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Arsenic is decreased in target organs during viral infection in mice

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Abstract

Arsenic (As), a potentially toxic trace element, has been shown to influence viral replication and resistance to microbial infection. However, the impact of infection on the normal As status in target organs involved in the disease process has not been studied to date. In the present study, As was measured through inductively coupled plasma mass spectrometry in the plasma, liver, spleen, kidney, heart, pancreas and brain at days 1 and 3 of coxsackievirus B3 infection in female Balb/c mice. The severity of the infection was assessed from clinical signs of disease. The infection changed plasma As in a biphasic pattern with a small increase (n.s.) at day 1 that turned into a decreasing trend (13%, $p<0.05$) by day 3. In the liver, spleen, heart, pancreas and kidney As was unchanged at day 1 but, at day 3, it had decreased by 71% ($p<0.01$), 64% ($p<0.01$), 55% ($p<0.01$), 63% ($p<0.01$) and 73% ($p<0.01$), respectively. In the brain, As went unchanged. The pathophysiological interpretation of these findings requires further research.

Keywords: Arsenic; Organ; Virus

Introduction

Several trace elements are essential for biological processes while others are known only for their toxicity [1]. Arsenic (As) occurs naturally in various concentrations in soils, plants and animals, including man [2]. It is an environmental agent considered as a high priority toxic substance largely because of its carcinogenic potential [3,4]. Although, or rather because As has the potential to act as a poison, it has been in medicinal use for more than 2400 years [4].

The primary biochemical mechanism of As toxicity is binding of the element to cellular sulfhydryl groups, resulting in inhibition of numerous cellular enzyme systems [2,3]. Several proteins with high cysteine content and accessible thiol groups are candidates for interactions with As [4]. It seems that the toxic/therapeutic effects of As are mediated, at least in part, by redox-sensitive proteins and enzymes [4]. Some effects of As on the immune system have been studied, but it is not known whether and to what extent it is involved in the inflammatory processes in the target tissues and organs of infectious micro-organisms.

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Although classified as a human carcinogen, arsenic trioxide (As_2O_3) is an effective and relatively safe drug in the treatment of acute promyelocytic leukemia [5]. Several studies confirm that As_2O_3 may be a valuable therapeutic tool in certain other cancer forms as well [5,6]. Moreover, early results show that the replication of bacteriophages [7] and the virus of hand-foot-and-mouth disease, i.e. coxsackievirus as used in the present study [8], were inhibited by treatment of bacteria and virus tissue cultures, respectively, with As. Before the advent of antibiotics, As was used in the treatment of syphilis during approximately 40 years [4] but, because of its carcinogenic potential, its only accepted use today relative to treating infectious diseases is in trypanosomiasis involving the central nervous system.

One characteristic host response during the early stage of an acute infectious disease is an increased synthesis of metal-binding proteins in several target organs involved in the infectious process [9,10]. An effect associated with this acute-phase response is altered dynamics of several essential and non-essential trace elements, resulting in changed plasma and tissue concentrations [9,11–13]. Although it is believed that certain trace elements are crucial for the host defence [9,14], including the development of inflammation [15,16], as well as for the virulence of micro-organisms [17,18], the biological purpose of most of these infection-induced changes remains virtually unknown.

Almost all humans contract several coxsackievirus 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. The murine infectious model of coxsackie B virus type 3 (CB3) has a pathogenesis that closely resembles CB3 myocarditis in humans. It is characterized by a short lasting viremic phase, during which viruses infect the target cells, primarily in the heart and pancreas [19]. The virus causes pancreatitis [20] and cardiomyocyte damage by apoptosis, lipid accumulation and necrosis [21,22]. Therapy with the essential trace element Se [16], as well as with immune stimulatory [23], and antiviral drugs [22,24] has partly been successful in providing protection to the heart. Thus, there is a need for new and more efficient therapeutic strategies in this and other viral infections and trace elements, including As, may be potential agents. However, the impact of infection on the normal As status in target organs of infection is unknown.

In the present study, As concentrations in the serum, liver, spleen, kidney, heart, pancreas and brain were sequentially and concomitantly studied during early CB3 infection using a well-described murine model of the human CB3 virus infection. The intention was to study the dynamics of As in target tissues of this common viral infection.

Material and methods

Mice

Adult female Balb/c mice were purchased from Charles River and maintained at the Animal Department, Biomedical Centre, Uppsala University. The mice were randomly assigned to groups of similar initial mean body weight and housed individually at $23 \pm 1\,^{\circ}C$ on a 12 h light/dark cycle behind hygienic barriers with free access to food (R3; Ewos, Södertälje, Sweden) and water.

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 by the local Ethical Committee for Experimental Use at the Faculty of Medicine, Uppsala University.

Experimental design

During the early phase of CB3 infection, as was presently studied (i.e. days 1 and 3 post-inoculation), groups of ($n = 6$ in each group) non-infected control mice (group 1) and infected mice sacrificed at days 1 (group 2) and 3 (group 3) of the infection were studied for changes in existent tissue As concentrations. As was measured in all sampled tissues, i.e. the plasma, liver, spleen, kidneys, heart, pancreas and brain on day 3 of the infection. However, on day 1 of the disease, the As content was measured in all organs except for the kidneys and brain. Days 1 and 3 of disease were chosen to represent the early incubation period of the disease and the viremic phase of the disease, respectively.

Virus

A myocarditic strain of CB3 was used [12,13,16,21,23]. The virus was propagated in HeLa cells, which were grown in Eagle's minimal essential medium supplemented with 5% fetal calf serum and antibiotics. Virus titres were determined on HeLa cells as plaque-forming units (pfu) and a stock solution was stored at $-20\,^{\circ}C$ until use. The stock solution of 10^7–10^8 pfu/mL was diluted with phosphate buffered saline to get 10^5 pfu/mL.

Infection

On day 0 of the infection, each mouse was inoculated intraperitoneally with approximately 2×10^4 pfu of CB3 virus. In pre-studies of adult mice, this dose and route of administration had been shown to produce 30%