Drug-induced mitochondrial dysfunction and cardiotoxicity

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1Laboratory of Cardiovascular Physiology and Tissue Injury, National Institutes of Health/National Institute on Alcohol Abuse and Alcoholism, Bethesda, Maryland; 2Cardiometabolic Research Group, Department of Pharmacology and Pharmacotherapy, Semmelweis University, Budapest, Hungary; 3PharmaHungary Group, Szeged, Hungary; and 4Department of Intensive Care Medicine BH 08-621-University Hospital Medical Center, Lausanne, Switzerland

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Varga ZV, Ferdinandy P, Liaudet L, Pacher P. Drug-induced mitochondrial dysfunction and cardiotoxicity. Am J Physiol Heart Circ Physiol 309: H1453–H1467, 2015. First published September 18, 2015; doi:10.1152/ajpheart.00554.2015.—Mitochondria has an essential role in myocardial tissue homeostasis; thus deterioration in mitochondrial function eventually leads to cardiomyocyte and endothelial cell death and consequent cardiovascular dysfunction. Several chemical compounds and drugs have been known to directly or indirectly modulate cardiac mitochondrial function, which can account both for the toxicological and pharmacological properties of these substances. In many cases, toxicity problems appear only in the presence of additional cardiovascular disease conditions or develop months/years following the exposure, making the diagnosis difficult. Cardiotoxic agents affecting mitochondria include several widely used anticancer drugs [anthracyclines (Doxorubicin/Adriamycin), cisplatin, trastuzumab (Herceptin), arsenic trioxide (Trisenox), mitoxantrone (Novantrone), imatinib (Gleevec), bevacizumab (Avastin), sunitinib (Sutent), and sorafenib (Nexavar)], antiviral compound azidothymidine (AZT, Zidovudine) and several oral antidiabetics [e.g., rosiglitazone (Avandia)]. Illicit drugs such as alcohol, cocaine, methamphetamine, ecstasy, and synthetic cannabinoids (spice, K2) may also induce mitochondria-related cardiotoxicity. Mitochondrial toxicity develops due to various mechanisms involving interference with the mitochondrial respiratory chain (e.g., uncoupling) or inhibition of the important mitochondrial enzymes (oxidative phosphorylation, Szent-Györgyi-Krebs cycle, mitochondrial DNA replication, ADP/ATP translocator). The final phase of mitochondrial dysfunction induces loss of mitochondrial membrane potential and an increase in mitochondrial oxidative/nitrative stress, eventually culminating into cell death. This review aims to discuss the mechanisms of mitochondrion-mediated cardiotoxicity of commonly used drugs and some potential cardioprotective strategies to prevent these toxicities.

Cardiotoxicity from Drug Developmental Perspective

ADVERSE CARDIAC EFFECTS ARE THE LEADING CAUSE of drug discontinuation and failure of clinical trials. Cardiotoxicity accounted for 45% of all drugs withdrawn between 1994 and 2006, which was due mainly to cardiac ischemia-related and arrhythmogenic side effects (Table 1) (28). Primarily, cardiotoxic drugs may induce cardiovascular adverse effects in a predictable dose- and time-dependent manner (e.g., doxorubicin). In contrast, secondarily cardiotoxic drugs promote adverse consequences in an unpredictable manner, often in patients with cardiovascular comorbidities (e.g., rosiglitazone). Although the above-mentioned adverse effects of numerous widely used drugs have been recognized recently, the cellular mechanisms of their cardiotoxicities are poorly understood. Moreover, the predictive value of currently available toxicity screening methods is very poor, particularly in subjects with cardiovascular comorbidities.

Strikingly, almost 10% of drugs in the last four decades have been recalled from the clinical market worldwide due to cardiovascular safety concerns. Recently, there have been major cases when already marketed drugs were withdrawn or their clinical indications were heavily restricted due to cardiovascular safety concerns, i.e., significantly increased risk of acute myocardial infarction or cardiac fibrosis revealed in phase IV postmarketing clinical studies [e.g., selective COX2 inhibitor rofecoxib (Vioxx) used for the treatment of inflammatory conditions (2004); a serotonin 4 receptor agonist, tegaserod (Zelnorm/Zelmac), used in irritable bowel syndrome (2007); an anti-obesity drug, sibutramine (Meridia; 2010); and peroxisome proliferator-acti-
Mitochondrial Oxidative Stress and Dysfunction is a Common Mechanism in Cardiotoxic Effects

Cardiomyocytes utilize an enormous amount of adenosine triphosphate (ATP), being in a constant energy-consuming contractile state. To maintain constant ATP production, malfunctioning mitochondria are constantly replaced by newly synthesized organelles by processes involving mitochondrial biogenesis and replication and autophagy/mitophagy (34, 153). These processes work in a tightly regulated manner, with mitochondrial fusion and fission allowing the dynamic formation and remodeling of a reticulated mitochondrial network (2). Since mitochondria are responsible for the production of ATP agents that interfere with the physiological mitochondrial mitochondrial function are expected to induce depletion of ATP pool. Eventually, these processes may lead to subsequent myocardial dysfunction. There are several potential ways how drugs may induce mitochondrial dysfunction. Mitochondrial replication is a specific process that is required to maintain a “healthy” mitochondrial population. The antiviral nucleotide reverse transcriptase inhibitors are interfering with the action of the polymerase of the mitochondrial DNA, thereby inhibiting mitochondrial replication. This gradually reduces mitochondrial function in various tissues that will be apparent first in metabolically active organs such as the heart and the liver, resulting in cardiotoxicity and hepatotoxicity. Other drugs may directly interact with the electron transport chain (antidiabetic thiazolidinediones/glitazones, nonsteroidal anti-inflammatory drugs), resulting in uncoupling of electron transport from ATP production or directly induce oxidative stress in the mitochondria by redox cycling or by promoting iron accumulation and glutathion depletion [doxorubicin (Adriamycin); ethanol and acetaminophen (Tylenol); Fig. 1].

