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Virus induces metal-binding proteins and changed trace element balance in the brain during the course of a common human infection (coxsackievirus B3) in mice

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Abstract

Autopsy of the brain has shown a change in trace element balance in some virus-infected individuals, but it is not known whether this event was a result of the infection. In the present study coxsackievirus B3 (CVB3) adapted to Balb/c mice was used to study whether infection induces gene expression of the metal-binding/transporting proteins metallothionein (MT1 and MT3) and divalent-metal transporter 1 (DMT1) and whether it changes the balance of trace elements in the brain. Virus and MT1, MT3, and DMT1 were quantitatively measured by RT-PCR on days 3, 6 and 9 of the infection. Trace elements (13) were measured in serum and the brain by ICP-MS. High numbers of virus were found in the brain on days 3 and 6, but virus counts were decreased and present only in 50% of the mice on day 9. Gene expression of MT1 tended to increase on all days, whereas that of MT3 only showed a minor and not significant increase on day 3. No clear effect was observed in the expression of DMT1. The increase of MT3 was correlated to the brain concentration of Cu. The Cu/Zn ratio in serum increased as a response to the infection. There was a similar decrease in Cd in serum and the brain. On day 6 of the infection, Hg increased in the brain ($p<0.05$) and was positively correlated to a concomitant decrease ($p<0.05$) in serum. Virus numbers in the brain were on day 6 positively correlated ($p<0.05$) to As concentrations. Enteroviral infections may therefore be an underlying factor regarding the changes in essential as well as potentially toxic trace elements in the brain.

Keywords: Brain; MT1; MT3; DMT1; Trace elements; Virus

1. Introduction

A number of studies have demonstrated changed concentrations of single essential and non-essential trace elements in the brain, both during aging (Markesbery et al., 1984a,b) and in certain pathological conditions (Chazot and Broussolle, 1993; Dexter et al., 1989). In

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particular, changes in the concentrations of the essential elements iron (Fe), copper (Cu) and zinc (Zn) have been observed in relation to pathological conditions of the brain, such as Parkinson's disease (Dexter et al., 1989) and Alzheimer's dementia (Chazot and Broussolle, 1993; Waggoner et al., 1999). Moreover, autopsy of the brain has shown accumulation of Fe in HIV infected individuals, but it is not known whether this event was a result of the HIV infection (Boelaert et al., 1996).

In general, acute infectious diseases, regardless of etiology and target organs of microorganisms, are associated with an acute-phase reaction, including changed concentrations of Fe, Cu and Zn in the blood that are normalized when the infection is over (Beisel, 1998; Funseth et al., 2000a,b; Ilback et al., 2003a,b). These trace elements are crucial for the host defense (Pekarek and Engelhardt, 1981), including the development of inflammation (Milanino et al., 1993; Nystrom-Rosander et al., 2004) and for the growth and virulence of many microorganisms (Beck et al., 1994; Krenn et al., 2005; Shankar and Prasad, 1998; Weinberg, 1999).

Almost all humans contract several coxsackievirus, including coxsackievirus B3 (CVB3), and other enterovirus infections during their lifetime, the majority of which pass unrecognized or cause only minor illness of the upper respiratory or gastrointestinal tract. However, the enteroviruses are the most common cause of meningitis in many countries including the USA and an important although infrequent cause of encephalitis (Rotbart and Webster, 2001). The murine infectious model of CVB3 shows a disease development similar to that in humans (e.g., involvement of target organs, including the pancreas, heart, meninges and brain) (Fohlman et al., 1990; Ilback et al., 1990; Woodruff, 1980). During the viremic phase target organs are infected and the characteristic acute-phase reaction initiated, including extensive shifts in metabolic pathways, in order to meet the nutritional needs of the activated immune defense (Beisel, 1998; Ilback and Friman, 2007; Ilback et al., 1984).

Metal-binding proteins, including acute-phase proteins, serve as carriers for essential trace elements, such as Fe-binding ferritin (Beisel, 1998) and divalent-metal transporter 1 (DMT1) (Burdo et al., 2001; Ke et al., 2005), Cu-binding ceruloplasmin (Friman et al., 1982) and the Zn-binding metallothioneins (MTs) 1 to 4 (Ilback et al., 2004; Nordberg and Nordberg, 2000). Reciprocally, trace elements are required for the activity of a number of acute-phase proteins and immune cells that directly participate and interact in host defense processes (Beisel, 1998; Ilback and Friman, 2007). As a result, during ongoing infection there is a flux of trace elements between blood and other tissues, including the tissues that are involved in the disease. Although less is known about infection-induced changes in non-essential trace elements, some such changes have been described that may not be favorable to the host (e.g., an increased redistribution of cadmium (Cd) from the liver to the kidneys) (Ilback et al., 2004), as well as increased mercury (Hg) concentration in red blood cells (Frisk et al., 2007). Whether these and earlier described trace element changes in infection also involve the brain is not known, however.

One aim of the present study was to identify infection-associated changes of trace elements in the brain and serum during the course of a viral infection (CVB3) that commonly occurs in humans. Another aim was to study whether trace element changes in the brain are associated with the replication of virus and the gene expression of metal-binding/transporting proteins.

2. Materials and methods

2.1. Mice

Adult female Balb/c mice aged 8–10 weeks (Charles River) were maintained at the animal department, Biomedical Center (Uppsala, Sweden). The mice were randomly assigned to groups of similar initial mean body weight (19.7 ± 0.7 g) and housed at 23 ± 1 °C on a 12-hour light/dark cycle behind hygienic barriers with free access to food (R3; Ewos, Södertälje, Sweden) and water. Control and infected mice were studied simultaneously.

The animal experiments described in this publication took into account all ethical aspects of the welfare of animals following the recommendations in "Guide for the Care and Use of Laboratory Animals" of the Swedish National Board for Laboratory Animals (CFN). The study was approved (C127/4) by the local Ethical Committee for Experimental Use at the Faculty of Medicine, Uppsala University.

2.2. Virus

Myocarditic CVB3 was used (Fohlman et al., 1990; Ilback et al., 1998; Ilback et al., 1990). The virus was propagated in HeLa cells, which were grown in Eagle minimal essential medium supplemented with 5% fetal calf serum and antibiotics (kanamycin). Virus titers in HeLa cells were determined as plaque-forming units (pfu) and a stock solution was stored at −20 °C until use. The stock solution of 10^7–10^8 pfu/ml was diluted with phosphate-buffered saline to get 10^5 pfu/ml.