Mitocans as Novel Agents for Anticancer Therapy: An Overview

Vandana Panda¹*, Prashant Khambat¹, Sachin Patil²,³

¹Department of Pharmacology, Principal K. M. Kundnani College of Pharmacy, Mumbai, India; ²Department of Pharmaceutics, Principal K. M. Kundnani College of Pharmacy, Mumbai, India; ³Karlsruhe Institute of Technology, Institute of toxicology and Genetics, Karlsruhe, Germany.

Received March 10th, 2011; revised July 15th, 2011; accepted July 31th, 2011.

ABSTRACT

Many conventional anticancer drugs have associated lack of safety by their toxicity. Relatively faster mutations in tumor cells pose a significant obstacle in treatment of cancer. Recently, "Mitocans" have emerged as a novel class of anticancer agents selectively targeting tumor cells and thus, are much less toxic than conventional anticancer chemotherapeutic agents. Mitocans are drugs that act directly on mitochondria within the cell, thus causing changes in energy metabolism of the cell. Amongst these mitocans, α-Tocopheryl succinate or vitamin E analogs are studied very well by researchers. This review discusses mitochondrial drug targeting strategies and a variety of novel mitochondrial drug targets of mitocans, such as electron transport chain, mitochondrial permeability transition, Bcl-2 family proteins and mitochondrial DNA. The purpose of this review is to focus on the various classes of mitocans, the mechanisms by which these drugs specifically act on tumor cells and their applications in cancer chemotherapeutics.

Keywords: Mitocans, Mitochondria, Cancer, Apoptosis, Membrane Permeabilization, Energy Metabolism

1. Introduction

Despite a steady development in cancer chemotherapeutics in recent years, this millennium has been a witness to various types of cancers becoming a major cause for mortality. Various chemotherapeutic or chemopreventive drugs that target cell microtubules and cytoskeleton components have been used for cancer treatment over the years [1,2]. The treatment of cancer to date remains a significant challenge, particularly due to continuous mutations, which make the tumor cells resistant to established chemotherapeutic molecules or drugs. Hence, for resolving the current problem, newer approaches are to be sought and put into practice. As mitochondria are the “power house” of the cell and are essential for its survival, they are novel targets showing considerable promise for intervention and treatment of cancer [3-5]. Anticancer agents specifically targeting cancer cell mitochondria are referred to as “mitocans”. Mitocans act by destabilizing mitochondria and unleashing their apoptotic potential, leading to death of tumor cells or reducing their growth drastically [6,7]. They destabilize mitochondria and cause the cytosolic release of modulators of apoptosis [8]. Some drugs have the potential to affect mitochondrial function directly while others may have different primary drug targets at other cellular locations and alter mitochondrial function as a side effect [3,8]. Thus, mitochondria become a major target in cancer cells.

The purpose of this review is to present an update on the action of various therapeutically active substances affecting mitochondria in tumor cells. This review describes various drug molecules acting on the mitochondria (mitocans), different classes of mitocans and their mechanisms of action. We also discuss about the scope of these drugs with examples like betulinic acid and α-TOS, which are more researched drugs in this field.


Mitochondria play a central role in energy metabolism (energy generation process) within the cell [9]. Impaired respiratory chain functioning can occur due to mitochondrial DNA mutations, leading to decreased ATP production [10], free radical generation [11] and alterations in cellular calcium handling [12]. This altogether initiates peroxidation of biomolecules like mitochondrial DNA, proteins, lipids and alters the mitochondrial permeability by opening transition pores, leading to apoptotic cell death.
mitochondria especially at complex I or III, it may react with molecular oxygen to form the superoxide radical [15,16]. During the cellular metabolism, superoxide radicals are constantly generated and may be converted into hydrogen peroxide and/or other reactive oxygen species (ROS), which are highly reactive and toxic to the cells when accumulated to higher levels. These species need to be removed from cells using an antioxidant system which includes metabolic enzymes such as superoxide dismutase (SOD), peroxidases and other redox molecules [17]. Maintenance of appropriate levels of intracellular ROS is vital for redox balance and signalling cellular proliferation under physiological conditions [18]. However, overproduction of ROS or a decreased ability of the cell to remove ROS can result in increased levels of ROS, causing cell death due to lipid peroxidation [19], oxidative DNA changes and protein and enzyme inactivation. This cell damaging effect may also kill the cancer cells if ROS are inductively accumulated in malignant cells which are metabolically active and produce higher ROS. Malignant cells are already under oxidative stress and are more susceptible to further stress by exogenous ROS generating compounds [20-23]. It is well known that when oxidative stress due to ROS reaches a threshold, cancer cell apoptosis occurs as shown in case of 2-methylestradiol [24,25]. Once apoptosis signalling pathways are initiated, cytochrome C and apoptotic factors are released into cytoplasm. This is associated with changes in mitochondrial ultrastructure, membrane permeability and transmembrane potential [17].

