Free Radical Biology and Medicine 96 (2016) 273–289

Contents lists available at ScienceDirect

Free Radical Biology and Medicine

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

Hydrogen peroxide/ceramide/Akt signaling axis play a critical role in the antileukemic potential of sanguinarine

Anees Rahman [a], Faisal Thayyullathil [a], Siraj Pallichankandy [a], Sehamuddin Galadari [a,b,*]

[a] Cell Signaling Laboratory, Department of Biochemistry, College of Medicine and Health Sciences, UAE University, P.O. Box 17666, Al Ain, Abu Dhabi, United Arab Emirates
[b] Al Jalila Foundation Research Centre, P.O. Box 300100, Dubai, United Arab Emirates

ARTICLE INFO

Article history:
Received 18 December 2015
Received in revised form
27 April 2016
Accepted 1 May 2016
Available online 3 May 2016

Keywords:
Sanguinarine
Hydrogen peroxide
Ceramide
Akt
Apoptosis

ABSTRACT

Dysregulation of apoptosis is a prime hallmark of leukemia. Therefore, drugs which restore the sensitivity of leukemic cells to apoptotic stimuli are promising candidates in the treatment of leukemia. Recently, we have demonstrated that sanguinarine (SNG), a benzophenanthridine alkaloid, isolated from *Sanguinaria canadensis* induces ROS-dependent ERK1/2 activation and autophagic cell death in human malignant glioma cells (Pallichankandy et al., 2015; [43]). In this study, we investigated the antileukemic potential of SNG *in vitro*, and further examined the molecular mechanisms of SNG-induced cell death. In human leukemic cells, SNG activated apoptotic cell death pathway characterized by activation of caspase cascade, DNA fragmentation and down-regulation of anti-apoptotic proteins. Importantly, we have identified a crucial role for hydrogen peroxide (H_2O_2)-dependent ceramide (Cer) generation in the facilitation of SNG-induced apoptosis. Additionally, we have found that SNG inhibits Akt, a key anti-apoptotic protein kinase by dephosphorylating it at Ser^{473}, leading to the dephosphorylation of its downstream targets, GSK3β and mTOR. Interestingly, inhibition of Cer generation, using acid sphingomyelinase inhibitor, significantly reduced the SNG-induced Akt dephosphorylation and apoptosis, whereas, activation of Cer generation using inhibitors of acid ceramidase and glucosylceramide synthase enhanced it. Furthermore, using a group of ceramide activated protein phosphatases (CAPPs) inhibitor (calyculin A, Okadaic acid, and phosphatidic acid), the involvement of protein phosphatase 1 form of CAPP in SNG-induced Akt dephosphorylation and apoptosis was demonstrated. Altogether, these results underscore a critical role for H_2O_2-Cer-Akt signaling axis in the antileukemic action of SNG.

© 2016 Elsevier Inc. All rights reserved.

1. Introduction

Leukemia constitutes a group of cancers that arise from malignant transformation of blood and blood forming organs. Despite the availability of many therapeutic approaches, resistance to chemotherapeutic agents is a consistent limiting factor in the treatment of many leukemia patients [1,2]. Therefore, new therapeutic agents and strategies are needed for the management of

Abbreviations: 4EBP1, eukaryotic translation initiation factor 4E-binding protein 1; aCDase, acid ceramidase; Akt, Akt/protein kinase B; aSMase, acid sphingomyelinase; CalA, calyculin A; CAPP, ceramide activated protein phosphatase; CDase, ceramidase; Cer, ceramide; Desip, desipramine; DMSO, dimethyl sulfoxide; FB1, Fumonisin B1; FBS, fetal bovine serum; GCS, glucosylceramide synthase; GSH, glutathione; GSK3β, glycogen synthase kinase 3 beta; H_2O_2, hydrogen peroxide; OH^-, hydroxyl radical; IAP, inhibitor of apoptosis protein; MitoPY1, Mito Peroxy Yellow 1, mTOR, mammalian target of rapamycin; MTT, 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide; Myr, Myriocin; NAC, N-acetyl cysteine; nCDase, neutral ceramidase; nSMase, neutral sphingomyelinase; OA, Okadaic acid; OPA, o-phthaladialdehyde; PA, phosphatidic acid; PBS, Phosphate buffered saline; PDK1, phosphoinositide-dependent kinase1; PI, propidium iodide; PP1, protein phosphatase 1; PP2A, protein phosphatase 2A; ROS, reactive oxygen species; SMase, Sphingomyelinase; SNG, sanguinarine; SOD, superoxide dismutase; Sod Pyr, sodium pyruvate; UDP-glucose, uridine 5′-diphosphoglucose disodium salt4E-BP1, eukaryotic translation initiation factor 4E-binding protein 1; aCDase, acid ceramidase; Akt, Akt/protein kinase B; aSMase, acid sphingomyelinase; CalA, calyculin A; CAPP, ceramide activated protein phosphatase; CDase, ceramidase; Cer, ceramide; Desip, desipramine; DMSO, dimethyl sulfoxide; FB1, Fumonisin B1; FBS, fetal bovine serum; GCS, glucosylceramide synthase; GSH, glutathione; GSK3β, glycogen synthase kinase 3 beta; IAP, inhibitor of apoptosis protein; MitoPY1, Mito Peroxy Yellow 1, mTOR, mammalian target of rapamycin; MTT, 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide; Myr, Myriocin; NAC, N-acetyl cysteine; nCDase, neutral ceramidase; nSMase, neutral sphingomyelinase; OA, Okadaic acid; OPA, o-phthaladialdehyde; PA, phosphatidic acid; PBS, Phosphate buffered saline; PDK1, phosphoinositide-dependent kinase1; PI, propidium iodide; PP1, protein phosphatase 1; PP2A, protein phosphatase 2A; ROS, reactive oxygen species; SMase, Sphingomyelinase; SNG, sanguinarine; SOD, superoxide dismutase; Sod Pyr, sodium pyruvate; O_2^-, superoxide; UDP-glucose, uridine 5′-diphosphoglucose disodium salt

