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

Submitted 14 July 2015; accepted in final form 15 September 2015

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.

heart; heart failure; cardiomyopathy; toxicology; drug development; reactive oxygen species

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-
Cardiotoxic Drugs

Table 1. Latest drug discontinuations due to cardiotoxicity issues

<table>
<thead>
<tr>
<th>Drug Name</th>
<th>Drug Classification</th>
<th>Year of Recall</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fenfluramine</td>
<td>Anorectic</td>
<td>1997</td>
</tr>
<tr>
<td>Terfenadine</td>
<td>Antihistamine</td>
<td>1998</td>
</tr>
<tr>
<td>Sertindole</td>
<td>Antipsychotic</td>
<td>1998</td>
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<tr>
<td>Astemizole</td>
<td>Antihistamine</td>
<td>1999</td>
</tr>
<tr>
<td>Grepafloxacin</td>
<td>Antibiotic</td>
<td>1999</td>
</tr>
<tr>
<td>Cisapride</td>
<td>Prokinetic</td>
<td>2000</td>
</tr>
<tr>
<td>Droperidol</td>
<td>Tranquilizer</td>
<td>2001</td>
</tr>
<tr>
<td>Levomethadyl</td>
<td>Treatment of opiate dependance</td>
<td>2003</td>
</tr>
<tr>
<td>Rofecoxib</td>
<td>Nonsteroidal anti-inflammatory agent</td>
<td>2004</td>
</tr>
<tr>
<td>Tegaserol</td>
<td>Prokinetic</td>
<td>2007</td>
</tr>
<tr>
<td>Benfluorex</td>
<td>Anorectic</td>
<td>2009</td>
</tr>
<tr>
<td>Sibutramine</td>
<td>Anorectic</td>
<td>2010</td>
</tr>
<tr>
<td>Rosiglitazone</td>
<td>Antidiabetic</td>
<td>2010</td>
</tr>
</tbody>
</table>

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 glutathione depletion [doxorubicin (Adriamycin); ethanol and acetaminophen (Tylenol); Fig. 1].

Oxidative/nitrative modifications of mitochondrial proteins might play a crucial role in the development of myocardial dysfunction (see Table 2). Oxidative/nitrative 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-hydroxynonenal) (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 acacitine. 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).

Cardiotoxicity of Anticancer Drugs

Doxorubicin-induced cardiotoxicity: is mitochondrial oxidative stress a cause or consequence?. Doxorubicin was discovered in the late 1960s. It is an anticancer antibiotic belonging to the anthracycline family and was isolated from a culture of the Streptomyces peucetius (1).

Although the early results of doxorubicin clinical trials achieved great success (12) a few years later in patients who underwent doxorubicin treatment, development of cardiac toxicity and cardiomyopathy has been reported (74, 139). Despite this serious side effect, doxorubicin is clinically widely used and is still probably one of the most potent anti-cancer agents available for the clinical practice (47). Doxorubicin is commonly used to treat several types of tumors, such as in different forms of leukemia and lymphomas, soft-tissue sarcomas, and solid tumors. Patients subjected to doxorubicin usually exhibit typical symptoms of cytotoxic chemotherapy (nausea, vomiting, alopecia, myelosuppression, stomatitis, and gastrointestinal disturbances); nevertheless, the cardiotoxicity is completely different, being probably the most hazardous side effect associated with doxorubicin treatment. In the worst case it may reach 50% mortality for the highest cumulative dosages. This dose-dependent chronic doxorubicin-induced cardiotoxicity
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 the blockade of DNA transcription and replication.

