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Mini-review

Polyphenols as mitochondria-targeted anticancer drugs

Sylwia Gorlach, Jakub Fichna, Urszula Lewandowska *

Department of Biochemistry, Medical University of Lodz, Lodz, Poland

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ABSTRACT

Mitochondria are the respiratory and energetic centers of the cell where multiple intra- and extracellular signal transduction pathways converge leading to dysfunction of those organelles and, consequently, apoptotic or/and necrotic cell death. Mitochondria-targeted anticancer drugs are referred to as mitocans; they have recently been classified by Neuzil et al. (2013) according to their molecular mode of action into: hexokinase inhibitors; mimickers of the Bcl-2 homology-3 (BH3) domains; thiol redox inhibitors; deregulators of voltage-dependent anionic channel (VDAC)/adenine nucleotide translocase (ANT) complex; electron redox chain-targeting agents; lipophilic cations targeting the mitochondrial inner membrane; tricarboxylic acid cycle-targeting agents; and mitochondrial DNA-targeting agents. Polyphenols of plant origin and their synthetic or semisynthetic derivatives exhibit pleiotropic biological activities, including the above-mentioned modes of action characteristic of mitocans. Some of them have already been tested in clinical trials. Gossypol has served as a lead compound for developing more efficient BH3 mimetics such as ABT-737 and its orally available structural analog ABT-263 (Navitoclax). Furthermore, mitochondriotropic derivatives of phenolic compounds such as quercetin and resveratrol have been synthesized and reported to efficiently induce cancer cell death *in vitro*.

Introduction

A growing number of anticancer strategies are focused on mitochondria whose potential as targets for such strategies stems from, among others, the fact that they are invariably present in all tumor cells [1,2]. In contrast, even tumors of the same type originated from individual patients may differ in a number of mutations. Therefore, tumors are unlikely to be treated effectively with agents targeted at a single gene or at a single signal transduction pathway. Anticancer drugs acting on mitochondria are referred to as mitocans and they have recently been classified on the basis of their molecular mode of action by Neuzil et al. [2] into: (I) hexokinase inhibitors; (II) mimickers of the Bcl-2 homology-3 (BH3) domain; (III) thiol redox inhibitors; (IV) deregulators of voltage-dependent anionic channel (VDAC)/adenine nucleotide translocase (ANT) complex; (V) electron redox chain-targeting agents; (VI) lipophilic cations targeting the mitochondrial inner membrane; (VII) tricarboxylic acid (TCA) cycle-targeting agents; (VIII) mitochondrial DNA (mtDNA)-targeting agents.

Polyphenols are plant-derived compounds with pleiotropic biological activities; although they exhibit far more activities than antioxidative one, they are most widely known as antioxidants and therefore protectors against oxidative damages [3,4]. One of the mechanisms underlying the antioxidant activity of polyphenols may consist in decreasing mitochondrial membrane fluidity (as a result of their partitioning into the hydrophobic core of the membrane) and a subsequent decrease in the kinetics of free radical reactions [5]. On the other hand, phenolic compounds were reported to exhibit pro-oxidant activity *in vitro* and *in vivo* [6]. For instance, (−)-epigallocatechin-3-gallate (EGCG, the major bioactive constituent of green tea) induced oxidative stress in oral cancer and premalignant cells while in normal human gingival fibroblasts the flavanol acted as an antioxidant [7]. Research indicates that the pro-oxidant activity of polyphenols is likely to be responsible for their cytotoxic and proapoptotic effects in cancer cells [8,9]. Thus, phenolics can either protect normal cells from oxidative stress as antioxidant agents [10] or trigger necrotic death of cancer or premalignant cells by acting as cytotoxic pro-oxidants [11,12].

As mitochondria are the major cellular source of reactive oxygen species (ROS), redox-active compounds (such as polyphenols) can be targeted to those organelles to modulate the levels of ROS and the processes they induce, including the mitochondrial permeability transition and cell death. Importantly, cancer cells show higher intrinsic levels of ROS and therefore lower antioxidant capacity than normal cells, which renders them less resistant to agents that further enhance oxidative stress [2]. However, it should be emphasized that polyphenols also affect mitochondria through mechanisms that are not redox-based [2]. On the basis of the classification of mitochondria-targeted agents by Neuzil et al. [2], the present

Abbreviations: ANT, adenine nucleotide translocase; EGCG, (−)-epigallocatechin-3-gallate; ETC, electron transport chain; GSH, glutathione; GRX, glutaredoxin; PTPC, permeability transition pore complex; ROS, reactive oxygen species; TCA, tricarboxylic acid; TRX, thioredoxin; TRX-R, thioredoxin reductase; TPP, triphenylphosphonium; VDAC, voltage-dependent anionic channel.

