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Review

Increasing role of the cancer chemotherapeutic doxorubicin in cellular metabolism

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Abstract

Objectives The use of doxorubicin, a drug utilised for many years to treat a wide variety of cancers, has long been limited due to the significant toxicity that can occur not only during, but also years after treatment. It has multiple mechanisms of action including the intercalation of DNA, inhibition of topoisomerase II and the production of free radicals. We review the literature, with the aim of highlighting the role of drug concentration being an important determinant on the unfolding cell biological events that lead to cell stasis or death.
Methods The PubMed database was consulted to compile this review.
Key findings It has been found that the various mechanisms of action at the disposal of doxorubicin culminate in either cell death or cell growth arrest through various cell biological events, such as apoptosis, autophagy, senescence and necrosis. Which of these events is the eventual cause of cell death or growth arrest appears to vary depending on factors such as the patient, cell and cancer type, doxorubicin concentration and the duration of treatment.
Conclusions Further understanding of doxorubicin's influence on cell biological events could lead to an improvement in the drug's efficacy and reduce toxicity.

Introduction – background on doxorubicin

Doxorubicin is an anthracycline antibiotic, isolated from the *Streptomyces peucetius* species, and is used effectively in a variety of cancers. It has a broad spectrum of use, treating both adult and childhood cancers and encompassing solid tumours and haematological malignancies.[1,2] It is often used to treat acute leukaemia,[3] breast cancer[4] and childhood solid tumours,[5] non-Hodgkin lymphomas,[6] Hodgkin's disease[7] and soft tissue sarcomas.[8] Unfortunately, despite being highly effective, doxorubicin is also non-selective to cancer cells, so its use is significantly limited due to toxicity. This toxicity often affects the heart, brain, liver and kidneys, and the consequences of these toxicities can take many years to become apparent.[9] Cardiotoxicity tends to be the most prominent adverse event, and it is a major dose-limiting factor that results in cardiac hypertrophy. This may be an acute or chronic effect and can appear later in life many years after doxorubicin treatment has stopped. It is thought to be induced by the formation of reactive oxygen species (ROS) and iron oxidation, as this induces the mitochondria to release cytochrome *c*, leading to apoptosis and cell death.[10] The brain and liver are also damaged by apoptotic cell death, while nephropathy results from interference with complexes I and IV in the mitochondria. This leads to oxidative damage due to an increase in superoxides and decrease in Vitamin E and antioxidants in the kidney's mitochondria, damaging the glomerulus. It also impairs the immune system, increasing a patient's susceptibility to infection and weakening their healing ability.[9]

Doxorubicin's molecular structure comprises a tetracyclic ring with two of the groups being adjacent quinone–hydroquinones and a sugar, daunosamine, attached to ring A with a glycosidic bond.[5] The daunosamine sugar is responsible for the drug's water solubility, while the tetracycline group is water-insoluble.[1] Doxorubicin differs from daunorubicin, another commonly used anthracycline, by only one hydroxyl group which accounts for the two drugs' different spectra of activity. The antineoplastic action of doxorubicin is due to a combination of different mechanisms such as the intercalation of DNA and inhibition of topoisomerase II, which can result in cell death or cell growth arrest.[1]

The novelty of this discussion article lies in the fact that the ultimate cell biological events as a result of doxorubicin action appear to vary depending on factors such as the patient, cell and cancer type, doxorubicin concentration and the duration of treatment.

