Biochemical and Biophysical Research Communications 450 (2014) 1021–1026

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

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

Down-regulation of mitochondrial thymidine kinase 2 and deoxyguanosine kinase by didanosine: Implication for mitochondrial toxicities of anti-HIV nucleoside analogs

Ren Sun, Staffan Eriksson, Liya Wang *

Department of Anatomy, Physiology and Biochemistry, Swedish University of Agricultural Sciences, The Biomedical Centre, Box 575, SE-751 23 Uppsala, Sweden

ARTICLE INFO

Article history:
Received 16 June 2014
Available online 26 June 2014

Keywords:
Thymidine kinase 2
Deoxyguanosine kinase
Nucleoside analogs
ddI
Mitochondrial toxicity

ABSTRACT

Mitochondrial thymidine kinase 2 (TK2) and deoxyguanosine kinase (dGK) catalyze the initial rate limiting phosphorylation of deoxynucleosides and are essential enzymes for mitochondrial function. Chemotherapy using nucleoside analogs is often associated with mitochondrial toxicities. Here we showed that incubation of U2OS cells with didanosine (ddI, 2′,3′-dideoxyinosine), a purine nucleoside analog used in the highly active antiretroviral therapy (HAART), led to selective degradation of both mitochondrial TK2 and dGK while the cytosolic deoxycytidine kinase (dCK) and thymidine kinase 1 (TK1) were not affected. Addition of guanosine to the ddI-treated cells prevented the degradation of mitochondrial TK2 and dGK. The levels of intracellular reactive oxygen species and protein oxidation in ddI-treated and control cells were also measured. The results suggest that down-regulation of mitochondrial TK2 and dGK may be a mechanism of mitochondrial toxicity caused by antiviral and anticancer nucleoside analogs.

© 2014 Elsevier Inc. All rights reserved.

1. Introduction

Didanosine (ddI, 2′,3′-dideoxyinosine) is one of the first nucleoside reverse transcriptase inhibitor approved for use in anti-HIV therapy and it is still used in combination with other nucleoside or non-nucleoside reverse transcriptase inhibitors in highly active antiretroviral therapy (HAART) [1]. Treatment with nucleoside analogs is often associated with mitochondrial toxic side effect e.g., cardiomyopathy, neuropathy and lipodystrophy, especially common in patients treated with AZT (Zidovudine) and ddI. This has become apparent since HAART has significantly increased the life expectancy of HIV infected individuals [2,3]. ddI is toxic primarily to the nervous system, including peripheral neuropathy, headache and retinal toxicity; and the gastrointestinal system, including pancreatitis and hepatitis [4].

Unlike many other nucleoside analogs used in anti-HIV therapy, the initial phosphorylation of ddI is not catalyzed by cellular deoxynucleoside kinases, but rather by the 5′-nucleotidase/nucleoside phosphotransferase, using IMP (inosine monophosphate) as phosphate donor as depicted in Fig. 1 [5]. The product, ddIMP (2′,3′-dideoxyinosine 5′-monophosphate) is converted to ddAMP (2′,3′-deoxyadenosine 5′-monophosphate) by adenylosuccinate lyase and adenylosuccinate synthetase. ddAMP is then further metabolized to its active form ddATP (2′,3′-dideoxyadenosine triphosphate) by nucleoside mono- and diphosphate kinases [6]. Although ddA can be phosphorylated by cellular enzymes to ddATP, in cells the majority of ddA is rapidly deaminated to ddI by adenosine deaminase. In addition, ddI can be degraded by purine nucleoside phosphorylase to hypoxanthine. The mechanism of action of ddATP is inhibition of HIV reverse transcriptase by competing with natural occurring substrate e.g., dATP. In addition ddATP can act as chain terminator once incorporated into the nascent DNA chain by the HIV reverse transcriptase. ddATP can also inhibit mitochondrial DNA polymerase and thus causes mitochondrial toxicities (Fig. 1) [7,8].

