Pathophysiology 23 (2016) 237–242

Contents lists available at ScienceDirect

Pathophysiology

journal homepage: www.elsevier.com/locate/pathophys

Elevated cardiac 3-deoxyglucosone, a highly reactive intermediate in glycation reaction, in doxorubicin-induced cardiotoxicity in rats

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ARTICLE INFO

Article history:
Received 19 October 2015
Received in revised form 19 July 2016
Accepted 3 August 2016

Keywords:
3-Deoxyglucosone
Doxorubicin
Cardiotoxicity
Advanced glycation end-products

ABSTRACT

3-Deoxyglucosone (3-DG) is a highly reactive carbonyl intermediate in glycation reaction (also known as Maillard reaction) and plays an important role in diabetic complications. We investigated the potential involvement of 3-DG in doxorubicin (DXR)-induced cardiotoxicity. Male Crl:CD(SD) rats received intravenous injections of DXR at 2 mg/kg, once weekly, for 6 weeks, with/without daily intraperitoneal treatment with 3-DG scavenging agents, i.e., aminoguanidine (AG, 25 mg/kg/day) and pyridoxamine (PM, 60 mg/kg/day). Cardiac levels of 3-DG, thiobarbituric acid reactive substances (TBARS), fructosamine, and pentosidine, plasma glucose levels and cardiac troponin I (cTnI), echocardiography, and histopathology were assessed at 4 and 6 weeks after treatment. Cardiac 3-DG levels were significantly increased by DXR treatment at 4 and 6 weeks. Cardiac fructosamine levels and plasma glucose were not altered by DXR; however, TBARS levels in the heart were significantly increased at 4 and 6 weeks, suggesting that the enhanced generation of 3-DG is not attributed to any abnormal glycemic status, but may be related to oxidative stress by DXR. An advanced glycation end-product, pentosidine, was significantly increased by DXR treatment at 6 weeks. Intervention by AG and PM ameliorated the DXR-induced echocardiographic abnormalities, increased cTnI in plasma, and histopathological lesion as well as normalizing the elevation of 3-DG and pentosidine levels. These results suggest that 3-DG is generated by DXR and involved, at least in part, in the pathogenesis of DXR-cardiotoxicity through glycation reaction.

1. Introduction

Doxorubicin (DXR) is a potent chemotherapeutic agent used in the treatment of several cancers including solid tumors, leukemias and lymphomas [1]. Despite its high antitumor efficacy, the clinical value of DXR has been limited by cardiotoxicity associated with chronic treatment that leads to a form of congestive heart failure. Although numerous hypotheses for the mechanisms have been proposed, enhanced oxidative stress to the cardiomyocyte has been widely implicated as the primary cause for DXR-induced cardiomyopathy [2–5]. DXR undergoes one-electron reduction to a semiquinone radical by mitochondrial NADH dehydrogenase and microsomal NADPH-P450 reductase. The semiquinone radical is then re-oxidized by reacting with molecular oxygen, resulting in the formation of superoxide anion radicals and other reactive oxygen species.

3-Deoxyglucosone (3-DG) is a highly reactive carbonyl intermediate in the glycation reaction (also known as Maillard reaction) [6,7]. It plays an important role in the pathophysiology of diabetic complications [8,9]. In diabetes mellitus, hyperglycemia enhances the generation of 3-DG through fragmentation of Amadori product and degradation from an intermediate in the Polyol pathway. 3-DG then reacts with protein amino residues leading to the formation of stable and irreversible advanced glycation end-products (AGEs) such as carboxymethyllysine, pyrraline, and pentosidine, which affect intracellular and extracellular structure and function in many different cell types [10–12]. Abordo et al. suggested that oxidative stress also enhances the biogenesis of 3-DG and documented an intracellular accumulation of 3-DG in murine P388D1 macrophages exposed to hydrogen peroxide and 1-chloro-2,4-dinitrobenzene [13].

Abbreviations: DXR, doxorubicin; AGE, advanced glycation end-product; 3-DG, 3-deoxyglucosone; AG, aminoguanidine; PM, pyridoxamine; cTnI, cardiac troponin I; TBARS, thiobarbituric acid reactive substances; MDA, malondialdehyde.

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http://dx.doi.org/10.1016/j.pathophys.2016.08.001
0928-4680/

Based on the redox cycling ability of DXR and the reported evidence of enhanced formation of 3-DG under oxidative stress, it is logical to hypothesize that 3-DG is elevated by DXR and contributes to the pathogenesis of DXR cardiotoxicity. The purpose of this study was to evaluate cardiac 3-DG levels in a DXR-cardiomyopathy rat model and to further understand the mechanism of 3-DG involvement in DXR-cardiotoxicity by using 3-DG scavenging agents aminoguanidine (AG) [14,15] and pyridoxamine (PM) [16,17].