Intercalation of DNA

Doxorubicin has been shown to intercalate deoxyribonucleic acid (DNA) and bind to and subsequently inhibit DNA polymerase, both of which lead to an inhibition of DNA synthesis.[11,12] It also inhibits ribonucleic acid (RNA) synthesis and transcription through RNA polymerase inhibition, although studies suggest that there is a preference for DNA over RNA synthesis inhibition.[11,13] X-ray crystallography has shown that doxorubicin's planar chromophore inserts between DNA bases, and its position is then stabilised by hydrogen bonding.[14] Therefore, the chromophore's hydroxyl group and the daunosamine sugar's amino group are responsible for bonding to and intercalating DNA.[15] This intercalation complex results in double-stranded DNA breaks and fragmented nuclei with condensed chromatin, resulting in the induction of apoptosis.[14] There have also been studies showing that DNA synthesis inhibition with a directly cytotoxic effect only occurs when doxorubicin concentrations are higher than the concentrations seen in patients, which are generally below 5 μM, so there remains some uncertainty over whether it is responsible for tumour cell growth inhibition.[16] Concentrations above 4 μM were required to inhibit DNA synthesis in one study,[17] while others found that concentrations above 2 μM were sufficient.[18] To add more variability to the findings, other studies demonstrate that drug concentrations as low as 0.01 and 0.2 μM can be quite effective.[16,19] Collectively, these variant results indicate that more studies are needed to shed further light on this important matter. More recent studies suggest that DNA synthesis inhibition may be an early transient signalling event which leads to cell apoptosis due to upregulation of the p53 protein.[9] The role of the p53 protein is in protecting the genome from mutations. It regulates a part of cell apoptosis through competition with DNA repair mechanisms. Its importance is demonstrated in the fact that it is the most frequently altered gene in cancer cells, and its mutation results in both the arrest of its normal apoptotic function and an increase in oncogenic function.[20] DNA synthesis inhibition can result from an increase in p53 through the protein's ability to upregulate the cyclin-dependent kinase (cdk) p21 protein, which binds to proliferating cell nuclear antigen. This leads to a preferential inhibition of DNA polymerase and results in the termination of cell growth and DNA repair.[11,20] An increase in p53 also leads to the downregulation of the transcription factor E2F, which similarly results in the arrest of cell growth as E2F can no longer bind to target promotor regions of genes vital to cell growth such as dihydrofolate reductase, thymidine kinase, thymidine synthetase and DNA polymerase-α. Doxorubicin's ability to inhibit DNA synthesis inhibition may lead to cytostatic activity, but it is the drug's other actions, such as topoisomerase inhibition, that directly cause cell death.[11]

Inhibition of topoisomerase II

Topoisomerase II inhibition is another of doxorubicin's cytotoxic mechanisms, with the targeting of topoisomerase II resulting in DNA cleavage and cell death.[21] Topoisomerase II is an enzyme which regulates DNA's superhelical state, relaxing accumulated positive supercoils, as well as unlinking intertwined DNA strands. It is therefore necessary for the DNA replication process to complete.[22] During replication, topoisomerase II induces double-strand breaks in DNA, and topoisomerase II inhibitors such as doxorubicin then act by stabilising topoisomerase II-DNA covalent cleavage intermediates. DNA is then unable to proceed further in the replication process, and cell death occurs.[23,24] As the topoisomerase II-DNA-drug complex is reversed once doxorubicin dissociates from the complex, residence time of the drug in the complex is a key factor of doxorubicin's cytotoxicity. This mechanism of action may also increase doxorubicin's selectivity for cancer cells as topoisomerase II levels have been shown to be elevated in proliferating cells.[25] To achieve cytotoxic action from topoisomerase II inhibition, enzyme-DNA binding must have occurred, thus making drug efficacy dependent on enzyme concentration in the cell.[23,26] Further evidence that this is an important action contributing to doxorubicin's activity has been derived from studies demonstrating that decreased levels or altered functions of topoisomerase enzymes have resulted in anthracycline-resistant cancer cells.[27,28] There is also an accompanying decrease in DNA strand breaks in cells with reduced or altered topoisomerase II.[29] This suggests that the presence of topoisomerase II is an important facet of the drug's efficacy, and that alterations in topoisomerase gene expression in cancer cells may result in drug resistance.[1,11]

Free radical-related damage to DNA

Doxorubicin's ability to produce reactive-free radicals is another mechanism of activity, and this occurs as doxorubicin can act as an electron acceptor in a reaction catalysed by cytochrome P450 reductase in the presence of NADH dehydrogenase.[21] This results in doxorubicin being reduced, as its quinone group becomes a semiquinone-free radical. The semiquinone-free radical causes oxidative