Mitochondrial thymidine kinase 2 (TK2) and deoxyguanosine kinase (dGK) catalyze the initial rate-limiting phosphorylation of all four deoxynucleosides e.g., thymidine, deoxycytidine, deoxyguanosine and deoxyadenosine to their respective monophosphates and play an important role both for mitochondrial DNA (mtDNA) replication and nuclear DNA repair. Deficiency in either TK2 or dGK activity due to genetic mutations causes devastating mitochondrial DNA depletion syndrome (MDS), which is characterized by tissues specific mtDNA depletion or deletion [9,10]. Depletions of mtDNA have been reported in patients treated with either AZT (zidovudine, 3′-azido-2′,3′-dideoxythymidine) or ddI and are associated with myopathy, neuropathy, and cardiomyopathy,

* Corresponding author. Fax: +46 18 550762.
E-mail address: liya.wang@slu.se (L. Wang).

http://dx.doi.org/10.1016/j.bbrc.2014.06.098
0006-291X/© 2014 Elsevier Inc. All rights reserved.

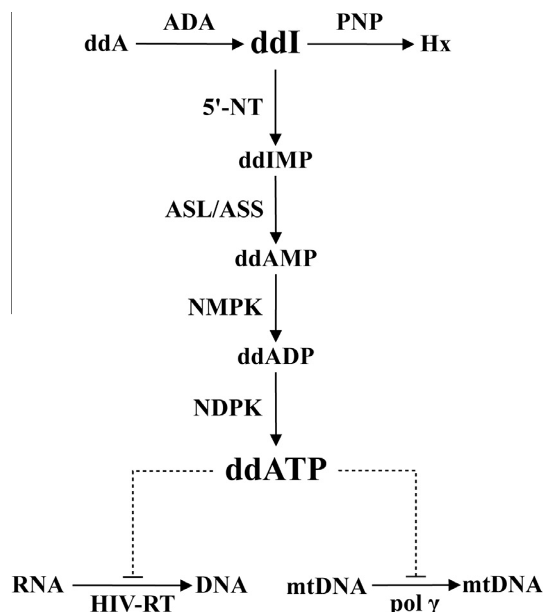

Fig. 1. Schematic representation of ddI metabolism and mechanism of action. ddI, 2′,3′-dideoxyinosine, ddA, 2′,3′-dideoxyadenosine, ddIMP, ddI monophosphate, ddAMP, ddA monophosphate, ddADP, ddA diphosphate, ddATP, ddA triphosphate; Hx, hypoxanthine. Enzymes: 5′-NT, 5′-nucleotidease/IMP phosphotransferase; ASL, adenylosuccinate lyase; ASS, adenylosuccinate synthetase; ADA, adenosine deaminase; NMPK, nucleoside monophosphate kinase; NDPK, nucleoside diphosphate kinase; HIV-RT, HIV reverse transcriptase; pol γ, mitochondrial DNA polymerase γ; PNP, purine nucleoside phosphorylase.

similar to what have been observed in the MDS patients with TK2 or dGK deficiency [2,11–13].

In this paper we describe our finding that treatment of cultured human cells with ddI caused severe reduction of mitochondrial dGK and TK2 levels, but had no effect on the cytosolic deoxycytidine kinase (dCK) or thymidine kinase 1 (TK1) levels. Co-incubation with guanosine reversed the effects of ddI on TK2 and dGK. The levels of intracellular reactive oxygen species (ROS) and protein carbonyl group were also determined.

2. Materials and methods

2.1. Materials

2′,3′-dideoxyinosine (ddI) was purchased from Carbosynth. Guanosine (Gua) was obtained from Sigma. Mouse monoclonal antibodies against cytochrome c oxidase subunit II (COX II) and subunit IV (COX IV), and anti α-tubulin antibody were purchased from Abcam. Polyclonal rabbit anti β-tubulin antibody was from Novus Biologicals. Polyclonal rabbit anti-human TK2 and dGK antibodies were produced using synthetic peptides chosen from the C-terminal sequences and affinity purified [14]. A mouse monoclonal anti-human TK1 antibody [15] obtained from AroCell AB (Uppsala, Sweden) and a polyclonal rabbit anti-human dCK antibody [16] were used to detect the TK1 and dCK proteins, respectively. All antibodies were diluted with recommended ratios in blocking buffer (0.05% Tween-20 and 5% non-fat milk in phosphate-buffered saline (PBS).

