Biochemical Pharmacology 80 (2010) 540–547

Contents lists available at ScienceDirect

Biochemical Pharmacology

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

A toxicoproteomic study on cardioprotective effects of pre-administration of docetaxel in a mouse model of adriamycin-induced cardiotoxicity

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ARTICLE INFO

Article history:
Received 19 March 2010
Accepted 30 April 2010

Keywords:
Adriamycin-induced cardiotoxicity
Docetaxel pre-administration
Fluorogenic derivatization-liquid chromatography–tandem mass spectrometry
Toxicoproteomics

ABSTRACT

Studies suggest that pre-administration of docetaxel (DOC) in adriamycin (ADR)-DOC combination anticancer therapy results in stronger antitumor effects and fewer ADR-induced cardiotoxic deaths in mouse model, yet no mechanism explaining this effect has been established. The aim of this study was to identify cellular processes in mouse heart tissue affected by different ADR/DOC dosing protocols using a toxicoproteomic approach. We applied fluorogenic derivatization-liquid chromatography–tandem mass spectrometry (FD-LC–MS/MS) – which consists of fluorogenic derivatization, separation and fluorescence detection by LC, and identification by LC–tandem mass spectrometry – to the proteomic analysis of heart tissue from control, intermittent-dosing (DOC-ADR), and simultaneous-dosing (ADR&DOC) groups. In DOC-ADR group, ADR was administered 12 h after DOC injection; in ADR&DOC group, both drugs were administered simultaneously; in control group, saline was administered at the same timing as ADR injection of other groups. Heart samples were isolated from all mice 1 week after the treatment. The highly reproducible and sensitive method (FD-LC–MS/MS) identified nine proteins that were differentially expressed in heart tissue of control and the two treatment groups; seven of these nine proteins participate in cellular energy production pathways, including glycolysis, the tricarboxylic acid cycle, and the mitochondrial electron transport chain. Significantly higher expression of glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was observed in the DOC-ADR group, the group with the fewer cardiotoxic deaths, than in the ADR&DOC group. Therefore, GAPDH may have potential as a drug target for protective intervention and a biomarker for evaluation of the cardioprotective effects in pre-clinical studies.

1. Introduction

Although adriamycin (ADR) is an anthracycline anticancer drug that has been widely applied in treating a range of cancers (e.g., lymphoma, leukemia, breast cancer, and ovarian cancer), severe cardiotoxicity and heart failure have been observed in ADR-treated cancer patients [1]. In clinical trials for metastatic breast cancer, an ADR and docetaxel (DOC) combination therapy is much more effective than the previous combination therapies (*i.e.*, ADR-cyclophosphamide and fluorouracil-ADR-cyclophosphamide) [2,3]. However, severe toxicities including myelosuppression and cardiotoxicity limit the clinical use of ADR/DOC combination therapy in many patients with breast cancer [2–4].

Many attempts have been made to reduce the adverse effects induced by anticancer drugs, and one such approach has been chronotherapy. Chronotherapy is defined as the administration of medications using biological rhythms to optimize therapeutic outcomes and/or control adverse effect. The chronopharmacology of many antitumor drugs has been studied in human and animals specifically to decrease adverse effects [5–16]. The individual toxicities of ADR and DOC apparently depend on dosing time in animals and human [8–13]. Among the chronopharmacologic studies, To et al. reported that the DOC-pretreated group, in which ADR was administered 12 h after DOC injection, exhibited not only stronger inhibition of tumor growth but also a significant

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doi:10.1016/j.bcp.2010.04.037

reduction in cardiotoxic deaths compared with all the other co-administration groups and with the ADR-alone group in mice [14]. This remarkable finding has been subsequently studied in detail using mouse models [15,16], and the reduction in toxic death was found to be DOC dose-dependent [16]; however, no mechanism explaining the effect of DOC pre-administration has been established.

Proteomics is the large-scale study of gene expression at the protein level and provides information on dynamic cellular performance. As an integration of proteomics, toxicology, and bioinformatics, toxicoproteomics mainly focuses on protein changes in cells or tissues with exposure to toxicants, including antitumor drugs [17,18].

In proteomic studies, comparative expression profiling of proteins has usually been performed using two-dimensional electrophoresis (2-DE) because this has been the method of choice. However, the 2-DE method has some drawbacks with regard to the reproducibility of the data. Importantly, 2-DE often cannot reproducibly resolve minute differences in protein expression levels between samples from different treatment groups. In an effort to overcome the limitation of 2-DE, Imai and co-workers developed an easily reproducible and highly sensitive proteomic approach, fluorogenic derivatization-liquid chromatography–tandem mass spectrometry (FD-LC–MS/MS) method [19,20]. This method involves fluorogenic derivatization of proteins, followed by high-performance liquid chromatography (HPLC) of the derivatized proteins, isolation of those proteins with differential expression between the treatment groups, enzymatic digestion of the isolated proteins, and identification of the isolated proteins utilizing LC–tandem MS with a database-searching algorithm. This method enables highly sensitive detection and high resolution of proteins at the femtomol level due to the fluorogenic derivatization which utilizes a non-fluorescent reagent to yield highly fluorescent products. The applicability of the method has been demonstrated in the analyses of extracts from *Caenorhabditis elegans*, mouse liver, breast cancer cell lines, mouse brain, and thoroughbred horse skeletal muscle, revealing the proteins related to early stage Parkinson's disease, hepatocarcinogenesis, metastatic breast cancer, aging, and training effects, respectively [21–26].

