be used as a measure of prophylactic control against viral diseases. In this work, we evaluated the immunostimulant effect of the Alpinone in primary culture of Atlantic salmon kidney cells throught of analysis of the levels of transcripts of key genes involved in the activation of the innate antiviral immune system
Materials and Methods: Cells from head kidney of Atlantic salmon were treated with 5 μg /mL, 10 μg / mL and 15 μg / mL of the Alpinone flavonoid. We used as negative control untreated cells and cells treated with DMSO corresponding to the solvent of the compound, and as positive control we used 10 μg / mL of Poly I: C. After 8, 24 and 48 hours of treatment with the Alpinone flavonoid, the total RNA extraction of the cultures was carried out, the cDNA synthesis of the samples and we analyzed the levels of transcripts of genes involved in the early antiviral response such as MX, IFNα, IRF3, MDA-5 and RIG-I by real-time PCR
Results: Alpinone flavonoid increases the transcript levels of the transcription factor IRF-3, the cytokine IFN-α, the antiviral protein Mx and RIG-1 receptors, while the TLR3 and TLR9 receptors they do not present changes in the levels of transcripts with respect to the control
Conclusion: Alpinone flavonoid increases the levels of transcripts of genes involved in the innate antiviral immune response, proposing this flavonoid as a potential candidate to be used as an antiviral agent for the treatment of diseases in salmonid fish. Acknowledgements: Fondecyt 11170984 & Fondecyt 1180265
keywords: Flavonoid, Alpinone, Imnunostimulant, Antiviral, Salmonid.

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Evidences of *Tenacibaculum maritimum* evading strategies against Senegalese sole (*Solea senegalensis*) primary head-kidney leucocytes

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Abstract

Tenacibaculum maritimum evading strategies are currently unknown and many aspects regarding the host-pathogen interaction are still not fully elucidated. Hence, the present study aimed to assess Senegalese sole cellular immune responses following stimulation with either live or UV killed *T. maritimum* through both functional (e.g. superoxide anion and nitric oxide production, leucocytes killing capacity) and gene expression approaches. Senegalese sole head-kidney leucocytes were isolated and exposed to several live or inactivated *T. maritimum* strains during 4 h, 12 h, 24 h and 48 h. In the present study, primary head-kidney leucocytes exposed to different live bacterial strains did not show significant changes in superoxide anion nor nitric oxide production, whereas UV killed *T. maritimum* strains seemed to stimulate leucocytes' nitric oxide release. Interestingly, leucocytes stimulated with both live and UV killed bacterial strains reacted with relatively low superoxide anion production. Regarding gene expression data, stimulation with live strains induced very low increase in interleukin-1β (*il1β*), hepcidin antimicrobial peptide (*hamp*), cyclooxygenase 2 (*cox2*) and g-type lysozyme (*glys*) transcripts at 4 h compared to non-stimulated cells, which decreased similarly until 48h. Although interleukin-10 (*il10*) expression levels presented a similar pattern, an upregulation was observed at 48 h post stimulation. In contrast, the expression levels of *il1β*, *cox2*, *hamp* and *il10* from host primary cell culture stimulated with inactivated bacterial strains increased more than those from leucocytes exposed to live bacteria and up to 100-fold. The downregulation of inflammatory, iron regulating genes and toll-like receptor 2, as well as the extensive destruction of phagocytes, in cells exposed to live bacteria could be considered as part of *T. maritimum* evading strategies.
keywords: Bacterial pathogens, innate immune response, respiratory burst, host defences.

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