Oxidative/nitrosative modifications of mitochondrial proteins might play a crucial role in the development of myocardial dysfunction (see Table 2). Oxidative/nitrosative modification may trigger potentially harmful events, including dissociation of catalytic subunits of enzymes, local or global unfolding, aggregation, or fragmentation, all promoting degradation of modified proteins leading to autophagy/mitophagy and endoplasmic reticulum stress (10, 17, 153). Oxidation/nitration might be directly triggered by reactive oxygen species (ROS)/reactive nitrogen species (RNS) or by products of secondary oxidation reactions formed during lipid peroxidation (e.g., malondialdehyde or 4-hydroxyxynonenal) (89, 174). The free radicals produced intramitochondrially can directly inactivate the electron transport complexes by interacting with the iron-sulfur cluster, or they may lead to activation of apoptosis-initiating pathways by inducing mitochondrial transition pore opening.

Inhibition of the tricarboxylic acid (TCA) cycle (also known as the citric acid cycle or Szent-Györgyi-Krebs cycle) also occurs due to excessive mitochondrial oxidants production by the oxidation of acacetin. Increased ROS generation in cardiomyocytes may trigger the activation of various mitochondrial-dependent and -independent cell death pathways involved in apoptotic and necrotic cell death [e.g., activation of caspases and poly(ADP-ribose) polymerases (PARP)] (115). Furthermore, superoxide in the mitochondria may react with nitric oxide to generate a highly reactive oxidant, peroxynitrite (110, 111), which may impair cellular function and lead to cell death (100) and/or dysfunction (114) in cardiomyocytes and endothelial cells via multiple interrelated mechanisms involving PARP (112) and matrix metalloprotease (MMP) activation (5). Mitochondrial proteins are particularly vulnerable to peroxynitrite-induced nitration, leading to irreversible functional loss (110, 119, 142).
can develop within 1 mo or even years after the treatment initiation (dosages >500 mg pro 1 m² body surface have 5% probability of inducing cardiac heart failure) (97). Recent studies also highlight the occurrence of late-onset cardiac dysfunction in adults who were treated with doxorubicin during their childhood; 5.8% of this population had severely reduced ejection fraction. However, systolic and diastolic dysfunction by sensitive modalities (strain rate imaging) was more prevalent (apparent in >30% of patients). Interestingly, survivors with metabolic syndrome were more prone to develop contractile dysfunction, suggesting a common molecular link in doxorubicin- and metabolic syndrome-induced contractile dysfunction (3).

The mechanism for doxorubicin-induced cardiotoxicity is controversial, and numerous hypotheses have been proposed in past decades. Initially, it was widely accepted that doxorubicin-induced cardiotoxicity is completely independent from its antineoplastic activity. This concept was in agreement with the fact that cardiomyocytes, as terminal, differentiated, nondividing cells, should not be sensitive to the primary antineoplastic activity, which is related to DNA transcription and replication. However, systolic and diastolic dysfunction by sensitive modalities (strain rate imaging) was more prevalent (apparent in >30% of patients). Interestingly, survivors with metabolic syndrome were more prone to develop contractile dysfunction, suggesting a common molecular link in doxorubicin- and metabolic syndrome-induced contractile dysfunction (3).