damage which results in cleavage or degradation of DNA, or deoxyribose, which induces DNA strand scissions.[30] The oxygen molecule also involved in this reaction produces reactive-free radicals including superoxides, hydroxyl radicals and peroxides, which cause further damage to DNA by oxidation.[11,21] During the metabolism of doxorubicin, the drug is reduced at C-13 to form doxorubicinol. Doxorubicin and doxorubicinol both then undergo acid-catalysed hydrolysis at their daunosamine sugar groups to form doxorubicinone and doxorubicinolone, respectively. Protonation at C-7 then removes the sugar, producing 7-deoxydoxorubicinone and 7-deoxydoxorubicinolone. The now twice-reduced doxorubicin becomes a C7-deoxyaglycone. A tautomer of this molecule is C-7-quinone-methide, which covalently binds to DNA and produces free radicals in close proximity to DNA.[1,2,9] There is also evidence of hydrogen peroxide formation due to an iron-mediated reaction catalysed by ferredoxin reductase, which also causes DNA damage. As studies show cytotoxic action both with and without iron, iron is not necessary for doxorubicin to cause free radical damage to DNA.[30] This mechanism is further evidenced by the obstruction of injury to DNA in the presence of free radical scavengers such as catalase, superoxide dismutase and dimethyl sulfoxide. This suggests that there may be DNA strand breakage due to free radicals, indicating that doxorubicin has mechanisms of action not associated with topoisomerase enzymes or intercalation. Studies do show however that this free radical-associated damage only occurs above clinically used drug concentrations, and that it is instead protein-associated strand breaks involving topoisomerase II inhibition that is responsible for cytotoxic action at clinically significant concentrations.[1] There have also been studies that have shown lipid peroxidation involving free radicals, affecting cell membranes.[31,32] Earlier studies, however, did not produce sufficient evidence that this would occur under true physiological conditions as they used concentrations of doxorubicin well above that of peak or steady-state drug concentrations seen in patients.[32,33] It was suggested that this may be due to the assay used to detect lipid peroxidation not being sensitive and selective enough, rather than it not contributing to drug activity. It has been advised that studies involving intact cells with doxorubicin concentrations >1–2 μM should be re-evaluated.[1] In recent years, studies have found that doxorubicin does induce lipid peroxidation and suggest that it is a factor contributing to doxorubicin's cardiotoxicity.[34,35]

Cell biological events induced by doxorubicin

More recent studies have suggested that doxorubicin-induced damage and fragmentation caused to DNA by the mechanisms discussed above, or by other mechanisms, may induce apoptosis or other cell biological events, leading to cell death or cell growth arrest.[11] In addition to apoptosis, there is evidence that autophagy, early or accelerated senescence and necrosis, may also be cell biological events key to doxorubicin's activity.[36] Studies have found that direct DNA synthesis inhibition and topoisomerase II inhibition do not always cause cytotoxicity, suggesting that there are other causes for the initiation of apoptosis.[37]

Apoptosis

Apoptosis, also known as programmed cell death, is usually considered to be a form of protection for the body. It is activated in response to damage or alterations in DNA, killing the cell to prevent replication and further mutations.[38,39] Apoptosis is a natural biological process involved in normal growth and development. In some cases, there may be partial apoptosis, a targeted form of apoptosis that preserves part of the cell for other physiological functions, such as the maturation of spermatocytes and mammalian erythrocytes.[38] Most, if not all cells contain the necessary material to trigger apoptosis, but these triggers, such as caspase enzymes, are inactive. Cell death appears to occur due to release from inhibition of these triggers, initiated by a variety of conditions that are deemed to be imperfect for the cell. As shown in Figure 1, although it can occur independent of caspases, a majority of the time, apoptosis occurs via caspase-dependent pathways that can be extrinsic or intrinsic. The extrinsic pathway is initiated by extracellular ligands such as the Fas ligand (FasL), tumour necrosis factor alpha (TNF-α) and TNF-related apoptosis-inducing ligand (TRAIL) binding to pro-apoptotic death receptors on the cell membrane.[38,40] These death receptors are coupled with procaspase-8 by Fas-associated death domain, activating caspase-8 which leads to the activation of caspase-3, -6 and -7, leading to apoptotic cell death.[40–42] Doxorubicin has been shown to induce the extrinsic apoptotic pathway through regulation of Fas. This may be due to doxorubicin downregulating the expression of soluble Fas, an inhibitor of FasL.[42,43] It has also been suggested that it may be due to activation of the calcium/calcineurin signalling pathway, which activates nuclear factor-activated T-cell 4 (NFATc4) and leads to upregulation of FasL.[44] Doxorubicin has also been shown to activate nuclear factor-kappa B (NF-κB) through ROS production, and this leads to the upregulation of various pro-apoptotic genes including FasL, c-Myc and p53.[42,45,46]

Apoptosis also has an intrinsic pathway, mediated by the anti-apoptotic B-cell leukaemia/lymphoma 2 (Bcl-2) protein family, members of which are bound to the surface of the mitochondria (Figure 1).[38] Doxorubicin initiates apoptotic cell death by inducing AMP-activated protein