* Corresponding author. Tel.: +48 42 272 57 14; fax: +48 42 213 05 18.
E-mail address: urszula.lewandowska@umed.lodz.pl (U. Lewandowska).

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mini-review presents a number of polyphenols and their derivatives (including mitochondriotropic derivatives) which exhibit activities characteristic of mitocans and could therefore be used as anticancer drugs or as lead structures for such drugs.

Class I mitocans: hexokinase inhibitors

Hexokinase converts glucose to glucose-6-phosphate, a substrate for metabolic pathways ultimately coupled with ATP generation. It is expressed at high levels in cancer cells and a direct correlation was established between its activity and tumor growth [2]. Among hallmarks of cancer cells, one could list enhanced glycolytic activity and impaired oxidative phosphorylation (the Warburg effect) [13]. Overexpressed hexokinase supports the highly glycolytic phenotype of cancer cells. It is associated with the cytosolic site of VDAC, a transmembrane protein in the mitochondrial outer membrane [14]. When ATP is newly synthesized by ATP synthase (located in the mitochondrial inner membrane), ANT together with VDAC move it to hexokinase active sites. Continuous phosphorylation of incoming glucose by overexpressed, mitochondria-bound hexokinase reduces the amount of ATP available for oxidative phosphorylation and, as a consequence, limits respiration in cancer cells. Moreover, the interaction between hexokinase and VDAC is recognized as critical for preventing induction of apoptosis in tumors [14].

Liver and kidney mitochondria of rats bearing dimethyl benzanthracene-induced mammary carcinoma were characterized by a marked increase in the activities of glycolytic enzymes (including hexokinase), with a simultaneous decrease in the activities of gluconeogenic enzymes [15]. Treatment of the rats with a polyphenol-rich extract from nut milk of *Semecarpus anacardium* (closely related to cashew/*Anacardium occidentale*) reversed those changes. A similar effect was observed in a murine model of an aggressive leukemia, induced by injection of BCR-ABL(+) 12B1 murine leukemia cells to the tail vein of BALB/c mice [16]. Treatment of the mice with an extract obtained from nut milk of *S. anacardium* cleared the leukemic cells from bone marrow and internal organs, resulting in a total regression of leukemia with no adverse side effects. An increase in the levels of glycolytic enzymes (including hexokinase) and a decrease in the levels of gluconeogenic enzymes were also reversed by the *S. anacardium* extract. According to the authors of the study, the observed effects of the treatment could be attributed to polyphenols (and possibly other compounds) present in the extract.

In human breast carcinoma MDA-MB-231 and MCF-7 cell lines, oroxylin A (an O-methylated flavone found in *Scutellaria baicalensis* and *Oroxylum indicum*) caused detachment of hexokinase from mitochondria, which resulted in inhibition of glycolysis [17]. In human colorectal cancer HCT116 and HT29 cell lines, both expression and activity of hexokinase were downregulated by curcumin, the main bioactive component of turmeric (*Curcuma longa*), in a concentration-dependent manner [18]. Furthermore, the phenolic compound induced phosphorylation of hexokinase by AKT (protein kinase B) and its subsequent dissociation from mitochondria, followed by mitochondria-mediated apoptosis.