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Fig. 1. Doxorubicin-induced mitochondrial dysfunction and the effect of trastuzumab on mitochondria-related survival pathways. Doxorubicin leads to marked induction of mitochondrial reactive oxygen species (ROS) production. It shows specific binding activity to the mitochondrial abundant cardiolipin, leading to selective mitochondrial accumulation. Doxorubicin is prone to redox cycling, thereby promoting ROS and reactive nitrogen species (RNS) production. There is also increased intramitochondrial free iron accumulation after doxorubicin exposition, giving rise to additional nonenzymatic ROS production by the Haber-Weiss reaction. The major consequences of uncontrolled ROS/RNS production are mitochondrial permeability transition pore (MPTP) opening and poly(ADP-ribose) polymerase (PARP) activation, converging to the propagation to cell death signaling mechanisms. In parallel with ROS/RNS induction, doxorubicin also interferes with the action of topoisomerase-2β, being involved in the regulation of peroxisome proliferator-activated receptor-γ coactivator-1α (PGC-1α) and thereby mitochondrial biogenesis- and metabolism-related pathways. Altogether, these doxorubicin-induced alterations profoundly alter both mitochondrial structure and function. The release of neuregulin-1 by the coronary endothelium activates human epidermal growth factor receptor (Her)4 (ErbB4) to dimerize with Her2 (ErbB2). The Her4/Her2 (ErbB4/ErbB2) dimer activates cardioprotective signaling pathways, including phosphatidylinositol 3-kinase (PI3K)/protein kinase B (Akt), ERK1/2, and focal adhesion kinase (FAK), which promote cell survival upon cellular stress. Trastuzumab blocks Her2 signaling and disrupts this cardioprotective machinery, resulting in loss of cytoprotective mechanisms. Furthermore, trastuzumab may trigger cellular oxidative stress and induce the expression and activation of proapoptotic proteins [e.g., Bcl-2-associated X protein (BAX)]. These events result in mitochondrial defects, leading to the opening of the MPTP and the activation of cell death pathways that precipitate myocardial dysfunction. BAD, Bcl-2-associated death promoter; mKATP, mitochondrial ATP-sensitive potassium channel.
drial abundant phospholipid cardioline. Mitochondrial DNA oxidation is cardioselective and cumulative (133) and might be a major contributor of heart failure development (71, 84). Once doxorubicin accumulates in mitochondria, it can initiate intramitochondrial ROS and RNS production (102) by various, mainly nonenzymatic mechanisms, leading to activation of cell death pathways (e.g., PARP-dependent cell death; see Refs. 112 and 113). One major hypothesis of doxorubicin-induced ROS formation implies the redox cycling-dependent production of superoxide anion described first by Davies and Dorsheon (21; also see Ref. 26) and confirmed by others (66, 121, 135). Increased ROS production subsequently induces activation of proinflammatory transcription factor NF-κB and inducible nitric oxide synthase (100). The diffusion-limited reaction of superoxide and nitric oxide forms peroxynitrite, a potent oxidant that further aggravates initiation of cell death (111). It has also been suggested that doxorubicin induces an alternative iron-mediated increase in ROS production (11, 103). According to this hypothesis, it is likely that mitochondrial accumulation of iron is detrimental, since doxorubicin-derived superoxide that will be eventually converted to H₂O₂ will form highly toxic hydroxyl radicals in the presence of iron by a reaction described as Haber-Weiss reaction. In addition, doxorubicin can interact with iron directly to form a complex that will result in iron cycling between the ferro [Fe(II)] and ferric [Fe(III)] forms and will lead to additional ROS production (49, 165). Doxorubicin-induced oxidative stress is further aggravated by the infectivity and/or inhibition of antioxidant mechanisms by doxorubicin. In line with this, overexpression of antioxidant enzyme systems [manganese superoxide dismutase (166), catalase (55), and metallothionein (39, 56)] alleviates doxorubicin cardiotoxicity.

Recently, the doxorubicin-related DNA transcription blockade has been also linked to mitochondrial dysfunction. The primary target of doxorubicin is the topoisomerase IIα expressed in many cancerous tissues (117). Detailed investigations shed light on the fact that cardiomyocytes express the other isozyme of topoisomerase 2, the Top2β (15). Accordingly, recent results suggest that doxorubicin-induced cardiotoxicity is not due solely to the ROS producing redox cycling reactions of doxorubicin. In the presence of Top2β (like in case of cardiac tissue), doxorubicin activates DNA response genes and consequently apoptosis pathways and further triggers marked alterations in the transcriptome, which selectively affects oxidative phosphorylation and mitochondrial biogenesis [downregulation of peroxisome proliferator-activated receptor-γ coactivator (PGC)-1α and PGC-1β] in cardiomyocytes, leading to mitochondrial oxidative stress and metabolic failure (42, 172) (Fig. 1). This explains the classical observation that doxorubicin causes both structural and functional mitochondrial abnormalities.

Targeting doxorubicin-induced cardiac pathological alterations in the clinical setting is really challenging. Although several drug candidates have been positively evaluated in animal models, only a few of them have been tested so far in clinical studies. It is also important to keep in mind that the drug used to treat/prevent doxorubicin-induced cardiomyopathy should not interfere with its antitumor activity.

Dexrazoxane is the most studied cardioprotective adjuvant for doxorubicin chemotherapy, which itself has antineoplastic properties. Due to a metabolite that chelates free iron (131), dexrazoxane alleviates doxorubicin-induced mitochondrial oxidative stress and the subsequent depletion of mitochondrial DNA (72). The clinical efficacy of dexrazoxane has been confirmed by Lipshultz et al. (81) in children with acute lymphoblastic leukemia undergoing doxorubicin treatment. They found that dexrazoxane attenuated doxorubicin-