3. Mitochondria as Pharmacological Target

Mitochondria provide a novel targeting site which can selectively kill cancer cells without affecting normal cells, unlike other anticancer agents [26]. For this reason, many drugs that target mitochondria are currently in clinical trials for treating cancer. There are several reports suggesting the possible role of mitochondria in various complex processes such as apoptosis and cardioprotection [3, 7,8,27]. Mitochondrial dysfunctions lead to various neurodegenerative disorders like Alzheimer’s disease [28-31], Parkinson’s disease [32-34], Huntington’s disease [35,36] and Amyotrophic lateral sclerosis [37-39], the so-called “mitochondrial diseases”. Thus, identification of mitochondria as a primary or secondary target of a drug may help us to understand better its mechanism of action and open new perspectives for its application in chemotherapy [40]. Various therapeutically active drugs like Paclitaxel (anticancer) [41-44], Cyclosporin A & Rapamycin (immunosuppressant) [45-47], antiviral nucleotide analogues [48-50], some sulfonylureas [51-53] and anesthetics [54,55] are known to act on the mitochondria. These drugs selectively disrupt energy metabolism in cancer cells leading to ROS generation and cell death. Because of their mitochondrial action and anticancer property, these drugs have been termed “mitocans”.

4. Cell Biology of Apoptosis

Apoptosis is a process that develops in 3 phases [6] as follows;

1) Initiation phase, which varies depending on the apoptosis-inducing agent and the biochemical pathway activated by it;

2) Commitment phase, which is common to different types of apoptosis, during which the cell “decides” to commit suicide;

3) Common degradation phase, which is characterized by activation of catabolic hydrolases (caspases and nucleases).

Before cells lyse, permeabilization of both, inner as well as the outer mitochondrial membrane takes place with dissipation of inner transmembrane potential ($\Delta \psi_m$) and/or release of apoptogenic proteins, such as cytochrome c and apoptosis-inducing factor (AIF) via the outer membrane [56-59]. This suggests that caspase activation may not always be required for apoptotic cell death to occur. Rather, cell death is intimately associated with the permeabilization of mitochondrial membranes [60]. A number of triggers such as Bax [61], Bak [62], c-Myc [62], PML [63], Fas-associated death domain [64], glucocorticoid receptor occupancy [58], tumor necrosis factor [65], growth factor withdrawal [66], CXCR4 (CXC chemokine receptor 4 also called fusin), cross-linking [67], and chemotherapeutic agents, such as etoposide [58], camptothecin [68], or cisplatin [69] induce cell death without activation of caspases and nucleases.

