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GH and IGF-I induction by passive immunisation of rainbow trout *Oncorhynchus mykiss* (Walbaum) using a somatostatin-14 antibody

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ABSTRACT

Inhibition of the growth axis by somatostatin was studied in juvenile rainbow trout using passive immunisation with a previously isolated somatostatin antibody (antiSS-14). Upon subcutaneous injection of laying hens (*Gallus domesticus*) with conjugated somatostatin-14 (SS-14), the antiSS-14 was isolated from egg yolk. Isolation of immunoglobulin was carried out at 40 days, revealing maximum IgY titre (IgY) at 3.22 ± 0.08 compared to control (0.1 ± 0.04) and highest total protein of 183 ± 2 mg ml^{-1} compared to the control (85 ± 3 mg ml^{-1}). To test the effects of antiSS-14 on the somatotropin axis, rainbow trout were passively immunised. Passive immunisation with extracted IgY at a dilution of 1:25 increased GH plasma concentrations after 30 min, reaching a maximum at 35 ± 7 ng ml^{-1} after 120 min. GH remained elevated for over a day, returning to baseline at 2 days. At 1:100, GH increased and returned to normal after 2 days. At 1:25, IGF-I plasma concentrations were increased at 30 min (277 ± 74 ng ml^{-1}, $p<0.05$), 120 min (212 ± 77 ng ml^{-1}, $p>0.05$), 240 min (359 ± 172 ng ml^{-1}, $p<0.05$) and 1 day (259 ± 117 ng ml^{-1}, $p<0.05$) compared to the control ($n=7$, Tukey test), returning to baseline levels after 2 days (145 ± 45 ng ml^{-1}). In the 1:100 treatment group, slightly elevated GH did not induce IGF-I, which fluctuated between 13 8 ± 15 ng ml^{-1} and 167 ± 35 ng ml^{-1} ($n=7$, $p>0.05$) compared to the control fish, ranging from 131 ± 10 ng ml^{-1} to 148 ± 6 ng ml^{-1}. Passive immunisation thus demonstrates up-regulation of plasma GH and IGF-I, suggesting the blockage of SS-14 mediated growth inhibition. The simplicity of antiSS-14 production in chicken eggs and the upregulation of GH and IGF-I suggests a potential use for growth promotion in aquaculture.

1. Introduction

In fish, growth is under the control of growth hormone (GH) secretion from the pituitary, regulating somatic growth, organ and tissue development and metabolic processes that influence somatic growth (Canosa et al., 2007). Most of these biological functions are mediated by plasma insulin-like growth factor I (IGF-I), released from the liver in response to circulating GH (Duan, 1998). This GH–IGF-I system, often referred to as the growth axis, is known to be further modulated by external cues such as temperature or photoperiod and is interlinked to other endocrine systems involving thyroid hormones, cortisol and insulin (Duan, 1998; Harvey, 1993). Nevertheless, it is generally accepted that somatic growth is mainly a consequence of GH secretion, which is under the neuroendocrine control of growth hormone releasing hormone (GHRH) and its antagonist somatostatin (Klein and Sheridan, 2008; Canosa et al., 2007). Consequently, a considerable number of studies have addressed GH secretion in order to enhance growth in aquaculture and fish production. Often, IGF-I is addressed as a growth parameter, both in plasma as well as its mRNA expression in the liver (Devlin et al., 2009; Fox et al., 2009; Duan, 1998). The major inhibitors of GH release are somatostatins (SSs), a functionally and structurally diverse family of peptides (Sheridan and Kittilson, 2004). In teleosts, in contrast to mammals, basal GH secretion is under the dominant inhibitory control of SSs (Canosa et al., 2007; Hall et al., 1986), characterising somatostatin down-regulation as a promising target for growth enhancement.