### Table 2. Oxidative and nitrative modifications of key mitochondrial proteins in various forms of cardiomyopathy

<table>
<thead>
<tr>
<th>Modified Protein</th>
<th>Modification</th>
<th>Function</th>
<th>Ref. Not(s.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3-Ketacyl-CoA thiolase</td>
<td>Oxidation</td>
<td>Fatty acid β-oxidation</td>
<td>23</td>
</tr>
<tr>
<td>Acetyl-CoA acetyl transferase</td>
<td>Oxidation</td>
<td>Fatty acid β-oxidation</td>
<td>23</td>
</tr>
<tr>
<td>Acyl-CoA dehydrogenase</td>
<td>Oxidation</td>
<td>Fatty acid β-oxidation</td>
<td>23</td>
</tr>
<tr>
<td>ATP synthase subunits</td>
<td>Nitrination</td>
<td>ATP synthesis</td>
<td>20, 82</td>
</tr>
<tr>
<td>BNIP3</td>
<td>Oxidation</td>
<td>Mitophagy, oxidative stress sensor</td>
<td>65</td>
</tr>
<tr>
<td>CaMK II</td>
<td>Oxidation</td>
<td>Mitochondrial stress response</td>
<td>44, 51</td>
</tr>
<tr>
<td>Carnitine palmitoyltransferase-1</td>
<td>Nitrination</td>
<td>Fatty acid transport</td>
<td>29</td>
</tr>
<tr>
<td>Complex I, III, and V</td>
<td>Oxidation</td>
<td>ATP synthesis</td>
<td>9, 18, 147</td>
</tr>
<tr>
<td>Complex I (24-kDa subunit)</td>
<td>Nitrination</td>
<td>ATP synthesis</td>
<td>149</td>
</tr>
<tr>
<td>Complex II</td>
<td>Oxidation</td>
<td>ATP synthesis</td>
<td>141</td>
</tr>
<tr>
<td>Complex III (38-kDa subunit)</td>
<td>Oxidation</td>
<td>ATP synthesis</td>
<td>158, 159</td>
</tr>
<tr>
<td>Connexin43</td>
<td>Nitrination</td>
<td>Mitochondrial potassium uptake</td>
<td>38, 52, 138</td>
</tr>
<tr>
<td>Creatine kinase</td>
<td>Nitrination</td>
<td>Maintenance of ATP pool</td>
<td>93, 94</td>
</tr>
<tr>
<td>Cytochrome c</td>
<td>Oxidation</td>
<td>Mitochondrial apoptosis</td>
<td>16</td>
</tr>
<tr>
<td>Enoyl-CoA hydratase</td>
<td>Oxidation</td>
<td>Fatty acid β-oxidation</td>
<td>23</td>
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<tr>
<td>mthHSP70, mitofin</td>
<td>Oxidation</td>
<td>Mitochondrial protein import</td>
<td>9</td>
</tr>
<tr>
<td>Peroxiredoxin 3</td>
<td>Nitrination</td>
<td>Antioxidant defense</td>
<td>149</td>
</tr>
<tr>
<td></td>
<td>Oxidation</td>
<td>Antioxidant defense</td>
<td>23, 67</td>
</tr>
<tr>
<td>Prohibitin</td>
<td>Nitrination</td>
<td>Unknown</td>
<td>85</td>
</tr>
<tr>
<td>Succinyl-CoA:3-oxoacid CoA transferase</td>
<td>Nitrination</td>
<td>Ketone body metabolism</td>
<td>149, 160</td>
</tr>
<tr>
<td>Superoxide dismutase-2</td>
<td>Nitrination</td>
<td>Antioxidant defense</td>
<td>23, 63</td>
</tr>
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<td></td>
<td>Oxidation</td>
<td></td>
<td>41</td>
</tr>
<tr>
<td>VDAC1</td>
<td>Nitrination</td>
<td>Mitochondrial apoptosis</td>
<td>150</td>
</tr>
</tbody>
</table>

BNIP3, Bcl-2 adenovirus E1B 19-kDa-interacting protein 3; VDAC1, voltage-dependent anion channel.
induced cardiac injury without compromising its antileukemic efficacy (81).

Since β-adrenergic receptor antagonists along with angiotensin-converting enzyme inhibitors are the most widely used drugs in the treatment of heart failure, their applicability in doxorubicin-treated patients is plausible. Accordingly, in patients taking carvedilol, a combined α- and β-receptor antagonist with antioxidant properties, left ventricular size remained constant, and diastolic function was preserved after doxorubicin treatment (53).

Consistently with the importance of mitochondrial ROS generation in the cardiotoxicity of doxorubicin, mitochondria-targeted antioxidants mito-tempol and MitoQ exerted cardioprotective effects in rodents without interfering with doxorubicin’s antitumor effect (24). Activation of the nuclear enzyme PARP due to doxorubicin-induced oxidative DNA injury and the consequent cell death are key events in doxorubicin-induced cardiotoxicity (112). Consequently, PARP inhibitors (e.g., olaparib), novel FDA-approved anticancer medications often combined with doxorubicin or cisplatin, are promising cardioprotective agents against doxorubicin-induced cardiomyopathy based on preclinical results (112).

Cisplatin. Cisplatin belongs to the alkylating group of broad-spectrum chemotherapeutic drugs used against various types of tumors [sarcomas, carcinomas (e.g., small cell lung cancer, ovarian), lymphomas, and germ cell tumors]. A significant factor limiting its applicability is acute and cumulative cardiotoxicity and nephrotoxicity (116), sharing similar cellular and molecular mechanisms (86, 99, 177). Cisplatin-induced cardiac dysfunction is associated with mitochondrial membrane depolarization along with ultrastructural abnormalities of the mitochondria. Following cisplatin treatment, cardiomyocytes also show signs of activation of the endoplasmic reticum stress response, increased caspase 3 activity, and increased rate of apoptosis (86). It is well documented that cisplatin promotes kidney injury by triggering mitochondrial ROS generation in renal tubular cells (177). It is highly plausible that cisplatin induces cardiotoxicity by similar mechanisms (involving increased mitochondrial oxidative stress). In accord with this, mitochondria targeted antioxidants might represent a promising approach to alleviate both cardiac and kidney injury in patients undergoing cisplatin chemotherapy (99).

Trastuzumab. Trastuzumab is a monoclonal antibody that inhibits the activation of the receptor tyrosine-protein kinase erbB-2/proto-oncogene neu (HER2/neu), thereby interfering with the growth of certain breast cancers. Extensive data have shown recently that HER2 has also an important role in embryonic heart development, and in the adult heart it is involved in cardiac protection (107). HER2-initiated signaling is essential for growth, survival, and inhibition of apoptosis of cardiomyocytes; therefore, in cases where the heart is subjected to biomechanical stress (e.g., hypoxia, myocardial injury, or concomitant anthracycline use), neuroregulin binds to HER2/HER4 heterodimers, thereby promoting cardiomyocyte survival via the activation of the phosphatidylinositide 3-kinase (PI3K) and MAPK pathways (106, 170). In addition, a decrease in HER2 and HER4 protein expression has been shown to be associated with the transition of compensated cardiac hypertrophy to heart failure (124). Furthermore, trastuzumab triggers cellular oxidative stress and induces the expression and activation of proapoptotic proteins. These events result in mitochondrial defects, leading to the opening of the mitochondrial permeability transition pore (MPTP) and the activation of cell death pathways, which precipitate myocardial dysfunction (Fig. 1) (6, 176).