With the use of cell-free systems where subcellular fractions (e.g., mitochondria, nuclei, and cytosol) are mixed together, Costantini et al. have demonstrated that apoptosis of mammalian cells develops in several steps in vitro [4]. Pro-apoptotic second messengers like ceramide, $Ca^{2+}$ and nitric oxide, whose nature depends on the apoptosis-inducing agent, accumulate in the cytosol during initiation phase. These agents then induce mitochondrial membrane permeabilization, allowing cells to enter the commitment phase. The apoptotic changes in the mitochondria include $\Delta \psi_m$ loss, transient swelling of the mitochondrial matrix, mechanical rupture of outer membrane and/or its nonspecific permeabilization by giant
protein-permeant pores, and release of soluble intermembrane proteins (SIMPs) like cytochrome c and adenylate kinase 2, through the outer membrane [59]. Once the mitochondrial membrane integrity is lost, a collapse in bioenergetic status, redox equilibrium and ion homeostasis takes place leading to cell death. The activation of proteases (caspases) and nucleases by SIMPs is necessary for acquisition of apoptotic morphology [59], the degradation step, to the point of no return of apoptotic process [70]. Different SIMPs provide a molecular link between mitochondrial membrane permeabilization and activation of catabolic hydrolases: cytochrome c (a heme protein that participates in caspase activation) [71], certain procaspases (in particular, procaspases 2 and 9, which, in some cell types, are selectively enriched in mitochondria) [72] and AIF [59,70]. AIF is a nuclear-encoded intermembrane flavoprotein that translocates to nucleus where it induces caspase-independent peripheral chromatin condensation and degradation of DNA into 50 Kbp fragments [70].

5. Mechanism of Action of Mitocans

The tumor cells are under increased intrinsic oxidative stress and are prone to apoptosis induced by the generated free radicals (Figure 1). Huang et al. reported a novel strategy to enhance superoxide generation and block mitochondrial electron transport chain in leukemic cells which in turn will increase the apoptosis induced by anticancer drugs [24]. This mechanism has already been proven using a specific mitochondrial electron transport chain complex I inhibitor, i.e. rotenone [73]. Partial inhibition of mitochondrial respiration enhances electron leakage from the transport chain, leading to an increased generation of superoxide radicals and thus leukemic cells become more sensitive to anticancer agents (i.e. those agents which act by free radical generation). The anti-leukemic agent As2O3 also inhibits mitochondrial respiratory function and increases generation of free radicals in cultured as well as primary leukemic cells [74]. Thus As2O3 can be used in combination with other anticancer agents which act by increasing free radical generation [17,75,76].

6. Molecular Mechanisms of Mitochondrial Membrane Permeabilization

The mechanism of mitochondrial membrane permeabilization is not completely understood. Some investigators have proposed that pro-apoptotic members of the Bcl-2 family are inserted in the outer membrane [77] where they oligomerize and form cytochrome c permeant pores in an autonomous fashion, not requiring the interaction with other mitochondrial membrane proteins [77,78]. However, Bax (pro-apoptotic Bcl-2 protein)-induced membrane permeabilization is inhibited by cyclosporin A (CsA) and bongkrekic acid (BA), two inhibitors of formation of the permeability transition pore, suggesting that sessile mitochondrial proteins (targets of CsA and BA) are involved in this process [78]. The permeability transition pore has a polyprotein structure that is formed at the contact sites between the inner and outer membranes [79,80]. One of the key proteins of the permeability transition pore complex (PTPC) is adenine nucleotide...
translocator (ANT). ANT, the target of BA, is the most abundant inner membrane protein [78]. ANT normally functions as a specific carrier protein for the exchange of adenosine triphosphate (ATP) and adenosine diphosphate (ADP), but it can become a nonspecific pore (Figures 2 and 3) [4].

The adenine nucleotide translocator (ANT) plays a dual role in the process of pore formation on the mitochondrial membrane as follows:

It functions as a highly specific transporter by exchanging adenosine diphosphate (ADP) and adenosine triphosphate (ATP) on the inner mitochondrial membrane.

The ANT can also become a pore, acting at several levels of conductance, specificity, and reversibility. Bcl-2 inhibits pore formation whereas Bax, atracyloside, palmitate, Ca$^{2+}$, thiol oxidants, and viral protein R (Vpr) from human immunodeficiency virus-1 favor pore formation.

Due to the presence of a pore protein, termed porin or voltage-dependent anion channel (VDAC), the outer mitochondrial membrane is permeable to polar molecules of up to five kDa and is impermeable to cytochrome c. However, VDAC can allow for leaking of cytochrome c by interacting with Bcl-2 and Bax, thus facilitating pore formation.