* Corresponding author at: Cell Signaling Laboratory, Department of Biochemistry, College of Medicine and Health Sciences, UAE University, P.O. Box 17666, Al Ain, Abu Dhabi, United Arab Emirates.

E-mail addresses: anees@uaeu.ac.ae (A. Rahman), t.faisal@uaeu.ac.ae (F. Thayyullathil), sirajpk@uaeu.ac.ae (S. Pallichankandy), sehamuddin@uaeu.ac.ae (S. Galadari).

http://dx.doi.org/10.1016/j.freeradbiomed.2016.05.001
0891-5849/© 2016 Elsevier Inc. All rights reserved.

this disease.

In recent years, the therapeutic potential of medicinal plants as a source of novel and promising anticancer agents have gained widespread attention amongst cancer biologists. Sanguinarine (SNG), a naturally occurring benzophenanthridine alkaloid, isolated from *Sanguinaria canadensis,* elicits an ample array of pharmacological activities such as antibacterial, antifungal, and anti-inflammatory properties [3–8]. In addition, SNG has been found to possess potent anticancer activities *via* its effect on diverse cellular events associated with initiation, promotion, and progression of different malignancies [9–14]. Interestingly, at lower concentrations, SNG is found to be more toxic to cancer cells compared to the normal cells [15]. Therefore, SNG has a potential to be developed as a promising anticancer agent. To date, research into the anticancer effect of SNG was predominantly focused on its ability to induce apoptotic cell death in multiple cancer models. However, the detailed signaling mechanism by which SNG induces tumor suppression in human leukemia has not yet been characterized.

Most of the cytotoxic drugs currently in use for the treatment of different cancers are shown to exert their effects through induction of a highly regulated programmed cell death mechanism known as apoptosis [16]. Apoptosis is characterized by distinct morphological changes and biochemical events regulated by a family of aspartate-specific cysteine proteases called caspases [16]. Evading apoptosis is one of the major hallmarks of leukemia progression and resistance to chemotherapy [17].

Reactive oxygen species (ROS) such as hydrogen peroxide (H_2O_2), superoxide (O_2^-), and hydroxyl radical (OH^-) are oxygen containing highly reactive free radical molecules mainly generated inside the mitochondria. Depending on the strength and duration of exposure, ROS can be both beneficial and harmful to the cells. Under normal physiological condition, a low level of ROS functions as a “redox messenger” that mediates growth adaptation and cell survival [18]. In contrast, excessive generation of ROS mediates cellular damage and promotes apoptotic cell death [18–20]. Despite having an effective antioxidant system, cancer cells generally maintain high levels of ROS when compared to the normal cells. This persistent pro-oxidative state can be exploited by several anticancer agents that cause further ROS production and ROS-mediated damage in cancer cells [19,21]. It should be noted that many of the currently used anticancer agents promote ROS generation on its *en route* to induce apoptosis [22]. For example, arsenic trioxide has been efficiently used for the treatment of acute promyelocytic leukemia, induces apoptotic cell death through the generation of ROS [23,24].

Ceramide (Cer), a multifunctional central molecule in the sphingolipid biosynthetic pathway, exerts potent tumor suppressive effect in a variety of cell types [25]. A diverse array of stressors, including oxidative stress, irradiation, heat shock, and anticancer drugs were reported to activate intracellular Cer generation leading to the induction of apoptosis [25,26]. Moreover, defects in Cer generation and metabolism have shown to be involved in tumor cell survival and resistance to chemotherapy, supporting the vital role of Cer as a tumor suppressor lipid [25]. Generation of Cer, directly or indirectly, through the regulation of different sphingolipid biosynthetic enzymes, such as sphingomyelinase (SMase) [27], ceramidase (CDase) [28], ceramide synthase [29], glucosylceramide synthase (GCS) [30], and sphingomyelin synthase [31] has been shown to play an important role in apoptosis.

