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Streptococcus agalactiae impairs cerebral bioenergetics in experimentally infected silver catfish

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ABSTRACT

It is becoming evident that bacterial infectious diseases affect brain energy metabolism, where alterations of enzymatic complexes of the mitochondrial respiratory chain and creatine kinase (CK) lead to an impairment of cerebral bioenergetics which contribute to disease pathogenesis in the central nervous system (CNS). Based on this evidence, the aim of this study was to evaluate whether alterations in the activity of complex IV of the respiratory chain and CK contribute to impairment of cerebral bioenergetics during *Streptococcus agalactiae* infection in silver catfish (*Rhamdia quelen*). The activity of complex IV of the respiratory chain in brain increased, while the CK activity decreased in infected animals compared to uninfected animals. Brain histopathology revealed inflammatory demyelination, gliosis of the brain and intercellular edema in infected animals. Based on this evidence, *S. agalactiae* infection causes an impairment in cerebral bioenergetics through the augmentation of complex IV activity, which may be considered an adaptive response to maintain proper functioning of the electron respiratory chain, as well as to ensure ongoing electron flow through the electron transport chain. Moreover, inhibition of cerebral CK activity contributes to lower availability of ATP, contributing to impairment of cerebral energy homeostasis. In summary, these alterations contribute to disease pathogenesis linked to the CNS.

1. Introduction

Marine and freshwater aquaculture is one of the fastest growing food-producing sectors worldwide, since it provides a source of protein and essential micronutrients for human health [1]. However, infectious diseases such as those caused by *Streptococcus agalactiae* [2] are one of the major limiting factors for production and productivity [3], which result in high economic losses for fish producers.

Streptococcus agalactiae (commonly known as group B streptococcus, GBS) is recognized as a serious causative agent of zoonosis with a broad host range, such as humans, cattle, dogs, cats and several fish species [4–6]. In fish, this disease is characterized by lethargy, anorexia, exophthalmia, corneal opacity and hemorrhage [7], affecting species such as Nile tilapia (*O. niloticus*) [6], carp (*Labeo rohita*) [8] and silver catfish (*Rhamdia quelen*) [9]. Of particular interest, it has been reported that the presence of bacteria in brain tissue caused by disruption and penetration of *S. agalactiae* through the blood–brain barrier (BBB) is linked with several alterations in the central nervous system (CNS), such as erratic swimming, dorsal rigidity, loss of equilibrium and lethargy, causing meningoencephalitis [10,11]. A recent study conducted by Baldissera et al. [12] demonstrated that alterations in cerebral bioenergetics through the activity of creatine kinase (CK) and

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complexes of the mitochondrial respiratory chain contribute to disease pathogenesis of the CNS, as well as to behavioral alterations during infectious diseases. Thus, our hypothesis is that impairment of bioenergetics may lead to brain damage during meningoencephalitis.

Mitochondrial abnormalities have been implicated in neuroinflammatory and neurodegenerative conditions, contributing to initiation and progression of some CNS dysfunctions [13], including abnormalities in oxidative phosphorylation complexes. Oxidative phosphorylation in the mitochondria provides biological energy for intracellular metabolic pathways, and it is particularly significant in brain cells because cerebral tissue is one of the most energy-consuming organs [14]. Cell energy is generated by four different oxidative phosphorylation complexes (complexes I–IV), which are localized in the inner or cristae of membranes and involved in biological and physiological energy for intracellular metabolic pathways, such as the PCr/CK system [15]. Recently, a study conducted by Baldissera et al. [16] demonstrated that alterations in the activity of complexes of the respiratory chain in gills contribute to disease pathogenesis in fish infected with *Pseudomonas aeruginosa*. Thus, our hypothesis is that alterations in the activity of complex IV of the respiratory chain are related to impairment of cerebral bioenergetics during *S. agalactiae* infection, contributing to the appearance of clinical signs and, consequently, to disease pathophysiology.

CK is a central controller of cellular energy homeostasis which catalyzes the reversible transfer of the N-phosphoryl group from phosphocreatine (PCr) to adenosine diphosphate (ADP) to regenerate adenosine triphosphate (ATP), exerting a key role in the energy homeostasis of cells with intermittently high and fluctuating energy requirements, such as neurons [17,18]. ATP is a relevant neuromodulator and neurotransmitter which exerts an important role in neural plasticity [19], and its decrease is associated with neurodegenerative disorders. Our hypothesis is that inhibition of cerebral CK activity may lead to an energy imbalance linked with ATP levels, contributing to disease pathogenesis in the CNS.