2.2. Cell culture conditions

U2OS (human osteosarcoma cell line, ATCC HTB-96™) cells were maintained in McCoy's 5A (Modified) medium (Gibco®, Cell Culture) supplemented with 10% fetal bovine serum (FBS, Gibco® Cell Culture), 100 U/ml penicillin and 0.1 mg/ml streptomycin at 37 °C with a humid atmosphere in the presence of 5% CO_2. All nucleosides were dissolved in dimethylsulfoxide (DMSO) as stock solutions and diluted in fresh cell culture medium prior to use. The final DMSO concentration in the complete medium was <0.05% (v/v).

2.3. Mitochondrial isolation and Western blot analysis

Mitochondria were prepared by differential centrifugation method as previously described [14,17]. For total cellular protein extraction, approximate 1×10^6 cells were re-suspended in 20 µl lysis buffer (50 mM Tris/HCl pH 7.6, 150 mM KCl, 5 mM $MgCl_2$, 5 mM DTT, 1× protease inhibitor cocktail (Roche), 0.5% NP-40, and 0.3 M sucrose) and cellular proteins were extracted by freezing and thawing thrice and sonication in an ice/water bath, followed by centrifugation at 16,000×g at 4 °C for 20 min. Protein concentration was determined by the Bradford method (Bio-Rad protein assay) using BSA as standard.

Mitochondrial proteins (30 µg/lane) and total cellular proteins (100 µg/lane) were subjected to 12% reducing SDS-polyacrylamide gel electrophoresis and transferred onto Immobilon polyvinylidene difluoride (PVDF, Millipore) membranes using a semi-dry transfer system. Membranes were incubated with blocking buffer at room temperature for one hour and then probed with the respective primary antibodies. The anti-mouse or anti-rabbit secondary antibodies conjugated with horseradish peroxidase (GE Healthcare) were applied to the membranes and the target proteins were detected by enhanced chemiluminescence immunodetection system (ECL kit; GE Healthcare). Band intensities were quantified by using the Quantify One/ImageLab software (Bio-rad).

2.4. Effects of ddI on mitochondrial TK2 and dGK and cytosolic TK1 and dCK levels

About 6×10^6 cells were seeded in 175 cm² tissue culture flasks and incubated in the presence of 20 µM ddI for 3 days. Mitochondria were then isolated and the levels of TK2 and dGK in mitochondrial extracts determined by western blot analysis with polyclonal rabbit anti human TK2 and dGK antibodies. In addition, guanosine (Gua) (20 µM) were supplemented to ddI treated cells as described above and mitochondria were then isolated and the levels of TK2 and dGK in mitochondrial extracts were determined [14].

To access the effects of ddI in the absence or presence of guanosine on the cytosolic TK1 and dCK levels, total cell lysates (~100 µg protein) prepared from ddI-treated and control cells were resolved on 12% SDS–PAGE and the levels of TK1 and dCK were determined by western blot analysis, using antibodies against human TK1 [15] and dCK [16].

2.5. Measurement of ROS levels and protein oxidation

Triplicates of 0.3×10^6 U2OS cells were seeded into individual wells of 6-welll flat-bottomed plates and incubated for three days in complete culture medium in the presence of various concentrations of ddI. To detect the intracellular ROS production, cells were treated with 5 µg/ml CM-H_2DCFDA/PBS for 15 min at 37 °C in dark. The cells were then trypsinized and re-suspended in PBS buffer. The intensity of oxidized CM-H_2DCFDA fluorescence was immediately measured by using a FACScan® flow cytometer/Cell Quest software (Becton Dickinson, CA, USA). The data for 10,000 events were collected for each sample and analyzed by the Flowjo software (Tree Star Inc, Asland, OR) and ROS levels were reported as the geometric mean florescence of the collected cells.

Protein oxidation (carbonylation) in total extracts of U2OS cells was evaluated by the Oxyblot assays, following the manufacturer's

Download English Version:

https://daneshyari.com/en/article/10754647

Download Persian Version:

https://daneshyari.com/article/10754647

Daneshyari.com