ANT, thus has two functions—that of a vital ATP/ADP carrier and that of a lethal pore (Figure 2) [81]. ANT interacts with another equally abundant protein of outer membrane, VDAC, as well as a soluble protein of the mitochondrial matrix, cyclophilin D, the target of CsA [80]. ANT and/or VDAC physically interact with Bcl-2 and Bax [78,79], the peripheral benzodiazepine receptor (PBR), as well as with several proteins involved

**Figure 2.** Role of ANT (adenine nucleotide translocator) in specific or nonspecific pore formation on the inner mitochondrial membrane.

**Figure 3.** Function of VDAC (voltage dependent anion channel) in pore formation on the outer mitochondrial membrane.
in the regulation of energy metabolism (e.g., hexokinase II and creatine kinase) [82]. It appears that the PTPC simultaneously controls the permeability of the outer membrane (pore forming protein: VDAC and/or Bax) [83] and that of the inner membrane (pore-forming protein: ANT and Bax) (Figure 3) and participates in energy metabolism (via the kinases and ANT). Because of the presence of multiple proteins, each of which influences pore opening, the PTPC senses a large number of metabolic conditions: redox couples (e.g., reduced versus oxidized glutathione, nicotinamide adenine dinucleotide [NAD+] versus nicotinamide adenine dinucleotide phosphate [NADPH], local ATP/ADP concentrations, different metabolites (e.g., glucose and creatine), ions (Ca2+ and Mg2+), the pH, and the Δψm. All of these factors determine the probability of membrane permeabilization by the PTPC [57]. Recombinant Bcl-2 as well as Bcl-xL (both of which are anti-apoptotic) have a direct inhibitory effect on the PTPC [79], as well as on ion channel formation by purified ANT [84] and VDAC [83]. In contrast, Bax (which is pro-apoptotic) aids ANT [79,84] or VDAC [83] to create large channels. The permeabilization of inner and/or outer mitochondrial membranes causes oxidation of reduced NADPH and glutathione, the depletion of ATP, the dissipation of Δψm and release of Ca2+ from the matrix leading to altered homeostasis of intracellular ions. All of these changes themselves increase the probability of PTPC opening [57]. This has two important implications: firstly, opening of PTPC in a self amplification loop co-ordinates lethal response among mitochondria within the same cells and secondly, the final result of PTPC opening does not depend on initiating a stimulus, be it a specific pro-apoptotic signal transduction cascade or nonspecific damage at the energy or redox levels [4,57,85].

PTPC (and its components) may not be the only mechanism by which mitochondrial membranes are permeabilized. Pro-apoptotic members of the Bcl-2 family, such as Bax or Bid, may cause outer membrane permeabilization without inducing an immediate dissipation [86]. Apoptosis without a complete Δψm loss has also been reported to occur in some cell lines, such as human promyelocytic leukemia cells (HL60) [87]. It is possible that Bax and other pro-apoptotic members of Bcl-2 family act independently by forming giant channels and/or acting on mitochondrial structures other than PTPC [83,88,89].

7. Pro-Apoptotic Signal-Transducing Molecules Acting on Mitochondria

7.1. Indirect Apoptotic Signals

Conventional anticancer agents, such as etoposide, doxorubicin, cisplatin, or paclitaxel (Taxol), trigger apoptosis by Inducing: 1) p53 expression [90,91]; 2) ceramide/GD3 pathway [92,93]; 3) CD95/CD95L ligand system [67,94] Affecting Bcl-2 like proteins [83,95] Compromising redox or energy balance [22]

Thus, these agents elicit mitochondrial permeabilization in an indirect fashion by induction of endogenous effectors that are involved in physiologic control of apoptosis. Following are important endogenous effectors that exert a direct effect on mitochondria and its PTPC.

7.2. Direct Apoptotic Signals

Redox Metabolism

De novo production of ROS resulting from over-expression of pro-apoptotic anti-oncogene p53 [90] or from treatment of cells with a second messenger such as ceramide [96] may cause changes in cellular redox potentials, depletion of reduced glutathione, or NADPH, all of which may induce or facilitate PTPC opening [57]. Peroxynitrite (formed by reaction of nitric oxide with superoxide anion) is also a potent PTPC trigger [97]. The mitochondrial mega-channel possesses several redox-sensitive sites, one of which is modulated by NADPH whereas a second is affected by mitochondrial matrix glutathione levels, is most likely located on ANT [98], and thiol oxidation will convert ANT to become a large nonspecific pore [99].