Anthracyclines induce a variable degree of myocardial damage even when administered at safe doses. The anthracycline-induced myocardial injury is associated with the concomitant activation of HER2 survival pathways within the cardiomyocytes as an attempt to prevent cardiomyopathy. Several in vitro, in vivo, and clinical studies have clearly shown that trastuzumab significantly aggravates anthracycline-induced cardiac damage, resulting in an extremely high incidence of symptomatic heart failure that reached 27% of treated patients (137). Although the incidence of symptomatic heart failure of trastuzumab-treated patients in monotherapy is significantly lower (~4%) (152), even trastuzumab alone exhibits inherent cardiac toxicity that is associated with significant alterations in the expression of myocardial genes essential for DNA repair and cardiac and mitochondrial function (31).

Arsenic trioxide. Arsenic trioxide is an antineoplastic drug, inducing changes in apoptotic signaling in cancer cells. It also appears that arsenic trioxide inhibits the PML-RAR fusion protein often detected in acute promyelocytic leukaemia. Although it induces dramatic remissions in patients with acute promyelocytic leukaemia, several clinical reports have shown that the treatment is associated with significant cardiotoxicity (QT prolongation, torsade de pointes, sudden death) (105, 151). Exposure of H9C2 cardiomyocytes to clinically relevant concentrations of the drug (2–10 μM) induced apoptosis, ROS formation, intracellular calcium overload, and caspase-3 activation (173). The negative impact of arsenic trioxide is further confirmed by in vivo studies showing a significant decrease in the maximum rate of rise in intraventricular pressure during ventricular contraction (maximal dP/dt) and significant increases in the end diastolic pressure. There is also impaired response to β-adrenergic stimulation (isoproterenol) (77). In a recent study, the proapoptotic effect of arsenic trioxide was confirmed, and the role of Parkin-dependent ubiquitin proteasome activation was described to be associated with arsenic trioxide-induced loss of mitochondrial membrane potentials (161).

Mitoxantrone. Mitoxantrone is a DNA topoisomerase inhibitor and nonanthracycline antineoplastic agent used for the treatment of various cancers (metastatic breast cancer, acute myeloid leukemia, non-Hodgkin’s lymphoma, acute lymphoblastic leukemia in children, and metastatic hormone-refractory prostate cancer). In addition to cancer therapy, it is widely used in multiple sclerosis, effectively slowing the progression and preventing the relapses in relapsing remitting and the progressive-relapsing form of the disease. Cardiomyopathy is a particularly concerning side effect of long-term mitoxantrone therapy, as it is usually severe, may occur years after treatment, and is irreversible (128, 140). Mitoxantrone induces striking energetic imbalance, as evidenced by decreased ATP levels, hyperpolarization of the mitochondrial membrane potential, and significant rise in the intracellular calcium levels in vitro. This is further complicated by late inhibition of ATP-synthase expression and activity with concomitant increase in ROS formation (126). It is now evident also that cardiac functional alterations are due to the presence of aberrant mitochondria, changes in mitochondrial complex IV and V activities, and
depletion of cardiac ATP levels (125). It cannot be also excluded that mitoxantrone as a DNA topoisomerase inhibitor may interact with the mitochondrial topoisomerase enzyme being critically involved in maintenance of mitochondrial integrity and cellular energy metabolism (27).

Imatinib mesylate. Imatinib mesylate is one of the first marketed drugs that has been developed for tyrosine kinase inhibition (inhibitor of the BCR/Abl fusion protein). Despite its cardiotoxic effect, imatinib mesylate represents a revolution in the management of patients with Philadelphia chromosome-positive chronic myelogenous leukemia, increasing the survival of patients by inducing complete cytogenetic response in more than 70% of patients treated (22, 57). In 2001 the FDA approved its clinical use, and the cardiotoxic effect of imatinib was described first only 5 years later, in 2006, by Kerkelä et al. (60). In several patients, sudden development of NYHA class 3–4 heart failure has been reported after a few months (7.2 ± 5.4) of imatinib therapy. The ultrastructural analysis revealed prominent membrane whorls in myocytes and an increased number of mitochondria and pleomorphic mitochondria with effaced cristae, which is indicative of increased mitochondrial biogenesis that is typically detectable in hearts with impaired mitochondrial energy production. In vitro studies on isolated cardiomyocytes revealed that imatinib produces a dose-dependent collapse in mitochondrial membrane potential. The inhibition of Abl kinase by imatinib resulted in increased endoplasmic reticulum stress, as detected by increased PRKR-like endoplasmic reticulum kinase (PERK) activation and phosphorylation of eukaryotic initiation factor-2α (eIF2α). PERK also influences mitochondrial proteostasis through translational attenuation of translocase of the inner membrane (the 23-kDa form TIM23), a major component of the mitochondrial protein import machinery. TIM23 is degraded after eIF2α phosphorylation, and thus unfolded proteins may accumulate in mitochondrial intermembrane space, challenging the mitochondrial proteostasis pathways and inducing mitochondrial death pathways (120). Therefore, alterations in ER stress pathways leading to mitochondrial function impairment seem to be essential in the cause of imatinib-induced cardiotoxicity (Fig. 2). Recent studies also confirm that the action of imatinib on the heart targets cardiomyocytes and involves mitochondrial impairment and cell death that can be aggravated further by the presence of oxidative stress (88).