Based on this evidence, the aim of this study was to evaluate whether alterations in the activity of complex IV of the respiratory chain and CK contribute to impairment of cerebral bioenergetics during *S. agalactiae* infection.

2. Materials and methods

2.1. Fish harvesting, maintenance of animals and water quality parameters

Healthy fish were collected for experimental purposes from a fish farm located in Rio Grande do Sul state (Brazil). The fish were transported alive and maintained in 250 L fiberglass tanks with continuous aeration and controlled water parameters (22–24 °C, pH 7.3–7.6, dissolved oxygen levels: 5.8–7.0 mg/mL) in fresh water for 10 days. Dissolved oxygen and temperature were measured with a YSI oxygen meter (model Y5512, Ohio, USA) and the pH with a DMPH-2 pH meter (Sao Paulo, Brazil). The total ammonia and non-ionized ammonia levels were determined according to Verdouw et al. [20] and Colt [21], respectively, as recently published in detail by Baldissera et al. [9]. The animals were fed to apparent satiation with commercial feed once a day. Any uneaten food, feces and other residues were removed daily 30 min after feeding.

2.2. Bacterial culture and inoculum preparation

Streptococcus agalactiae was isolated from a patient in southern Brazil and identified according to colony characteristics and biochemical tests, as recently published in detail by Baldissera et al. [9].

The bacterial culture was maintained frozen in glycerol, and was seeded in nutrient agar for 24 h. Following this, the bacterial culture was grown on sheep blood agar for use in this experimental model. The suspension of *S. agalactiae* was washed twice in sterile saline (NaCl 0.9%), turbidity (OD600) adjusted to 0.8–1.0 (equivalent to 7×10^7 CFU/mL) and used for infection, according to the protocol established by Baldissera et al. [9].

2.3. Animals and experimental study

Twelve juvenile silver catfish (60.5 ± 8 g; 22 ± 3 cm) were used as the experimental model for the assessment of enzymatic activity of respiratory chain complexes (complex IV), as well as the enzymatic activity of CK in brain tissue. The silver catfish were assigned to two groups with six animals each: uninfected animals (the negative control group) and experimentally infected animals (the positive control group) inoculated intragastrically with 300 μL of a bacterial suspension containing 7×10^7 CFU/mL, according to the protocol established by Baldissera et al. [9]. The negative control group received the same dose of sterile saline by the same route.

The methodology used in the experiment was approved by the Ethical and Animal Welfare Committee of the Universidade Federal de Santa Maria under protocol number 074/2014.

2.4. Sample collection and tissue homogenization

On day 7 post-infection (PI), all animals were euthanized by spinal cord section according to the Ethics Committee recommendations. Thereafter, the brain tissue was removed and dissected in a glass dish over ice and divided into two parts: the right hemisphere was used for the measurement of complex IV activity and to evaluate the brain histopathology, while the left hemisphere was used to measure CK activity. The hemispheres were washed in SET buffer (0.32 M sucrose, 1 mM EGTA, 10 mM Tris-HCl, pH 7.4) and homogenized (1: 10 w/v) in the same SET buffer with a Potter–Elvehjem glass homogenizer. The homogenates were centrifuged at 10,000×*g* for 15 min at 4 °C, and the supernatants were collected for determination of cerebral complex IV and CK activities. The supernatants were stored for no more than one week at −80 °C only when the assay was not carried out immediately.

2.5. Complex IV activity

Enzymatic activity of complex IV (cytochrome *c* oxidase) of the mitochondrial respiratory chain was measured in cerebral tissue according to the method described by Rustin et al. [22], as described in detail by Baldissera et al. [23]. The complex IV activity was calculated as nmol/min/mg of protein.

2.6. CK activity

Cerebral CK activity was assayed in the reaction mixture containing the following final concentrations: 10 mM Tris-HCl buffer, pH 7.5, 7 mM PCr, 1.5 mM $MgSO_4$, 0.625 mM N-dodecyl β-D-maltosidose and approximately 3 μg of protein in a final volume of 80 μL. After 10 min of pre-incubation at 37 °C, the reaction was started by the addition of 0.3 μmol of ADP and stopped after 10 min by the addition of 1 μmol of ρ-hydroxymercuribenzoic acid. The concentration of the reagents and the incubation time were chosen to assure linearity of the enzymatic reaction. Appropriate controls were carried out to measure chemical hydrolysis of PCr. The creatine level was estimated according to the colorimetric method of Hughes [24]. The color was developed by the addition of 0.1 mL of 2% α-naphthol and 0.1 mL of 0.05% diacetyl in a final volume of