7.3. Energy Metabolism

ADP and ATP are physiologic ligands of ANT and they function as endogenous inhibitors of PTPC [57,100] and/or pore formation induced by atractyloside [101], ROS, or thiol cross-linkers [84,93]. Their depletion, therefore, might facilitate PTPC opening. Matrix alkalinization and/or Δψm reduction also trigger PTPC opening [80]. Thus, uncoupling or inhibition of respiratory chain (which leads to a decrease in Δψm) may be expected to favour mitochondrial membrane permeabilization [102].

7.4. Lipid Messengers

Ceramide is generated in cells exposed to several apoptosis-inducing stimuli, including signalling via the Fas/Apo-1/CD95 receptor or tumor necrosis factor (TNF) receptor, nonspecific stress, or cytotoxic drugs [93]. When added to cells, ceramide induces mitochondrial membrane permeabilization [94,103] but not the PTPC opening [103]. To induce apoptosis, ceramide must be converted into ganglioside GD3 in the Golgi apparatus. GD3 then translocates to mitochondria and causes PTPC opening [94].

7.5. Cytosolic Calcium

Ca2+ ions are among the most efficient triggers of the PTPC. At supraphysiologic doses (> 10 mM), Ca2+ suf-
fices to induce permeability transition whereas at lower doses, it facilitates induction of permeability transition (PT) by other stimuli [57,104].

7.6. Pro-Apoptotic Members of Bcl-2 Family

On induction of apoptosis, several pro-apoptotic Bcl-2 family members can translocate from the cytosol (Bax, Bid, and Bad) or from the microtubules (Bim) to mitochondria, where they can incorporate into the outer membrane and undergo a conformational change [77, 105]. The mechanism of Bax translocation to mitochondria involves activation by JNK through phosphorylation of 14-3-3, a cytoplasmic anchor of Bax [106]. However, Bid translocation likely involves its cleavage by caspase 8 [107], and Bad translocation may involve its dephosphorylation, causing its release from cytosolic 14-3-3 protein [108]. Pro-apoptotic signalling may also lead to the inactivation of anti-apoptotic members of Bcl-2 family. Inactivation of Bcl-2 is achieved by chemotherapeutic agents (such as paclitaxel), which act on microtubule assembly and cause its hyperphosphorylation and, simultaneously, favour opening of PT pore [109]. In addition, Bcl-2 can be cleaved by caspase 3 in a reaction that yields a pro-apoptotic product [110].

7.7. Caspases

Ligation of some receptors can lead to a rapid proteolytic activation of caspases within seconds or minutes. Caspases can act on members of the Bcl-2 family (e.g., caspase 8 cleaves BID, caspase 1 cleaves Bcl-xL, and caspase 3 cleaves Bcl-2), thereby activating pro-apoptotic members of the Bcl-2 family (Bid) or inactivating anti-apoptotic members (Bcl-2 and Bcl-xL) [60,65,66,72, 87,110].

8. Chemotherapeutic Agents Acting Directly on Mitochondria

Mitocans affect mitochondria-associated activities such as hexokinase inhibition, activation of mitochondrial permeability transition pore (MPTP), inhibition of Bcl-2 anti-apoptotic proteins, and blocking the electron transport/respiratory chain [3,4,6,7,17,89,111-114]. The classification of mitocans is shown in Table 1 [5], and examples of some of the prominent members of these groups are discussed below.