2. Materials and methods

2.1. Experimental protocol

The study was conducted in facilities approved by the Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC) International, and all experimental procedures were pre-approved by the internal animal ethics committee. A total of 66 male Crl:CD(SD) rats, age 8 weeks, were divided into 6 groups by body weight-balanced randomization prior to the initiation of experiments. Groups consisted of control (n = 10), aminoguanidine (AG: n = 10), pyridoxamine (PM: n = 10), DXR (n = 12), DXR+AG (n = 12), and DXR+PM (n = 12). Rats in the control, AG, and PM groups received saline intravenously (i.v.), and those in the DXR, DXR+AG, and DXR+PM groups received 2 mg/kg DXR, i.v. once per week. In addition, saline, AG (25 mg/kg/day), or PM (60 mg/kg/day) was injected intraperitoneally (i.p.) daily for 6 weeks, from the initiation of the experiments. The dose levels of AG and PM were selected based on the reports by Matsumoto et al. [18] and Muellenbach et al. [19], respectively; and, these dose levels were confirmed not to be associated with any abnormal clinical signs or any changes in hematological or serum biochemical examinations in the present study (data not shown). All animals were checked for mortality and clinical signs throughout the experimental period. 5–6 rats/group were necropsied under isoflurane anesthesia at weeks 4 and 6. Prior to necropsy, animals were fasted overnight, anesthetized with isoflurane, and bled from the orbital sinus into EDTA-treated tubes for determination of plasma glucose and cTnI. The heart was then rapidly excised, washed with cold physiological saline, and blotted dry. A portion of the heart was homogenized in phosphate buffered saline with 0.05% Tween 20 (pH 7.4) at 4 °C using a Polytron PT-MR 3100 tissue homogenizer (Kinematica Inc., NY, USA). Homogenates were then centrifuged at 600 × *g* for 10 min. Total protein concentrations in the supernatant were determined using Protein Assay Lowry Kit (Nacalai Tesque, Inc., Kyoto, Japan). The supernatant was used for the 3-DG, fructosamine, TBARS, and pentosidine assays. The remainder of the heart was stored in 10% neutral buffered formalin for histopathological examination.

2.2. Determination of cardiac 3-DG

3-DG levels were determined by derivatization with *o*-phenylenediamine and electrospray ionization liquid chromatography/mass spectrometry of the resulting quinoxaline according to the method devised by Nakayama et al. [20].

2.3. Determination of cardiac fructosamine

Cardiac fructosamine was determined by a colorimetric method using Modular Analytics P (Roche Diagnostics, Tokyo, Japan).

2.4. Determination of plasma glucose

Plasma glucose levels were determined by hexokinase method using Modular Analytics P (Roche Diagnostics, Tokyo, Japan).

2.5. Assay for thiobarbituric acid reactive substances (TBARS)

TBARS was determined using the method devised by Buege & Aust [21] and expressed as nmol of malondialdehyde (MDA) equivalents per mg protein.

2.6. ELISA for cardiac pentosidine

The pentosidine levels in the heart were determined using a commercially available competitive enzyme-linked immunosorbent assay (FSK pentosidine ELISA kit, Fushimi Pharmaceutical Co. Ltd., Kagawa, Japan) [22].

2.7. Echocardiography

Rats were anesthetized with Inactin® hydrate (40 mg/kg, iv). Left ventricular (LV) end-systolic and end-diastolic diameters were measured at the level of the papillary muscles using two-dimensional guided M-mode imaging (ECCOCEE SSA-340A, Toshiba Medical Systems Corporation, Tochigi, Japan) at 4 and 6 weeks. LV fractional shortening percentage (FS%) was assessed as the index of left ventricular contractility and was calculated as (LV end-diastolic diameter [LVDd] minus LV end-systolic diameter [LVDs])/LVDd × 100.

2.8. ELISA for plasma cTnI

Plasma levels of cTnI were determined using a commercially available sandwich enzyme-linked immunosorbent assay (High Sensitivity Rat Cardiac Troponin-I ELISA Kit, Life Diagnostics, Inc., PA, USA).

2.9. Histopathological examination

Formalin-fixed, paraffin-embedded heart samples were sectioned at 4 μm, stained with hematoxylin and eosin, and examined microscopically. The histopathological findings were graded as follows: very slight (+), slight (++), and moderate (+++). The examination was performed by Masayuki Kemi, D.V.M., Ph.D. who has extensive experience in preclinical toxicologic pathology evaluation of drugs and is a pathologist certified by Japanese Society of Toxicologic Pathology.

2.10. Statistical analysis

All data are presented as the means ±SE. For 3-DG, plasma glucose, fructosamine, TBARS, and pentosidine, statistical comparisons were first performed using Student's T-test between the control and DXR-alone groups. If there were statistically significant changes in the DXR-alone group, then one-way analysis of variance was executed followed by the Tukey-Kramer test among all groups to evaluate an ameliorative effect of the 3-DG scavenging agents. For echocardiography and cTnI, statistical comparisons were performed among all groups using one-way analysis of variance followed by the Tukey-Kramer test. A probability of $p < 0.05$ served as the criterion for statistical significance.

3. Results

All animals survived to the scheduled termination; and, there were no abnormal clinical signs in any groups throughout the experimental period.

Cardiac 3-DG levels were significantly increased 2.3- and 2.7-fold at 4 and 6 weeks of treatment, respectively, in the DXR-treated group compared to the control (Fig. 1).