Class II mitocans: mimickers of the Bcl-2 homology-3 (BH3) domain

The BCL-2 family comprises anti- and proapoptotic proteins which share one or more of four characteristic Bcl-2 homology (BH) domains, BH1 to BH4. The antiapoptotic proteins include BCL-2, BCL-xL, BCL-W, and MCL-1 [19]. The proapoptotic members of the family are divided into multidomain BAX-like proteins, also known as effector proapoptotic proteins (such as BAX and BAK), and BH3-only proteins (e.g., BIM, BID, and PUMA) [19]. BAX and BAK contain the BH3 domain and therefore are able to interact with BH-3 only proteins which act as their activators in response to cellular stress (such as chemotherapy). For instance, BAX and BIM were reported to associate in the cytosol and then translocate to the mitochondrial outer membrane where they assemble into pore-like complexes [19]. The pore formation results in a release of apoptotic factors (including cytochrome c) from the intermembrane space into the cytosol, leading to activation of the post-mitochondrial apoptotic signaling and cell death. Cancer cells often overexpress antiapoptotic BH3-interacting proteins, which prevents the oligomerization of the proapoptotic proteins necessary for the pore formation and, as a consequence, protects the cells from apoptosis and renders them resistant to chemotherapeutic drugs [2]. Accordingly, overexpression of antiapoptotic members of the BCL-2 family, associated with poor overall survival, has been observed in approximately 80% of B-cell lymphomas [20] and over 80% of multiple myelomas [21]. For this reason, small molecules (referred to as BH3 mimetics) targeting the interaction between the anti- and proapoptotic proteins from the BCL-2 family have been investigated as potential anticancer drugs [2].

One of such molecules is gossypol, a polyphenolic compound isolated from the seeds of the cotton plant (*Gossypium*) as a racemic mixture; the R-(−) enantiomer acts as a BH3 mimetic [2]. Gossypol was reported to induce apoptosis in human myeloma OPM2 cell line through displacing BH3-only proteins from Bcl-2 [21]. Furthermore, it inhibited interleukin-6 signaling at the level of JAK2 activation, which resulted in downregulation of antiapoptotic MCL-1 and impairment of the antiapoptotic function of BCL-2 by its dephosphorylation at serine 70. In earlier studies, AT-101 (an orally bioavailable solvate of (−)-gossypol and acetic acid) synergistically enhanced the activity of cytotoxic agents against lymphoma and multiple myeloma cell lines *in vitro*, with IC_{50} between 1 and 10 μM for a diverse panel of B-cell lymphomas [20]. Importantly, AT-101 proved to be both safe and effective in a murine model of drug-resistant B-cell lymphoma, enhancing the efficacy of the conventional therapy [20].

In the ClinicalTrials.gov database (https://clinicaltrials.gov, website accessed on April 29, 2015), 24 clinical studies are registered for AT-101, tested alone or in combination with chemotherapeutic drugs. Among the studies, 16 are completed and 3 are currently recruiting patients suffering from leukemia (phase I/II trial), laryngeal cancer (phase II), or non-small cell lung cancer (phase III). The completed studies are mainly phase II trials which enrolled patients with prostate cancer (including hormone refractory prostate cancer), chronic lymphocytic leukemia, lymphoma, non-small cell and small cell lung cancer, esophageal cancer, laryngeal cancer, adrenocortical cancer, glioblastoma, or squamous cell carcinoma of the head and neck.

It is worth emphasizing that gossypol has served as a lead compound for developing more efficient BH3 mimetics such as ABT-737 and its orally available analog ABT-263 (Navitoclax). Among 23 clinical studies with ABT-263 registered in the ClinicalTrials.gov database (website accessed on April 29, 2015) and regarding various types of cancers, 4 trials are currently recruiting patients and 3 trials are not yet recruiting. 15 Studies registered as completed were predominantly phase I trials; they also included one phase I/II study on small cell lung cancer and two phase II studies on chronic lymphocytic leukemia. ABT-737 was tested in a phase II trial aimed at an *ex vivo* evaluation of apoptosis-inducing ability of this gossypol derivative and platin (alone and in combination) in samples of high grade serous ovarian carcinoma (ClinicalTrials.gov identifier: NCT01440504). A recent paper revealed that ABT-737 induced apoptosis as a single agent in fresh samples of high grade serous ovarian carcinoma; its efficacy was not improved by the addition of carboplatin [22]. This BH3-mimetic showed promise as a monotherapy in a specific subgroup of tumors characterized by expression of BIM (a proapoptotic BH3-only protein) and, preferably, a low expression of phospho-ERK1/2 or MCL-1. According to the authors of the study, it seems that in ovarian cancers MCL-1 has to be downregulated for ABT-737 to be effective.

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