The first class of Mitocans is hexokinase inhibitors which selectively induce apoptosis in cancer cells that metabolize anaerobically [115]. The glucose metabolites, 2-deoxyglucose (2DG), oxamate, and 3-bromopyruvate are hexokinase-inhibiting mitocans [116]. In tumor cells, hexokinase isoforms HK-I and HK-II bind to voltage-dependent anion channel (VDAC) on the outer mitochondrial membrane to inhibit cytochrome c release, and protect against apoptotic cell death [117]. Use of hexokinase inhibitory drugs should enhance the efficacy of conventional cancer chemotherapeutics and radiation regimens that focus on aerobic cancer cells. In addition, hexokinase inhibitors may be used in conjunction with anti-angiogenic agents to limit oxygen supply to tumor cells [5,117-121].

The second class of mitocans includes novel small molecule inhibitors of Bcl-2 and Bcl-xL anti-apoptotic proteins that are overexpressed in cancer cells [122]. The BH4 (Bcl-2 homology 4) helix-containing anti-apoptotic proteins Bcl-2 and Bcl-xL both share a hydrophobic groove on their surface whose function is to bind the BH3 amphipathic helix of pro-apoptotic family members, thereby preventing apoptosis. This hydrophobic groove also binds a range of small molecules, including the natural compound gossypol, that result in blocking of BH3 binding [123]. Thus, small molecules blocking Bcl-2 and Bcl-xL will enable BH3 family of pro-apoptotic inducers to then freely bind to their relevant targets and induce apoptosis. The BH3 mimetics are only recently entering clinical trial in patients with cancer [113,124-126].

Mitocans from the arsenite class of compounds (trivalent inorganic salt formed by arsenic trioxide) have been

<table>
<thead>
<tr>
<th>Class</th>
<th>Type</th>
<th>Examples</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Hexokinase inhibitors</td>
<td>3-Bromopyruvate, 2-Deoxyglucose, Oxamate</td>
</tr>
<tr>
<td>II</td>
<td>Bcl-2/Bcl-xL mimetics</td>
<td>Gossypol, α-Tocopheryl succinate, Antimycin A</td>
</tr>
<tr>
<td>III</td>
<td>Thiol redox inhibitors</td>
<td>Isothiocyanates, Arsenites, Arsenic trioxide</td>
</tr>
<tr>
<td>IV</td>
<td>VDAC/ANT targeting drugs</td>
<td>Lonidamine, Retinoid analogs such as CD437</td>
</tr>
<tr>
<td>V</td>
<td>Electron transport chain targeting drugs</td>
<td>4-Hydroxy retinamide, Tamoxifen, Antimycin A</td>
</tr>
<tr>
<td>VI</td>
<td>Lipophilic cations targeting inner membrane</td>
<td>Rhodamine-123, MKT-077, (KLAKKLAK); peptide, Mastoparan, Viral protein-R of HIV-1</td>
</tr>
<tr>
<td>VII</td>
<td>Drugs targeting other sites</td>
<td>Resveratrol (ATPase), Betulinic acid</td>
</tr>
</tbody>
</table>

Table 1. Classification of mitocans.
Mitocans as Novel Agents for Anticancer Therapy: An Overview

used medically for many years to treat cancers and are effective against hematological and other malignancies [74, 75, 127]. Hence, arsenic oxides and their derivatives have become established as effective treatments for acute promyelocytic leukemia and are in trials for other hematological cancers, including myelodysplastic syndromes, multiple myeloma, and chronic myelogenous leukemia [128]. The arsenite compounds are likely to modulate critical cysteine residues in the ANT channel, thereby inhibiting its activity [129, 130].

Another group of mitocans is represented by the ANT channel-inhibiting drugs such as lonidamine. Lonidamine (an indazole carboxylate derivative) induces activation of MPTP and causes mitochondrial permeabilization by binding and affecting ANT in mitochondrial inner membrane of tumor cells [129, 131]. Although lonidamine is a potent anti-proliferative anticancer drug, a recent clinical trial have shown it to have little or no additional benefit over conventional therapies and, as a result, has not been pursued further as a broad-spectrum anticancer therapy [132].