Cardiotoxic effect of antiangiogenic drugs. Antiangiogenic drugs inhibiting vascular endothelial growth factor (VEGF) and hypoxia-inducible factor 1-related signaling have been approved for the treatment of advanced carcinomas of the lung, breast, colon, and rectum (59). After their initiation in the clinical practice, several cardiovascular side effects involving left ventricular dysfunction and subsequent heart failure have been reported (13, 19, 129). Myocardial dysfunction develops partially on the basis of the heart’s dependence on adequate angiogenesis; however, other mitochondria-related signaling pathways also play important roles in the observed pathology (164).

Bevacizumab (Avastin) is a recombinant humanized monoclonal antibody that blocks angiogenesis by inhibiting VEGF-A. The development of cardiomyopathy and heart failure, however, is a rare event in bevacizumab-treated patients (2%; see Ref. 95). Small-molecule, multitargeted receptor tyrosine kinase inhibitors have been developed to inhibit divergent pathways involved in tumor cell survival (VEGF,

Fig. 2. Mechanisms of imatinib cardiotoxicity. The small molecule inhibitor of the Bcr/Abl (fusion kinase of the break point cluster region of chromosome 22 and the Abelson1 gene of chromosome 9) kinase imatinib induces mitochondrial dysfunction by interfering with mitochondrial protein import machinery. Increased PRKR-like endoplasmic reticulum kinase (PERK) activation in the endoplasmic reticulum leads to the phosphorylation eukaryotic initiation factor 2α (eIF2α) and to translational attenuation of translocase of the inner membrane (TIM) 23-kDa form that is essential for protein import into the mitochondrial matrix. Impaired protein import will negatively affect major mitochondrial metabolic pathways, including mitochondrial DNA synthesis, the Krebs cycle, β-oxidation, and hem synthesis. TOM, translocase of the outer membrane.
platelet-derived growth factor, c-kit, RET proto-oncogene, RAF proto-oncogene serine/threonine-protein kinase-1, and Fms-like tyrosine kinase 3). Sunitinib (Sutent) and sorafenib (Nexavar) are the most widely used drugs with potent antiangiogenic activity that are currently approved for the treatment of metastatic renal cell carcinoma and for imatinib-resistant gastrointestinal stromal cell tumors. Sunitinib is reported to be cardiotoxic, deteriorating myocardial contractility in up to 28% of treated patients (19). The mechanism of sunitinib cardiotoxicity can be explained by inhibition of off-target pathways such as the ribosomal S6 kinase and AMP-activated protein kinase (43, 61), both of which are involved in mitochondrial energy homeostasis and quality control (175).

**Cardiotoxicity of Antiviral Drugs**

In attempt to control HIV infection, multiple antiviral drug combinations have been developed. The majority of highly active antiretroviral therapy regimens involve nucleoside analogs that inhibit the reverse transcriptase of the virus, such as zidovudine [azidothymidine (AZT)]. However, long-term treatment with AZT may cause cardiomyopathy as a result of mitochondrial toxicity. AZT-triphosphate interferes with the mitochondrial DNA polymerase-γ, the enzyme responsible for mitochondrial DNA replication (75) (Fig. 3). It has also been suggested that AZT may directly inhibit important mitochondrial transport mechanisms such as the mitochondrial ADP/ATP translocator (8) and the mitochondrial deoxynucleotide carrier (25). Although energy depletion by both direct inhibitions of ADP/ATP translocation and mitochondrial replication contributes to cardiac dysfunction, it is now evident that AZT induces increased mitochondrial ROS production as well (36). It is supported by the fact that AZT-induced cardiomyopathy is prevented in mitochondrial superoxide dismutase transgenic mice. In addition, catalase targeted directly into the mitochondria also prevents the AZT-induced oxidative stress and cardiomyopathy development (64). In line with these results, we have reported a sudden increase in mitochondrial ROS production in AZT-treated human cardiomyocytes that was associated with subsequent activation of major cell death pathways (caspase-3 and -7 as well as PARP) (36) being involved in cardiomyopathy development.

**Cardiotoxicity of Antidiabetics**

Diabetes is a major risk factor and comorbidity for heart diseases, including ischemic heart disease and diabetic cardiomyopathy (32, 153). Therefore, many of the cardiac disease patients are treated with oral antidiabetics on top of general medications for ischemic heart disease. However, some of the antidiabetic drugs, especially thiazolidinediones and sulfonylureas, have been shown to exert potential cardiotoxicity.