Somatostatins are a family of phylogenetically ancient neuropeptides found in all vertebrate species (Nelson and Sheridan, 2005; Conlon et al., 1997). First isolated as a 14-amino acid peptide, several SSs have been identified, comprising NH_2-terminal extensions of SS-14 or minor modifications originating from a duplicated gene (Conlon et al., 1997). Although they possess an array of physiological functions, comprising neuromodulation, osmoregulation, the control of growth, development, and metabolism, they are best known as an

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antagonist of growth hormone (Patel, 1992). Furthermore, somatostatins inhibit the transcription of IGF-I mRNA as well as the release of IGF-I protein from hepatocytes, decrease the expression of GH receptors (GHR), and induce GHR internalisation (Very and Sheridan, 2007).

In principle, it has been shown that accelerated growth can be achieved by neutralisation of SS-mediated inhibition, thereby increasing GH and IGF-I secretion. For example, intraperitoneal injection of a SS-14 antagonist induced GH secretion and subsequently increased plasma IGF-I (Xiao and Lin, 2003). Since SS-14 is a potent natural inhibitor of GH secretion in fish, blocking its actions by passive immunisation could provide a growth-promoting effect for fish production in aquaculture.

In passive immunisation, specific antibodies (AB) bind to the target which consequently modulates their biological action. The application of passive immunisation for blocking SS has been reported in domestic livestock such as cattle, pigs, sheep and chickens (Dawson et al., 1997; Elsaesser and Drath, 1995; Vankessel et al., 1993). Although growth responses were variable from study to study, immunoneutralisation of SS in fishes indicated significant growth promotion. Peterson et al. (2003) reported that passive immunisation of rainbow trout with polyclonal antiSS-14 increased GH levels over time, but effects on growth performance and food conversion ratio (FCR) were highly dependent on Ab titres. In several studies, efficiency of immunoneutralisation has been suggested to be highly specific for the Ab used (Diez et al., 1992; Mayer et al., 1994). Opportunities to improve passive immunisation include reduction of reported meat toughness (domestic livestock), avoidance of adverse effects on treated animals, and – most importantly – improving the effectiveness of growth enhancement. With regard to the latter, use of new vaccines is most promising. For new vaccines, growth promotion has to be evaluated carefully, since an AB may even prolong the half-life of the target and thus enhance its action. It was shown that the anabolic efficacy of GH in sheep and cows could be increased through the co-administration of monoclonal AB that binds GH, suggesting an increase in GH half-life or AB-facilitated receptor binding (Bomford and Aston, 1990; Pell et al., 1989). Similar studies were reported for IGF-I AB (Pell et al., 2000; Hill et al., 1997).

Yolk derived from the eggs of immunised chickens has been recognised as an excellent source of polyclonal AB for over a decade (Tini et al., 2002; Gassmann et al., 1990). As a consequence, production of AB in chicken eggs offers a simple, easy-to-perform and cost effective method compared to classical protocols using living mammals such as rabbits: Characterised by the large amounts of relatively inexpensive yolk IgY and with regard to animal ethic issues, this approach seems highly desirable for passive immunisation strategies (Chalghoumi et al., 2009; Schade et al., 2005). In addition, simple standardised IgY extraction methods have been established (Akita and Nakai, 1992), but optimisation and adaptation are commonly required. An egg yolk derived antiSS-14 together with a specific immunisation strategy, seems a practical approach in establishing immunoneutralisation of an inhibitor target protein such as SS-14. The production of chicken-derived SS-14 Ab – upon evaluation – offers a practical approach for the use of "designer eggs" for growth promotion in aquaculture. The objective of the present study was to examine the effects of such an egg derived polyclonal antiSS-14 on GH and IGF-I concentrations in serum during a short-term period (48 h). This information is needed to establish an immunoneutralisation strategy for a long-term application.