CD437 (6[3-adamantyl-4-hydroxyphenyl]-2-naphthalene carboxylic acid) is a new synthetic retinoid acid receptor g (RARg) agonist inducing apoptosis of human breast, lung, cervical, and ovarian carcinomas, melanoma, prostate cancer, neuroblastoma, and acute promyelocytic leukemia (APL). Several mechanisms of induction of cell death process have been reported; activation of AP-1 complex; increase of p53, p21, and Bax; decrease of Bcl-xL; cell-cycle arrest; and activation of caspase 3 and caspase 7 [132, 133]. Moreover, in intact cells CD437-dependent caspase activation is preceded by release of cytochrome C from mitochondria. CD437 also causes membrane permeabilization and this effect is prevented by PTPC inhibitors CsA and BA. Since CD437-mediated cell killing is suppressed by CsA and BA, it appears plausible that CD437 exerts its cytotoxic effects via PTPC [133-136].

Attempts have been made to use cationic lipophilic toxins like MKT-077, as anti-cancer agents. MKT-077, a cationic rhodacyanine dye, is selectively toxic to carcinoma cells in vitro and in vivo [137], perhaps owing to higher ∆ψm in carcinoma cells versus normal cells. Two mechanisms accounting for mitochondrial toxicity of MKT-077 have been proposed [138, 139], namely, a selective MKT-077-driven depletion of mitochondrial DNA in carcinoma cells but not in normal epithelial cells and inhibition of mitochondrial respiration with a decrease in the activities of succinate–cytochrome c reductase and cytochrome oxidase. All of these effects are enhanced by photoactivation. Because of its selectivity toward tumor cells, MKT-077 is currently being evaluated in phase I clinical trials [137, 140]. Another cationic lipophilic dye, chloromethyl-X-rosamine, also acts as a photosensitizer [141].

Betulinic acid, a pentacyclic triterpene, is a novel experimental anticancer drug. It possesses anti-tumor activity in vitro and in vivo in melanoma, neuroectodermal tumors, and glioma cell lines. Fulda et al. have shown that betulinic acid induces apoptosis via direct mitochondrial alterations [142]. In betulinic acid-induced apoptosis, perturbation of mitochondrial function, including loss of mitochondrial permeability transition, precedes other key features of apoptosis such as activation of caspase cascade and nuclear fragmentation via the liberation of soluble factors, such as cytochrome C or AIF [142]. Bcl-2 and Bcl-xL block all mitochondrial and cellular manifestations of apoptosis induced by betulinic acid, as does bongkrekic acid, an inhibitor of PTPC [143, 144].

The redox-silent analogs of vitamin E (VE) are a new group of mitocans which unlike antioxidant VE, selectively induce apoptosis in malignant cells via mitochondria-dependent apoptotic signalling [120]. Alpha tocopheryl succinate (α-TOS), a prominent example of this class is postulated to act by two mechanisms: the major one, in which it inhibits oxidative respiration at the level of complex II (CII), and an auxiliary role, which involves its binding to Bcl-2 and Bcl-xL to allow Bax to form mitochondrial membrane channels [92]. Thus, upon inhibiting the activity of CII, α-TOS impairs transfer of electrons along the redox chain, which leads to generation of ROS, such as the superoxide anion radicals. In the cytosol, ROS oxidize cysteine residues on Bax monomers to form disulfide bridges between monomers of Bax, causing a conformational change and dimerisation that is followed by Bax mobilisation to the mitochondrial outer membrane to form (mega) channels. In the mitochondria, ROS trigger the oxidase activity of cytochrome C that leads to oxidative modification of cardiolipin (CL) with ensuing release of cytochrome C from its binding to the mitochondrial inner membrane phospholipid, followed by cytochrome C extrusion via the Bax channel to cytosol [92, 145]. α-TOS may also complex with BH3 binding hydrophobic groove of Bcl-2 and Bcl-xL, preventing capture of pro-apoptotic Bax homologue Bak by anti-apoptotic Bcl-2 and Bcl-xL. As a result, there is increase in Bak, which is required to form channels in mitochondrial outer membrane, leading to induction of apoptosis especially in prostate cancer cells [125]. In particular, hemisuccinate and two proximal isoprenyl units of side chain were shown to play a critical role in ligand anchoring and Bcl-2 protein-ligand complex formation. From the relationship of α-TOS with UbQ, it is speculated that Bcl-2 and Bcl-xL bind both ubiquinone and quinine related structures. In fact, many small chemi-
cal molecules that have been found recently to bind these proteins are likely to mimic ubiquinones [5]. For example, antimycin A, a well-described inhibitor of quinone-binding site on cytochrome bc1 in mitochondrial respiratory chain, has also been shown to compete for binding with BH3 for hydrophobic groove of either Bcl-2 or Bcl-xL [146]. Furthermore, a 2-methoxy antimycin A derivative with no inhibitory effects on the respiratory chain retains selectivity for Bcl-xL. Hence, a role for quinones such as UbQ in binding anti-apoptotic Bcl-2-related family members, affecting their ability to form dimer with BH3 pro-apoptotic family members, seems very likely [5].