The serine/threonine kinase, Akt/protein kinase B (Akt) has emerged as an important and versatile cellular survival and proliferative protein. Akt has been reported to be constitutively activated in a wide variety of cancers [32,33]. Akt is activated by phosphoinositide-dependent kinase1 (PDK1)-dependent phosphorylation at Thr^{308} or Ser^{473}. Once activated, Akt attenuates apoptosis and promotes cell survival by catalyzing the phosphorylation of a number of downstream targets [32]. Simultaneously, ceramide activated protein phosphatases (CAPPs), such as protein phosphatase 1 (PP1) and protein phosphatase 2A (PP2A), were shown to dephosphorylate Akt at Ser^{473} which ultimately leads to the inactivation of Akt and induction of apoptosis [34–37].

In the present study, we investigated the antileukemic effect of SNG in Jurkat and Molt-4 human leukemic cells *in vitro*. We have found that SNG efficiently inhibited the growth of these cells by inducing apoptosis. Furthermore, we also evaluated the molecular signaling pathways of SNG-induced apoptosis. Indeed, this is the first report to demonstrate that SNG induces apoptosis which is regulated by the H_2O_2-mediated Cer up-regulation leading to Akt dephosphorylation. Our studies will provide the groundwork for future investigation of SNG to be developed in to a pro-apoptotic drug for the treatment of leukemia.

2. Materials and methods

2.1. Chemicals and antibodies

Sanguinarine, glutathione (GSH), GSH assay kit, N-acetyl cysteine (NAC), 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide (MTT), bovine liver catalase, superoxide dismutase (SOD), L-ascorbic acid, α-lipoic acid, dimethyl sulfoxide (DMSO), Mito Peroxy Yellow 1 (MitoPY1), uridine 5′-diphosphoglucose disodium salt (UDP-glucose), phosphatidylcholine, o-phthaladialdehyde (OPA), phosphatidic acid (PA), desipramine (Desip), anti-rabbit IgG, and anti-mouse IgG were purchased from Sigma Chemical Co. (St. Louis, MO, USA). D-erythro-C_{12}-NBD-Cer was a kind gift from Prof. YA Hannun (Department of Biochemistry and cell biology, Stony Brook Cancer Center, NY, USA). Oxidation sensitive DCFH-DA, C_6-NBD-Cer, C_6-NBD-sphingomyelin, and Amplex red H_2O_2 assay kit were obtained from Molecular Probes (Eugene, OR, USA). Phosphate buffered saline (PBS), trypsin-EDTA and fetal bovine serum (FBS), penicillin/streptomycin, RPMI 1640 GlutaMAX, and sodium pyruvate (Sod Pyr) were bought from Gibco (Grand Island, NY, USA). Fumonisin B1 (FB1), myriocin (Myr), U0126, C_6-Cer, and Z-VAD-FMK were from Enzo Life Sciences (San Diego, CA, USA). MnTBAP, anti-actin, anti-GSK3αβ, anti-Bcl-2, anti-Mcl-1, anti-BAD, anti-BAX, and donkey anti-goat IgG antibodies were from Santa Cruz Biotechnology Inc. (Santa Cruz, CA, USA). Anti-caspase-9, anti-caspase-8, anti-caspase-3, anti-PARP, anti-Akt, anti-phospho Akt (Ser^{473}), anti-BID, anti-PI3K p110 alpha, anti-PI3K p85, anti-phospho PDK1 (Ser^{241}), anti-phospho mTOR (Ser^{2448}), mTOR, anti-phospho 4EBP1($Thr^{37/46}$), anti-4EBP1, and anti-phospho GSK3β (Ser^9) were from Cell Signaling Technology (Beverly, MA, USA). Anti-XIAP, anti-cIAP-1, and anti-cIAP-2 were from BD Biosciences (East Rutherford, NJ, USA).

2.2. Leukemic cell lines, cell culture conditions and drug treatment

Human leukemic Jurkat and Molt-4 cells (ATCC, VA, USA) were grown in RPMI 1640 GlutaMAX medium supplemented with 10% v/v heat-inactivated FBS, 50 IU/mL penicillin, and 50 μg/mL streptomycin in an incubator containing humidified atmosphere of 95% air and 5% CO_2 at 37 °C. Sanguinarine was dissolved in DMSO at a concentration of 10 mM and was stored in a dark colored bottle at −20 °C. The stock was diluted to the required concentration with DMSO when needed. Prior to the SNG treatment, cells were grown to about 80% confluence, and then exposed to desired concentration of SNG for required time period. Cells grown in a medium containing an equivalent amount of DMSO without SNG served as control.

Download English Version:

https://daneshyari.com/en/article/8267782

Download Persian Version:

https://daneshyari.com/article/8267782

Daneshyari.com