Selenium Supplementation Decreases Coxsackievirus Heart Disease During Murine AIDS

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Abstract

Coxsackievirus B3 (CVB3) induces myocarditis, especially in the immunodeficient or immature. To investigate whether CVB3 induced pronounced cardiomyopathy during the severe immune dysfunction of murine acquired immunodeficiency syndrome (AIDS), female C57BL/6 mice were infected with LP-BM5 retrovirus and then coinfected with CVB3. C57BL/6 mice, essentially resistant to CVB3-induced cardiomyopathy, became susceptible to this cardiomyopathy because of the immune dysfunction caused by murine AIDS. This susceptibility suggests that retrovirus infection causes conditions favoring the CVB3 induction of cardiac lesions. Mice were fed a diet supplemented with selenium (Se) at nine times the recommended daily dose for mice (0.933 mg/kg of diet). Heart tissues were analyzed histopathologically 12 d after CVB3 challenge. Mice experiencing concurrent retrovirus and CVB3 infection had a high degree of cardiac lesions that were consistent with myopathy compared to that in uninfected mice (p < 0.05). Se supplementation during murine retrovirus infection significantly diminished the pathogenesis caused by concurrent CVB3 infection in mice that had murine AIDS. There was a significant increase in the survival of dually retrovirus and CVB3-infected mice that were fed Se, compared to that of identically treated mice that were not fed Se. Hepatic lipid peroxides were significantly diminished in the Se-supplemented mice as compared to those in immunodeficient mice without supplementation (p < 0.1). Increased oxidation occurred in mice that had murine AIDS, which was also shown by reduced tissue vitamin E. Therefore, the reduction of retrovirally induced oxidation and inflammation by Se produced conditions that improved the survival of mice during CVB3 infection.

Key Words: Coxsackievirus B3; selenium; murine AIDS; retrovirus.

Introduction

Selenium (Se) is an essential trace mineral that has a fundamental role in human and animal health. Se influences both the cellular and humoral arms of the immune response (1–5). Marked decreases in plasma Se are seen in patients who are admitted into intensive care units for systemic inflammatory response syndrome, sepsis, or direct ischemia/ perfusion (5). Adequate Se intake protects hosts against viral
or bacterial infections (1,3,6–8). Therefore, Se may enhance resistance to infection via a more efficient T-helper-1/T-helper-2 (Th1/Th2) response. Patients who have human immunodeficiency virus (HIV) have low levels of Se, and Se supplementation is associated with the improvement of T-cell function, reduced apoptosis, and an increase in patient survival (9). Micronutrient and, specifically, Se deficiencies accentuate immunodeficiency in AIDS while affecting the pathogenicity of several viruses (4). Therefore, the mechanisms for greater survival caused by Se supplementation were investigated.

Murine acquired immunodeficiency syndrome (AIDS) induced by LP-BM5 murine leukemia retrovirus has been useful in studying retrovirus-induced immunodeficiency (10). Murine AIDS, like human AIDS, has a wide array of immunological abnormalities that include B- and T-cell functional deficiencies, causing a loss of T-cells from thymus and mucosal surfaces. Our objective was to determine whether Se supplementation would ameliorate the myocarditis and prolonged survival caused by CVB3 coinfection in murine AIDS.

Material and Methods

Mice

Four-week-old female C57BL/6 mice were obtained from Charles River Laboratories (Wilmingon, DE, USA). Mice were housed four per cage at the University of Arizona Central Animal Facility, which is fully approved by the American Association for Accreditation of Laboratory Animal Care. Animals were maintained under protocols approved by the University of Arizona Committee on Animal Research. The housing facility was kept at 70°F and 35–40% relative humid-ity and had a 12-h light/dark cycle. Mice were fed specific diets and had water ad libitum. Mice were randomly assigned to one of the following groups: retrovirus infected, retrovirus infected with Se-supplementation, retrovirus + CVB3 infected, and retrovirus + CBV3 infected with Se supplementation.

LP-BM5 Murine Leukemia Infection

The retrovirus-infected mice received an intraperitoneal treatment of 0.1 mL inoculum containing the virus in Minimum Essential Medium (MEM) with an esotropic titer (XC) of 4.5 log_{10} (plaque-forming units) × 10^{-3}/L, which induces disease within a time-course comparable to that previously published (11). The retrovirus infection period was 105 d before CVB3 infection (12). The infection of C57BL/6 mice that had the murine AIDS retrovirus led to rapid induction of immunologic disorders and had many similarities to human AIDS (10).

Selenium Supplementation

Mice were divided into four groups and fed either a Se-supplemented or Se-adequate diet. The AIN-76A diet was fed to the Se-adequate control group and had mineral mix corresponding to 0.1 mg Se/kg diet (Salt Mix #200000, DYETS). Se-supplemented groups were fed 0.933 mg of Se/kg diet, 0.833 mg Se in the form of sodium selenite, and 0.1 mg from mineral mix. Vitamin Mix #300050 was added to both diets at 1%. A ninefold increase of Se in the diet does not show a risk of toxicity (13,14).

CVB3 Infection

After 15 wk of Se supplementation, mice were inoculated intraperitoneally with 3 × 10^5 tissue culture infectious dose-50 (TCID_{50}) of CVB3 strain 59 in 0.1 ml of MEM. In short, cardiovirulent CVB3 strain 59 stocks were propagated in HeLa cell monolayers in MEM supplemented with 10% fetal bovine serum and 50 mg/L gentamicin (Gibco-BRL, Gaithersburg, MD) at 37°C in a humidified 5% CO₂ atmosphere. The virus was titered by TCID_{50} (15). Mice were euthanized after 12 d of CVB3 infection.

Histopathology

At indicated times after inoculation, mice were euthanized and their hearts were removed for study. Hearts were rinsed in saline and transversely cut in half. One-half of each heart was immediately placed into Histochoice Tissue Fixative (AMRESCO, Solon, OH, USA) and stored at room temperature. Fixated hearts were sectioned (6 µm) on a Zeiss HM 505 N cryostat (Carl Zeiss, Thornwood, NY, USA) and stained with hematoxylin and eosin. The severity of inflammatory lesions within the myocardium was graded by a pathologist who had no knowledge of the other experimental variables. Grading was done in a semiquan-itative manner according to the relative degree (from heart to heart) of mononuclear cell infiltration and the extent of necrosis.