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Original Contribution

Phenylbutyrate, a histone deacetylase inhibitor, protects against Adriamycin-induced cardiac injury

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Abstract

Cardiac injury is a major complication for oxidative-stress-generating anticancer agents exemplified by Adriamycin (ADR). Recently, several histone deacetylase inhibitors (HDACIs) including phenylbutyrate (PBA) have shown promise in the treatment of cancer with little known toxicity to normal tissues. PBA has been shown to protect against oxidative stress in normal tissues. Here, we examined whether PBA might protect heart against ADR toxicity in a mouse model. The mice were i.p. injected with ADR (20 mg/kg). PBA (400 mg/kg/day) was i.p. injected 1 day before and daily after the ADR injection for 2 days. We found that PBA significantly decreased the ADR-associated elevation of serum lactate dehydrogenase and creatine kinase activities and diminished ADR-induced ultrastructual damages of cardiac tissue by more than 70%. Importantly, PBA completely rescued ADR-caused reduction of cardiac functions exemplified by ejection fraction and fraction shortening, and increased cardiac manganese superoxide dismutase (MnSOD) protein and activity. Our results reveal a previously unrecognized role of HDACIs in protecting against ADR-induced cardiac injury and suggest that PBA may exert its cardioprotective effect, in part, by the increase of MnSOD. Thus, combining HDACIs with ADR could add a new mechanism to fight cancer while simultaneously decrease ADR-induced cardiotoxicity.

Keywords: Sodium phenylbutyrate; Adriamycin; Oxidative stress; Histone deacetylase inhibitor; Heart; Mitochondria; Antioxidant

Introduction

Adriamycin (ADR) is a potent anticancer drug that is used in treating both hematological and solid tumors [1]. However, severe cardiomyopathy and heart failure have been observed in ADR-treated cancer patients [2], which limit the clinical dosage of ADR in cancer treatments, i.e., 450 mg/m^2 body surface [1]. The anticancer activity and cardiotoxicity of ADR are mediated via different mechanisms [3]. DNA intercalation and DNA topoisomerase II inhibition underscore ADR anticancer activity [4]. Oxidative stress is generally held as the mediating mechanism in the multiple biological processes leading to ADR cardiotoxicity, e.g., redox-mediated superoxide radical production [5,6], tissue-specific mitochondrial DNA damage [7], and disturbances of calcium [8,9] or iron [10,11] homeostasis. Structurally, ADR is a quinone, which can generate a large amount of $O_2^{\bullet -}$ via a redox cycling reaction catalyzed by several endogenous reductases [5] and endothelial isoform of nitric oxide synthase [6]. $O_2^{\bullet -}$ in turn gives rise to a variety of more active reactive oxygen species, including H_2O_2, $^{\bullet}OH$, and $ONOO^-$, which trigger further oxidation of biomolecules [12]. Despite its side effects, ADR remains an important component in most chemotherapeutic regimens, due to its efficacy in treating a broad spectrum of cancers. Numerous research projects have focused on prevention of ADR-induced cardiac

Abbreviations: ADR, adriamycin; HAT, histone acetyltransferase; HDAC, histone deacetylase; HDACI, HDAC inhibitor; PBA, phenylbutyric acid; LDH, lactate dehydrogenase; CK, creatine kinase; MnSOD, manganese superoxide dismutase; DETAPAC, diethylenetriaminepentaacetic acid; TSA, trichostatin A; NBT, nitroblue tetrazolium; ER, endoplasmic reticulum

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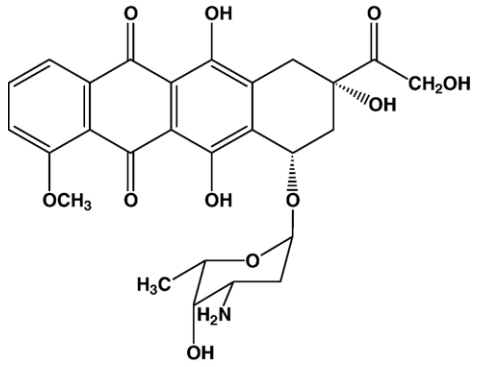
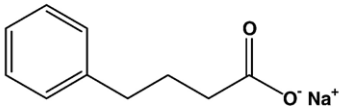


Adriamycin



Sodium phenylbutyrate

Fig. 1. Chemical structures of Adriamycin (ADR) and sodium phenylbutyrate (PBA).

injuries [2]. Coadministration of cardioprotective agents, which do not attenuate the anticancer activity of ADR, is one approach [13–17].

