GONADOTROPIN SOMATIC GENE THERAPY RESULTS IN LONG LASTING CIRCULATION OF BIOLOGICALLY ACTIVE HORMONES IN AN AQUACULTURED MARINE FISH

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Introduction
The use of recombinant or native proteins to solve serum protein deficiencies is widespread in humans. This is also the case of gonadotropins, which have been used in reproductive management of a variety of species. In many cases native proteins are easily cleared from the circulation and therefore the development of recombinant peptides with longer circulating half-life has been offered as an alternative. A different approach is the use of gene-based therapies. Somatic gene transfer is based on the introduction and expression of a foreign gene into an adult tissue without being incorporated in the genome. In mammals, the use of this technique is mainly focused on gene therapy (Hojman, 2010). In fish, its development has been mainly directed to DNA vaccination. However, its application as a method for in vivo delivery of gene products into fish blood has hardly been explored (Lorenzen et al., 2000).

The control of fish reproduction is important from an applied point of view, and can be a limiting step in fish culture. An efficient control depends on a deep knowledge of the basic processes governing the initiation and cyclic progression of the reproductive events. The follicle-stimulating hormone (Fsh) and the luteinizing hormone (Lh) play central roles in vertebrate reproduction. These gonadotropins bind and activate specific receptors (Fshr and Lhr) located in the fish gonads, and their action begins a signalling cascade controlling different steps of gametogenesis and steroidogenesis.

Thus, our aim was to investigate the use of somatic gene transfer for systemic treatment with reproductive hormones using the European sea bass (Dicentrarchus labrax) as model, and to propose it as an alternative to the continuous administration of the corresponding protein in gene function studies or biotechnological applications.

Materials and methods
Recombinant genes coding for single-chain analogs of the sea bass Fsh and Lh (scFsh and scLh) were injected into the skeletal muscle of this fish. Positive control animals were injected with the corresponding single-chain hormones generated in an in vitro cell system (Molés et al., 2011). The ability of the fish muscle to produce and secrete the encoded proteins into the bloodstream was evaluated by measuring plasma Lh and Fsh levels with homologous assays developed by our group (Mateos et al., 2006: Molés et al., 2012). The physiological impact of the recombinant hormones produced by the fish muscle cells was assessed in reproductively active males for the injections of the scLh-coding gene and their effect on sperm production was recorded. The injections of the scFsh-coding gene were tested in prepurberal juvenile males to evaluate the ability of scFsh in promoting spermatogenesis. Sex steroid levels, expression of marker and target genes and cell proliferation and gonad maturation were evaluated.

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Fig. 1. Schematic representation of the somatic gene transfer method.

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Results
The availability of the genes coding for the sea bass gonadotropin subunits enabled us to generate recombinant gonadotropins in different eukaryotic expression systems (mammalian and insect cells) and DNA constructs, including the production of single-chain gonadotropins. All these recombinant hormones are functionally active, but their production rates and in vivo stability showed remarkable differences depending on the system used. Ligand activation studies showed that each sea bass receptor responds exclusively to its specific gonadotropin. However, sea bass Lhr could be activated by mammalian LH and FSH, while sea bass Fshr was only activated by mammalian FSH.

Next, somatic gene transfer was evaluated for in vivo delivery of gene products into fish blood. Thus, injection of gonadotropin-coding genes in muscle resulted in high circulating levels of active gonadotropins. The injections of adult mature males produced an active Lh able to increase sperm production without affecting its quality in terms of density. The injection of the coding gene provoked longer-lasting and higher plasma Lh levels than the direct injection of the recombinant scLh produced in vitro, and both hormones were equally active. On the other hand, the injections of either recombinant scFsh or its coding gene increased plasmatic levels of Fsh, but with different performance. The administration of scFsh generated an acute increase of the plasmatic levels of this hormone and a quick response, followed by a fast clearance from the bloodstream, while the increase in plasma Fsh levels attained by injection of its coding gene was progressive and lasted longer. Correspondingly, the injection of the scFsh coding gene was more efficient in triggering spermatogenesis in this juvenile fish.

Discussion and conclusions
We have developed a series of tools to study and control gonadotropin actions in the European sea bass. This species is a good model due to the already existing knowledge on its reproductive patterns and the availability of other tools for physiological studies.

We found a promiscuous activation of sea bass Lhr when using mammalian hormones, which is in line with findings in other fish receptors, and recommends caution in the biotechnological application of heterologous hormones, if these have not been previously evaluated.

The availability and use of homologous recombinant gonadotropins offers a unique tool for studying gonadotropin actions in a given species. One step further is the direct administration of the gonadotropin coding genes to the fish. All together, the use of gene therapy for hormone replacement in fish is a real alternative to the production of recombinant gonadotropins for in vivo use, due to the low cost of production and the high persistence of the injected DNA, and has a broad range of potential applications such as its use in out-of-season breeding programs or reproductive dysfunctions in fish species.

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References