Therefore, the majority of studies focused on the involvement of overt mitochondrial abnormalities and components. It has been shown that doxorubicin forms adducts with mitochondrial DNA and binds to other biomolecules like the mitochon-
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. No(s.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3-Ketoacyl-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>
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<tr>
<td>Connexin43</td>
<td>Nitrination</td>
<td>Mitochondrial potassium uptake</td>
<td>38, 52, 138</td>
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<tr>
<td>Creatine kinase</td>
<td>Nitrination</td>
<td>Maintenance of ATP pool</td>
<td>93, 94, 162</td>
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<tr>
<td>Cytochrome c</td>
<td>Nitrination</td>
<td>Mitochondrial apoptosis</td>
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<tr>
<td>Enoyl-CoA hydratase</td>
<td>Oxidation</td>
<td>Fatty acid β-oxidation</td>
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<tr>
<td>mHSP70, mitofilin</td>
<td>Oxidation</td>
<td>Mitochondrial protein import</td>
<td>9</td>
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<tr>
<td>Peroxiredoxin 3</td>
<td>Nitrination</td>
<td>Antioxidant defense</td>
<td>149</td>
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<tr>
<td></td>
<td>Oxidation</td>
<td></td>
<td>23, 67</td>
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<tr>
<td>Prohibitin</td>
<td>Nitrination</td>
<td>Unknown</td>
<td>85</td>
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<tr>
<td>Succinyl-CoA:3-oxoacid CoA transferase</td>
<td>Oxidation</td>
<td>Ketone body metabolism</td>
<td>149, 160</td>
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<tr>
<td>Superoxide dismutase-2</td>
<td>Oxidation</td>
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<td>Nitrination</td>
<td></td>
<td>41</td>
</tr>
<tr>
<td>VDAC1</td>
<td>Nitrination</td>
<td>Mitochondrial apoptosis</td>
<td>150</td>
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</tbody>
</table>

BNIP3, Bcl-2/adenovirus E1B 19-kDa-interacting protein 3; VDAC1, voltage-dependent anion channel.

Doxorubicin-related DNA oxidation is cardiacoselective 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 reductive cycling-dependent production of superoxide anion described first by Davies and Dorsam (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 ferri [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-
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 ventricle 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 reticulum 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 phosphatidylinositol 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 in-
tegrity 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 sur-
vival 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-depen-
dent collapse in mitochondrial membrane potential. The inhibi-
tion of Abl kinase by imatinib resulted in increased endo-
plasmic reticulum stress, as detected by increased PRKR-like
endoplasmic reticulum kinase (PERK) activation and phos-
phorylation of eukaryotic initiation factor-2α (eIF2α). PERK
also influences mitochondrial protein import machinery.

**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 mono-
clonal antibody that blocks angiogenesis by inhibiting
VEGF-A. The development of cardiomyopathy and heart fail-
ure, 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 di-
vergent pathways involved in tumor cell survival (VEGF,
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 an 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 glitazones 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...
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\textsubscript{ATP}, 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 PPAR\gamma coactivator-1\alpha (PGC-1\alpha) (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\textsubscript{ATP} 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 sarcoplasmic and mitochondrial K\textsubscript{ATP} channels. Sarcoplasmic K\textsubscript{ATP} blockade may lead to action potential shortening causing tachyarrhythmias. Mitochondrial K\textsubscript{ATP} 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 cardio-
myopathy (30, 37).

The toxic effect is explained by three closely related hypoth-
eses (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 synthe-
sized 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 pro-
duction 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 phosphoryla-
tion (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 metab-
olism by cytochrome p450 2E1 isoenzyme (45, 171). Mitochon-
drial oxidative damage in ethanol-treated rats also con-
tributes to myocardial fibrotic changes (155). Alcohol-induced
oxidative stress is further enhanced by aggravated angiotensin
II signaling, resulting in myocardial NADPH oxidase upregu-
lation, oxidative stress, inflammation, and fibrosis (146), which
are known contributors of myocardial dysfunction (154). Eth-
anol 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 posttrans-
lational 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 irre-
versible 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 meta-
bolism of the excess of catecholamines (79). The transfor-
mation 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.
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 Verga 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 myocyteolysis 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, troponymosin-α1 chain, myosin light polypeptide, and myosin regulatory light chain), mitochondrial proteins (ubiquinonocytochrome 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 anticancer 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/nitrative 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.

ACKNOWLEDGMENTS

We are indebted to Dr. George Kunos, the Scientific Director of the National Institute on Alcohol Abuse and Alcoholism (NIAAA), for continuous support.

GRANTS

This study was supported by the Intramural Research Program of the of National Institutes of Health/NIAAA (to P. Pacher). Z. V. Varga was supported by the Rosztoczy Foundation.

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

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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