The mitochondrially targeted form of α-TOS called MitoVES is a recent development in the VE class of mitocans. Mitochondrially targeted vitamin E succinate (MitoVES) is modified so that it is preferentially localized into an ideal position across the interface of the mitochondrial inner membrane and matrix, greatly enhancing its pro-apoptotic and anti-cancer activity. Using genetically manipulated cells, MitoVES causes apoptosis and generation of reactive oxygen species (ROS) in CII-proficient malignant cells but not their CII-dysfunctional counterparts. The agent has no effect either on the enzymatic activity of CI or on electron transfer from CI to CIII [147].

Mastoparan, a peptide isolated from wasp venom, is the first peptide known to induce mitochondrial membrane permeabilization and apoptosis via a CsA-inhibitable mechanism [148]. This peptide has an α-helical structure and possesses some positive charges that are distributed on one side of the helix. A similar peptide (KLAKLAKKLAKLAK or (KLAKLAK)2 (K = lysine, L = alanine, and A = leucine) has been found recently to disrupt mitochondrial membranes when it is added to purified mitochondria, although the mechanisms of this effect have not been elucidated [149]. The pro-apoptotic 96 amino acid protein viral protein R (Vpr) from human immunodeficiency virus-1 contains an α-helix with several cationic charges that concentrate on same side of helix [150]. Vpr induces apoptosis via a direct effect on the mitochondrial PTCP, causes dissipation of Δψm as well as the mitochondrial release of apoptogenic proteins, such as cytochrome C or AIF.

9. Conclusions

The search for a selective and efficient anticancer agent for treating neoplastic diseases has yet to deliver a universally suitable compound. Recently, a novel target for potential anticancer agents, the mitochondrion, was discovered which shows a considerable promise for future clinical applications. Anticancer agents specifically targeting cancer cell mitochondria are the “mitocans”, which achieve cancer cell death by mitochondrial mediated apoptosis. But, further clinical studies are required for mitocans to be used as established anticancer therapies or in conjunction with existing chemotherapies. As mitocans have shown limited side effects on normal “healthy” cells in vivo, they offer great potential in cancer therapy.

REFERENCES

Mitocans as Novel Agents for Anticancer Therapy: An Overview


[34] J. M. A. Oliveira, M. B. Jekabsons, S. Chen, A. Lin, A. C. Rego, J. Gonçalves, L. M. Ellerby and D. G. Nicholls, “Mitochondrial Dysfunction in Huntington’s Disease:
doi:10.1111/j.1471-4159.2006.04361.x


doi:10.1016/j.bbadis.2009.08.012

doi:10.1093/brain/awf167

doi:10.1002/mus.20489

doi:10.3390/ijms10052252

doi:10.1002/jcb.20006


doi:10.1074/jbc.M106802200

doi:10.1158/0008-5472.CAN-08-1418


doi:10.1074/jbc.272.34.21104

doi:10.1021/bi00050a027

doi:10.1152/ajpcell.00314.2006

doi:10.1074/jbc.M106743200


doi:10.1002/dmrr.621

doi:10.1016/S0006-2952(97)00136-6


doi:10.1097/00000542-200207000-00004

doi:10.1074/jbc.M109.065664

doi:10.1126/science.1117105


Mitocans as Novel Agents for Anticancer Therapy: An Overview


Mitocans as Novel Agents for Anticancer Therapy: An Overview


