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Original article

Diverse stage-dependent effects of glucocorticoids in a murine model of viral myocarditis

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ABSTRACT

Background: The effects of glucocorticoids on viral myocarditis are contentious. The aim of the present study was to determine whether there is a "window of opportunity" for glucocorticoid treatment in a mouse model of acute viral myocarditis induced by Coxsackie group B3 virus (CVB3).
Methods: A/J (H-2a) mice were randomly assigned to one of four experimental groups: (1) viral infection without dexamethasone (DEX) treatment; (2) treatment with 0.75 mg/kg, i.p., DEX each day for 5 days prior to viral infection; (3) 0.75 mg/kg, i.p., DEX treatment for 5 days immediately after viral infection; and (4) 0.75 mg/kg, i.p., DEX treatment for 5 days starting on day 7 after infection.
Results: DEX administration before or immediately after viral infection improved survival and attenuated left ventricular dilatation, systolic dysfunction, fibrosis, and infiltration of immune cells in the post-infectious heart. In contrast, late administration of DEX reduced survival (as determined on day 14), and was associated with sustained increases in cardiac tumor necrosis factor-α and interferon-γ levels. The beneficial effects of early DEX administration on survival were completely abrogated by coadministration of a selective cyclooxygenase (COX)-2 inhibitor (NS-398; 5 mg/kg per day, p.o.). Notably, the virus titer in the post-infectious heart was significantly suppressed by DEX, but coadministration of NS-398 at the time of viral infection abolished the suppressive effects of DEX and, in fact, increased virus titers.
Conclusions: Early administration of DEX is beneficial in the treatment of fulminant viral myocarditis, whereas late administration of DEX is harmful. The beneficial effects of DEX on survival were completely abolished by simultaneous administration of a selective COX-2 inhibitor. Hence, we speculate that a direct action of DEX on cardiomyocytes, rather than anti-inflammatory effects of DEX on immune cells, confers resistance to myocardial damage induced by viral infection.

Introduction

Long-term morbidity and mortality of viral myocarditis are related to abnormal immune responses and inflammation, thus immunosuppression was considered beneficial in the treatment of myocarditis. However, two randomized clinical trials [1,2] failed to confirm any beneficial effects of immunosuppressive therapy (consisting of prednisone alone or in combination with either cyclosporin or azathioprine) in patients with myocarditis. On the basis of these findings, there is now a consensus among cardiologists that immunosuppressive agents should not be prescribed routinely for the treatment of viral myocarditis. Nevertheless, steroids are often administered in addition to hemodynamic support for patients with fulminant myocarditis, with this treatment regimen reported to be successful [3–5].

The ability of glucocorticoids (GCs) to limit the acute inflammatory response is well accepted. GCs act on leukocytes and endothelial cells to attenuate leukocyte–endothelial cell interactions and to reduce the generation and release of proinflammatory cytokines and mediators. However, little is known about the genomic actions of GCs on cardiomyocytes. The glucocorticoid receptor (GR) is expressed abundantly in cardiomyocytes, and so steroid therapy may directly affect the gene expression profile of cardiomyocytes. Previously, we demonstrated that selective activation of the GR upregulated cyclooxygenase (COX)-2 and prostaglandin (PG) biosynthesis in cardiomyocytes, and that this phenomenon was responsible for GR-mediated cardioprotection against ischemia–reperfusion injury [6]. The importance of PGs in

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the pathology of inflammation is evidenced by the success of COX-2 inhibitors in mitigating such conditions. However, PGs seem to have protective effects in the heart, where COX-2 inhibitors have been reported to worsen a variety of pathophysiological conditions in mice, including ischemia–reperfusion injury [7], left ventricular remodeling after myocardial infarction [8], doxorubicin-induced myocardial injury [9], and acute viral myocarditis [10]. The use of nonsteroidal anti-inflammatory drugs during myocarditis has also been associated with increased mortality [11].

The aim of the present study was therefore to determine whether there is a "window of opportunity" for the use of GCs in the treatment of acute viral myocarditis and, if so, to evaluate the role of COX-2 activity in the cardioprotection conferred by GC treatment.

Methods

This study was approved by the Institutional Animal Care and Use Committee of Yamaguchi University and was performed in accordance with the guidelines of the American Heart Association and Yamaguchi University School of Medicine, as well as Law No. 105 and Notification No. 6 of the Japanese government.

Animals and experimental protocols

Coxsackievirus B3 (CVB3; Nancy strain), an important human pathogen that induces acute viral myocarditis, was obtained from American Type Culture Collection and stored at −80 °C until use. Three-week-old, inbred, certified virus-free A/J (H-2a) male mice were purchased from Japan SLC (Shizuoka, Japan). A/J (H-2a) mice are susceptible to CVB3-induced myocarditis and develop a severe course of acute viral myocarditis. A/J (H-2a) mice that were not infected with the virus served as the control group. All mice were initially infected by i.p. injection of 2×10^4 plaque-forming units (p.f.u.) CVB3 in 0.2 mL saline.