2. Materials and methods

2.1. Anti SS-14 production

Six white leghorn chicken *Gallus domesticus* (Hy-Line W-36, Italy), approximately 44 weeks of age (peak of egg production), were kept at the facilities of the Faculty of Animal Sciences (Tehran, Iran) at 18–24 °C and a 14 h:10 h light-to-dark regime and fed ad libitum. SS-14 (American peptide company, USA) and human serum globulin (HSG, Sigma, St. Louis, USA) were conjugated with glutaraldehyde (Sigma, St. Louis, USA) as described by Arimura et al. (1975) and stored at −80 °C until used. Immunisation was carried out with 0.2 mg conjugated SS-14 dissolved in 1 ml of Complete Freund's Adjuvant (CFA) on day 0 as first injection followed by 3 booster injections at 14 days, 21 days and 28 days with 0.1 mg conjugated SS-14 in 1 ml of incomplete Freund's Adjuvant (IFA). The control group was subcutaneously injected with 1 ml of normal saline (0.91% w:v NaCl). IgY titre in the eggs was monitored before the first injection (0 day), the days of booster injections (14 days, 21 days, and 28 days), and on 31 days, 35 days, 40 days, 50 days, 60 days, and 67 days. For IgY purification, egg yolk was separated from the egg white and washed with distilled water. The yolk membrane was carefully removed and PBS (0.01 M, pH 7.6) was added (1:2 egg:PBS v/v). To separate lipids from the yolk, PEG 6000 (3.5% total volume) was added under stirring (10–15 min). Upon centrifugation (4400 g at 4 °C, 30 min) supernatant was filtered (Whatman Grade No. 42, Quantitative Filter Paper). PEG 6000 was added (8.5% total volume, under stirring, 20 min), followed by centrifugation at 12,000 g (10 min, 4 °C). The IgY pellet was resuspended in an equal volume of PBS and an additional purification step with PEG 6000 (12.5% filtered volume) was applied. Finally, the pellet was resuspended in PBS (1 pellet: 6 PBS v/v) overnight at 4 °C.

For the ELISA evaluation, egg extract from 0 day, 28 days and 31 days were selected randomly and a serial dilution of the serum was used to determine the optimal antibody-antigen dilution: Plates were coated with 100 µl SS-14 at 2, 4, 8, 16 µg ml^{-1} carbonate buffer (0.05 M, pH 9.5), incubated at 4 °C overnight, followed by three washings (0.01 M PBS; pH 7 + 0.05% Tween 20). Then, 250 µl blocking agent (5% skim milk in 0.01 M PBS) was added. After incubation at 37 °C for 75 min, three successive washings were performed, followed by the addition of 100 µl rabbit anti-chicken horse radish peroxidase (HRP) conjugate (1:15,000 dilution), incubation at 37 °C for 75 min, and three washings. 100 µl of BM blue POD substrate (Roche, Germany) were added and incubated at room temperature (15 min). The reaction was stopped with 50 µl H_2SO_4 (1 N). Absorbance was read at 450 nm using an ELISA microplate reader (BIO RAD-680). Samples with high optical densities were pooled and total protein content of this IgY stock was determined (Lowry et al., 1951).

2.2. Passive fish immunisation

To reduce the impact of gonad maturation, gynogenetic juvenile rainbow trout *Oncorhynchus mykiss* with an average weight of 66 ± 3.54 g were acclimatised for 3 weeks in 1000 L square tanks supplied with ground water in flow-through at approx. 15 L/min at 13 ± 0.6 °C under a natural photoperiod (O_2 10.2 ± 0.2 mg; pH 7.46 ± 0.14, NO_2^- <0.03 mg/L, 0.1–0.2 NH_3/NH_4^+). To allow fast access for immunisation, fish were kept in a net cage (0.8 m 0.4 m 0.65 m), 45 fish per cage (approximately 15 kg/m^3), two cages per tank. Two hundred and seventy fish were distributed randomly, providing a replicate of each group, and fed twice daily with commercial trout pellets. Fish were injected interperitoneally upon anaesthesia (70 ppm MS222) with 0.1 ml of a) 1:25 IgY (5 mg/ml) PBS solution (high titre immunisation), b) 1:100 IgY (1.25 mg/ml) PBS solution (low titre immunisation) or c) PBS solution (0.01 M, pH, 7.6).

2.3. Blood sampling

For hormone quantification, 1 ml blood was sampled from the caudal vein with a heparinised 1 ml syringe at 30 min, 120 min, 240 min, 1 day and 2 days after immunisation. 7 fish were randomly sampled before immunisation to establish a baseline for plasma GH