Rosiglitazone, a thiazolidinedione class of antidiabetic compound acting as an insulin sensitizer via activation of PPAR receptors (122), has been shown by meta-analyses to increase cardiovascular risk. In 2007, a meta-analysis of four randomized controlled trials of rosiglitazone used for at least 12 mo for prevention or treatment of type 2 diabetes showed that rosiglitazone was associated with a significantly increased risk of myocardial infarction and heart failure, although no increase in risk of cardiovascular mortality was observed (136). A recent metaanalysis of the cardiovascular outcomes in 16 studies, including 810,000 thiazolidinedione users, confirmed that the use of rosiglitazone is associated with significantly higher odds

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Fig. 3. Mechanisms of zidovudin [azidothymidine (AZT)] cardiotoxicity. AZT interferes with mitochondrial DNA polymerase-γ, the enzyme responsible for mtDNA replication. Since the mitochondrial DNA (mtDNA) codes the proteins of the electron transport chain (ETC), inhibition of mtDNA replication will result in mitochondrial energetic imbalance. AZT may directly inhibit the mitochondrial ADP/ATP translocator, inhibiting the transport of ATP produced from oxidative phosphorylation and the substrate ADP to be transported from the cytoplasm to the mitochondrial matrix.
of congestive heart failure, myocardial infarction, and death relative to pioglitazone users (83). The mechanism by which thiazolidinedione antidiabetics, especially rosiglitazone, may exert cardiotoxicity is still not exactly known, but several mechanisms were suspected in preclinical studies (Fig. 4). Both rosiglitazone and pioglitazone have been shown to block K<sub>ATP</sub>, thereby leading to increased incidence of ventricular fibrillation during ischemia in pigs (127). Adverse electrophysiological changes in mice, rats, and canine cardiac myocytes have been also shown due to rosiglitazone treatment in vitro (143, 144). Inhibition of mitochondrial respiration has been shown by PPAR agonists troglitazone and darglitazone in isolated mitochondria of the rat liver (104). By a toxico-proteomics approach, other mitochondrial off-targets of troglitazone that may lead to impaired mitochondrial glutathione import and increased oxidative stress have been found (73). In another toxico-proteomics study, off-target affinity of glitazones was identified with various targets, including components of mitochondrial energy metabolism (46). Troglitazone has also been shown to induce cytotoxicity and mitochondrial toxicity at least in part by promoting the degradation of PPARγ coactivator-1α (PGC-1α) (78). More interestingly, troglitazone has been shown to activate mitochondrial permeability transitions pore (MPTP) in isolated rat liver mitochondria (109). Since MPTP inhibition is a common downstream target of most cardioprotective signals, activation of MPTP by troglitazone may lead to deteriorated ischemic tolerance of the heart, i.e., hidden cardiotoxicity (32).

The K<sub>ATP</sub> blocker sulfonylureas show potential cardiotoxicity. A meta-analysis of all trials with a duration of at least 6 mo comparing a sulfonylurea with a nonsulfonylurea agent in type 2 diabetes concluded that the use of sulfonylureas was associated with increased mortality and a higher risk of stroke, whereas the overall incidence of major cardiovascular events was not affected (96). Other meta-analyses showed that patients receiving sulfonylureas had increased all-cause and cardiovascular mortality risks (35, 118). The mechanism by which sulfonylureas may exert cardiotoxicity is the inhibition of both sarcolemmal and mitochondrial K<sub>ATP</sub> channels. Sarcolemmal K<sub>ATP</sub> blockade may lead to action potential shortening causing tachyarrhythmias. Mitochondrial K<sub>ATP</sub> blockade may lead to oxidative stress, thereby leading to mitochondrial dysfunction (32, 33).

Cardiotoxicity of Recreational Drugs: Alcohol, Cocaine, Methamphetamine, Ecstasy, and Cannabinoids

Cardiotoxicity in alcohol abuse. Alcohol abuse impairs myocardial contractility, leading to systolic dysfunction and...
dilation of the ventricles, which is known as alcoholic cardiomyopathy (30, 37).

The toxic effect is explained by three closely related hypotheses (Fig. 5). Alcohol may exert cardiotoxic effect by directly damaging cardiac mitochondria since the metabolizing enzyme, the alcohol dehydrogenase, is almost absent from cardiomyocytes (4). In addition, acetaldehyde, the metabolic by-product synthesized in the liver during oxidative alcohol catabolism, may further trigger the damage of cardiomyocytes by reducing the synthesis of myocardial proteins (68, 130) and thereby disturbing calcium homeostasis and inducing endoplasmic reticulum stress (40, 76). Nonoxidative metabolism of alcohol usually results the production of fatty acid ethyl esters that have been shown to be produced in the heart and to induce mitochondrial dysfunction through uncoupling of mitochondrial oxidative phosphorylation (69). In addition, alcohol-induced cardiac toxicity may also involve the inhibition of the mitochondrial respiratory chain by impairing the function of tricarboxylic acid cycle (Szent-Györgyi-Krebs cycle) enzymes (92). Alcohol induces oxidative stress mainly in the mitochondria due to its metabolism by cytochrome p450 2E1 isoenzyme (45, 171). Mitochondrial oxidative damage in ethanol-treated rats also contributes to myocardial fibrotic changes (155). Alcohol-induced oxidative stress is further enhanced by aggravated angiotensin II signaling, resulting in myocardial NADPH oxidase upregulation, oxidative stress, inflammation, and fibrosis (146), which are known contributors of myocardial dysfunction (154). Ethanol also may activate inducible nitric oxide synthase (partially by increasing endotoxin load from the gastrointestinal tract; see Ref. 7) to produce nitric oxide, which when reacting with superoxide forms the highly reactive substance peroxynitrite, which can further impair the function of mitochondrial function by posttranslational protein modifications (110, 153). There is also evidence in animal models of chronic alcoholism for decreased number of mitochondria and impaired expression of pivotal mitochondrial components like the master regulator of mitochondrial biogenesis, PGC-1α (48, 90). The combination of these different toxic effects both contributes to the pathology and potentially culminates in the decrease of myocardial contractility and function.