Acetylation homeostasis is a major epigenetic mechanism in cancer development and heart dysfunction [18–20], which is tightly regulated by the opposing histone acetyltransferases (HATs) and histone deacetylases (HDACs) [21,22]. Acetylation of histones opens chromatin structure for gene transcription and stabilizes and activates transcription factors for an increased activation of target genes [21]. Acetylation is negatively regulated by HDACs. In mammals, there are four classes of HDACs (class I, IIa, IIb, III, and IV) categorized based on homology to yeast HDACs [23]. Acetylation homeostasis can be easily modulated by the ever-growing members of HDAC inhibitors (HDACIs) [21,24], which are currently categorized into six structurally distinct groups: short-chain fatty acids, hydroxamates, cyclic tetrapeptides, benzamides, electrophilic ketone, and miscellaneous [23,25].

Phenylbutyric acid (PBA) is a short-chain fatty acid that has been clinically tested as an anticancer drug [26]. Toward normal tissues it not only shows little toxicity but also provides protections against various stimuli [27–32]. The anticancer activity of PBA is generally attributed to its function as an HDACI [23,26]. However, multiple activities can be assigned to its protection of normal tissues, such as the activities of an HDACI [28], a chemical chaperone [27,29,30], and an ammonia sink [31,32].

Based on the previous reports that PBA protects normal tissues against oxidative stress [27,28], we envisioned a possibility that PBA might protect heart from ADR injury. In this study, we investigated the effects of PBA on acute ADR cardiotoxicity in wild-type C57BL/6 mice by echocardiographic characterization, ultrastructural pathology analysis, and measurement of serum lactate dehydrogenase (LDH) and creatine kinase (CK) activities. We also examined the protein and activity levels of the primary antioxidant enzyme, manganese superoxide dismutase (MnSOD), in PBA-treated cardiac tissues.

Materials and methods

Reagents

Reagents of the highest grade available were purchased from Sigma (St. Louis, MO, USA), unless otherwise specified. Adriamycin (doxorubicin hydrochloride; Fig. 1) was purchased from Pharmacia, Inc. (Kalamazoo, MI, USA). PBA was obtained from Scandinavian Formulas Inc. (Sellesville, PA, USA).

Mice and treatment

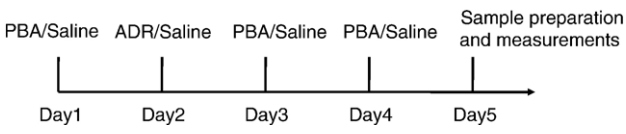
Wild-type male mice of inbred strain C57BL/6, 8–12 weeks of age and 22–28 g of body weight, were randomly divided into four groups, saline, ADR, PBA, and ADR+PBA, and treated according to Scheme 1. Further characterizations were performed after the treatments. Animal experiments were performed in accordance with NIH policies on the humane care and use of laboratory animals and approved by the Institutional Animal Care and Use Committee, University of Kentucky.

Echocardiography

Five mice were used in the saline and PBA treatments and seven were used in the ADR and PBA+ADR treatments. After the treatments, the mice were anesthetized with 2% isoflurane and echocardiographic images were taken with a Vevo 660 high-resolution Imaging system (VisualSonic, Toronto, Ontario, Canada) equipped with a high-frequency 30-MHz probe. Short-axis-motion-mode (M-mode) images were recorded at the papillary muscle level for cardiac function analysis.

Ultrastructural examination of cardiac tissues

Ultrastructural injury in cardiac tissues was evaluated by electron microscopic analysis of four mice from each treatment group using methods described by Yen et al. [33] with minor



Scheme 1. Treatment schedule. C57BL/6 mice were divided into four groups: saline, ADR, PBA, and ADR+PBA, and treated with i.p. injection ADR (20 mg/kg) or PBA (400 mg/kg/day). The same volume of saline was used in control treatment.