Following inoculation, mice were randomly assigned to one of four treatment groups ($n = 10$ mice per group) and injected with either 0.75 mg/kg, i.p., dexamethasone (DEX) or a similar volume of the vehicle control (2% dimethyl sulfoxide). As indicated in Fig. 1A, the four treatment groups were: (1) virus infection without DEX administration (no DEX treatment); (2) DEX treatment for the 5 days prior to virus infection (DEX preinfection); (3) DEX treatment for the 5 days immediately after virus infection (DEX early postinfection); and (4) DEX treatment for 5 days starting on day 7 after infection (DEX delayed postinfection).

Infected mice were isolated, five per cage, in a special unit for 14 days. After 14 days, the mice were anesthetized with 5% pentobarbital (0.015 mL/g) and their hearts removed. The hearts were cut in half along the midline, with one half fixed in 10% buffered formalin and embedded in paraffin according to standard procedures, and the other half were plunged into liquid nitrogen and then stored until use.

In some experiments (Fig. 1B), mice were co-administered 5 mg/kg per day, p.o., NS-398 (a selective COX-2 inhibitor; EMD Biosciences, Darmstadt, Germany; Fig. 1B), starting at the same time as viral inoculation [10].

Morphometry and histopathology

Paraffin-embedded ventricular tissues were cut into 4-μm sections and stained with hematoxylin–eosin and Azan solution. Left ventricular dimensions and wall thickness were measured using the transverse section of the middle portion of each ventricle. The cavity dimensions and the wall thickness of the left ventricle were calculated using the methods described by Takata et al. [12].

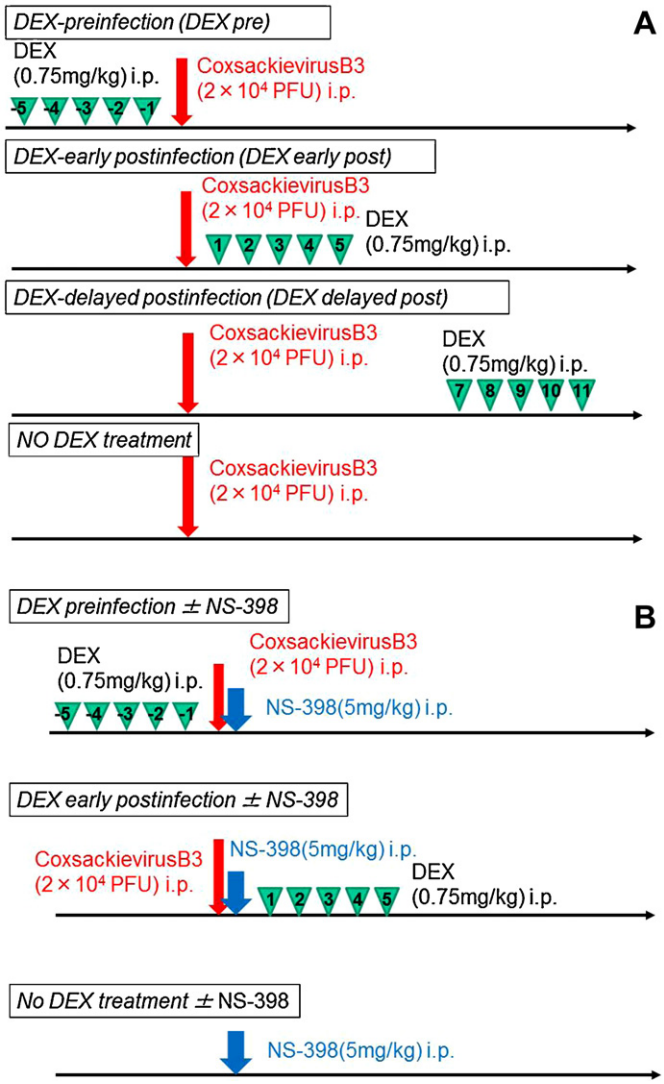

Fig. 1. (A) Diagram showing the different treatment protocols. Mice were randomly assigned to one of four experimental groups: (1) viral infection without dexamethasone (DEX) treatment (no treatment); (2) daily DEX treatment for the 5 days prior to viral infection (DEX preinfection); (3) daily DEX treatment for the 5 days immediately after viral infection (DEX early postinfection); and (4) DEX treatment for 5 days starting on day 7 after infection (DEX delayed postinfection). All surviving mice were killed for histological evaluation 14 days after viral inoculation. (B) Diagram showing the experimental protocol for simultaneous administration of NS-398 or vehicle (0.9% normal saline) with virus inoculation. PFU, plaque-forming units.

Immunohistochemistry

Ventricular tissues frozen in OCT compound (Miles, Elkhart, IN, USA) and stored at −80 °C were used for immunoenzymatic staining. Frozen samples were cut into 4-μm sections and stained using a DAKO LSAB kit (Dako, Carpinteria, CA, USA) according to the manufacturer's instructions. The primary antibody used was against murine lymphocytes (anti-mouse CD45; R&D, Minneapolis, MN, USA). The intensity of immunostaining was graded semiquantitatively on a three-point scale as follows: 1+, positive staining in <10% of cells; 2+, positive staining in 10–30% of cells; and 3+, positive staining in >30% of cells [13,14].