The aim of this study was to identify the cellular processes affected by the pre-administration of DOC in ADR/DOC combination therapies using a toxicoproteomic approach based on FD-LC–MS/MS. The present study reported the differential analysis of mouse heart tissues isolated from control, intermittent-dosing (DOC-ADR), and simultaneous-dosing (ADR&DOC) groups.

2. Material and methods

2.1. Preparation of dosing drugs

ADR, supplied by Kyowa Hakko Kogyo Co., Ltd. (Tokyo, Japan), was dissolved in saline; the concentration was 2 mg/ml. DOC (Taxotere®, Sanofi-aventis, Bridgewater, NJ, USA) was dissolved in ethanol; 5% glucose in water was added to obtain the ratio of ethanol and glucose solution (3:97, v/v) and the final concentration of DOC was 1.25 mg/ml.

2.2. Animal treatment and tissue processing

Male ICR mice (5-weeks old) were purchased from Japan SLC (Nagasaki, Japan). Mice were housed 3–4 per cage under standardized light–dark cycle conditions (light on 7:00–19:00) at a room temperature of 24 ± 1 °C with free access to food and water. Animal care and experimental procedures were performed in accordance with the Guide for the Care and Use of Laboratory Animals

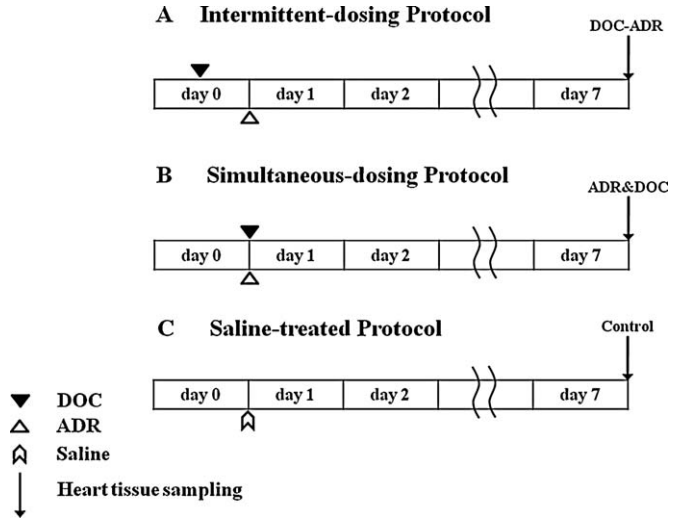

Fig. 1. Experimental protocol and time-line for the *in vivo* studies. *Abbreviations*: DOC, docetaxel (12.5 mg/kg i.v.); ADR, adriamycin (20 mg/kg i.v.). Heart tissue sampling: animal sacrifice, removal heart and processing.

(National Institute of Health) with approval from the Institutional Animal Care and Use Committee of Graduate School of Biomedical Sciences, Nagasaki University. Mice were divided into the intermittent-dosing group (DOC-ADR), in which ADR was administered 12 h after DOC injection; the simultaneous-dosing group (ADR&DOC), in which both drugs were administered simultaneously; and the saline-treated group (control), in which saline was administered at the same timing as ADR injection of the other groups. The anticancer drugs were intravenously administered once (20 mg/kg of ADR and 12.5 mg/kg of DOC) (Fig. 1). The ADR dose had been shown to be cardiotoxic in mouse [27]. The DOC dose had been shown to provide the strongest cardioprotection in the intermittent-dosing mouse group [16]. Also, when dosing intervals (6, 12, 24 h) between DOC and ADR treatments were changed, 12-h interval group showed the lowest toxic death rate [16]. Heart samples were isolated from all mice 1 week after ADR was administered in the ADR&DOC and DOC-ADR groups. All heart samples were immediately rinsed with phosphate buffer saline and frozen at −196 °C. All heart samples were homogenated using the Frozen Cell Crasher (Microtec Co., Ltd., Chiba, Japan). At least four mice were used in each experimental group (i.e., control, DOC-ADR and ADR&DOC), and all data was subjected to statistical analysis.

2.3. Preparation of samples and determination of total proteins

Homogenated heart tissues (50 mg) were suspended in 250 μl of 10 mM 3-[(3-cholamidopropyl)dimethylammonio]propanesulfonate (CHAPS) solution (Dojindo Laboratories, Kumamoto, Japan), and were centrifuged at $5000 \times g$ for 15 min at 4 °C. The supernatant was then collected and stored as a soluble fraction at −80 °C until use. The total protein content of the supernatant was determined with the Quick Start Bradford Protein assay kit (Bio-Rad Laboratory, Inc., Hercules, CA, USA) with bovine serum albumin as a standard protein by following the written instructions. After determination of total protein content, the supernatant was diluted with CHAPS solution to 2.4 mg total protein/ml and used as a starting protein sample.

2.4. FD-LC–MS/MS method

A 10-μl volume of sample was mixed with 42.5 μl of a mixture of 0.83 mM tris(2-carboxyethyl)phosphine hydrochloride (Tokyo Chemical Industry, Tokyo, Japan), 3.33 mM ethylenediamine-