Cardiotoxicity in cocaine abuse. Cocaine abuse causes irreversible structural and functional abnormalities in the heart, resulting in chronic reduction in left ventricular contractility and increased incidence of arrhythmias. Additionally, coronary vasoconstriction and atherosclerosis develops, which makes cocaine users more susceptible to myocardial infarction (123).

The main cause of cardiac side effects in cocaine abuse is overstimulation of the adrenergic system. Most of the toxic effects of cocaine on the molecular level are mediated by oxidative stress or mitochondrial dysfunction caused by metabolism of the excess of catecholamines (79). The transformation of catecholamines into “aminochromes” occurs, which may undergo redox cycling after entering the mitochondria.

![Fig. 5. Mechanisms of ethanol cardiotoxicity. Ethanol will increase mitochondrial ROS production by complex mechanisms. On the one hand, ethanol and acetaldehyde will inhibit the function of the Krebs cycle and the ETC, whereas in parallel there is increased intramitochondrial NADH production by aldehyde dehydrogenase 2 (ALDH2). To bypass the inhibited ETC, NADH may be used for ROS/RNS production, leading to MPT opening. Inhibition of PGC-1α by ethanol leads to deteriorated mitochondrial biogenesis and oxidative metabolism. CYP2E1, cytochrome P450 2E1.](http://ajpheart.physiology.org/doi/10.220.32.246)
This leads to the generation of significant amounts of oxygen-derived free radicals. There is also evidence for inhibition of complex I by cocaine (169) and for xanthine oxidase-dependent increase in mitochondrial ROS production after cocaine exposure (157). In turn, calcium overload and oxidative stress promote mitochondrial permeability transition and cardiomyocyte cell death via activation of both the apoptotic and necrotic pathways (70). The central role of mitochondrial oxidative stress in cocaine-induced cardiotoxicity is also supported by the results of Vergeade et al. (156) showing the attenuation of cardiotoxicity by MitoQ, a mitochondrial-targeted antioxidant.

Cardiotoxicity in methamphetamine and ecstasy abuse. Methamphetamine abuse is a significant problem with a steeply increasing frequency of use worldwide. Chronic methamphetamine use is associated with focal contraction band necrosis in association with cellular degeneration and myocytolysis in the heart. After chronic administration, cardiac hypertrophy, intracellular vacuolization, and fibrosis can be also observed (167). The direct mechanisms by which methamphetamine exerts its harmful effects on the heart are not known in detail. Nevertheless, increased mitochondrial superoxide production (91), increased mitochondrial protein tyrosine residue nitration (see Table 2) (85), and induction of Fas- and mitochondria-dependent apoptosis have been reported recently (80).

Ecstasy is a substituted amphetamine, producing structural and functional alterations in the myocardium that are associated with increased oxidative stress. It has been reported that ecstasy significantly increases nitrotyrosine content in the heart. In addition, a detailed proteomic analysis revealed increased nitration of contractile proteins (troponin-T, tropomysosin-α1 chain, myosin light polypeptide, and myosin regulatory light chain), mitochondrial proteins (ubiquinoncytochrome c reductase and ATP synthase), and sarcoplasmic reticulum calcium ATPase (134).

Cardiotoxicity in cannabinoid abuse. Synthetic or designer cannabinoid compounds are getting popular, especially among young people. These illicit drugs are among the most frequently used ones, since these mixtures can still be purchased easily due to the lack of legal restrictions (manufacturers are constantly changing and substituting different chemicals in their mixtures). Several case reports were published recently, showing occurrence of life-threatening complications induced by synthetic cannabinoids involving cardiotoxicity (168), acute kidney injury (14), or cerebral ischemia (145). The majority of studies suggest cannabinoid receptor 1 (CB1)-dependent toxicity (148, 163), which is in line with our recent results showing the role of increased endocannabinoid levels (98) and overactivated CB1 signaling (101) in doxorubicin-treated hearts. Our results also suggest that peripherally restricted CB1 receptor antagonism might be a promising strategy to alleviate cardiac dysfunction and reduce doxorubicin-induced apoptosis in the myocardium (97).

Conclusions and Therapeutic Perspectives

Collectively, multiple lines of evidence briefly discussed in this synopsis strongly suggest that the cardiotoxicity of multiple commonly used antineoplastic drugs, antiviral compounds, and antidiabetic or illicit drugs of abuse such as alcohol, cocaine, methamphetamine, ecstasy, and synthetic cannabinoids involves direct or indirect mitochondria-related toxicity, which is comprised of interference with the mitochondrial respiratory chain (e.g., uncoupling) or inhibition of the important mitochondrial enzymes (oxidative phosphorylation, Szent-Györgyi-Krebs cycle, mitochondrial DNA replication, ADP/ATP translocator), eventually leading to loss of mitochondrial membrane potential, an increase in mitochondrial oxidative/nitritative stress, and cell demise. More thorough understanding of the common mechanisms of mitochondrial cardiovascular toxicities is required to develop sensitive and high-throughput mitochondrial toxicity screening methods and in vivo models to better predict the unforeseen cardiotoxicity issues with novel compounds as well as devise novel cardioprotective strategies based on more selective targeting of specific mitochondrial processes for prevention of the above-discussed severe cardiovascular adverse consequences of common drugs.

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DISCLOSURES

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AUTHOR CONTRIBUTIONS

Z.V.V. prepared figures; Z.V.V., P.F., L.L., and P.P. drafted manuscript; Z.V.V., P.F., L.L., and P.P. edited and revised manuscript; Z.V.V., P.F., L.L., and P.P. approved final version of manuscript.

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