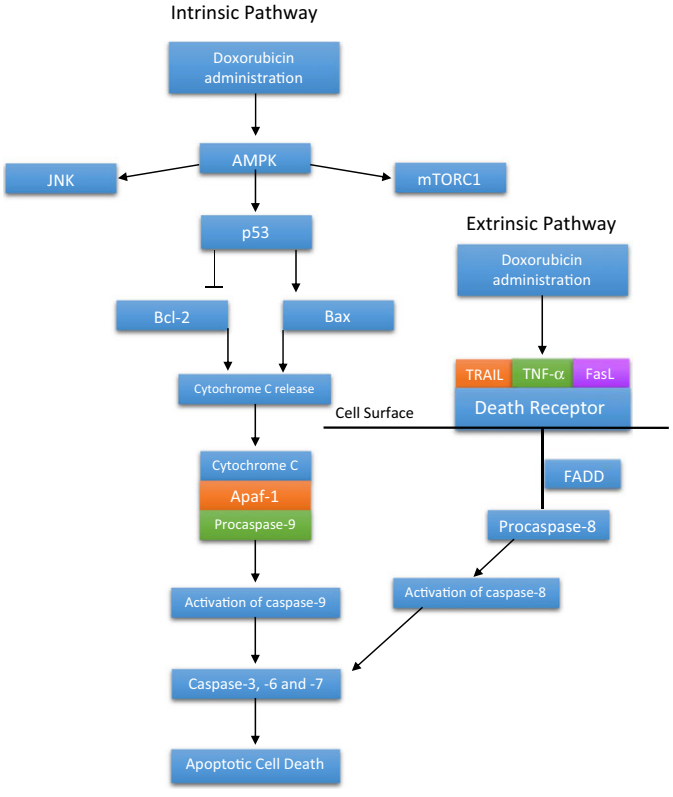

Figure 1 Doxorubicin's induction of both the extrinsic and the intrinsic apoptosis pathways. Doxorubicin can initiate the extrinsic pathway by upregulating FasL and inducing the activation of caspase-8, leading to the activation of caspase-3, -6 and -7, and therefore apoptotic cell death. Doxorubicin initiates the intrinsic pathway by upregulating AMPK which results in the upregulation of p53, JNK and mTORC1. The inhibition of anti-apoptotic Bcl-2 and increase in pro-apoptotic Bax induce the release of cytochrome *c* from the mitochondria which then binds to Apaf-1 and procaspase-9. Activated caspase-9 then leaves the complex, activating caspase-3, -6 and -7, resulting in apoptotic cell death. AMPK, AMP-activated protein kinase; Apaf-1, apoptotic protease-activating factor-1; Bax, Bcl-2-associated X; Bcl-2, B-cell leukaemia/lymphoma 2; FADD, Fas-associated death domain; FasL, Fas ligand; JNK, c-Jun N-terminal kinase; mTORC1, mammalian target for rapamycin complex 1; TNF-α, tumour necrosis factor alpha; and TRAIL, TNF-related apoptosis-inducing ligand.

kinase (AMPK), which activates p53 and c-Jun N-terminal kinase (JNK), and inactivates mammalian target for rapamycin complex 1 (mTORC1). The p53 protein induces a downregulation of the anti-apoptotic Bcl-2 protein and upregulation of the pro-apoptotic Bcl-2-associated X (Bax) protein. JNK also involved in mediating the expression of Bcl-2 and Bax and is important in the regulation of apoptosis as a result.[47] The Bcl-2/Bax ratio, which is altered by doxorubicin activating AMPK, is a key determining factor of whether a cell dies by apoptosis or survives.[48] As with doxorubicin's other actions, drug concentration is likely the decisive factor in which these outcomes occur, with a high concentration necessary to cause cell death.[9] The alteration in the balance of Bcl-2/Bax leads to the release of cytochrome *c* from the mitochondria which forms an apoptosome complex with apoptotic protease-activating factor-1 (Apaf-1) and procaspase-9. The now activated caspase-9 leaves the complex and activates caspase-3, -6 and -7, and from this point on there is no option but for the cell to continue down the apoptotic pathway and die.[9,41,42] Apoptotic cell death is characterised by nuclear condensation and fragmentation, and cleavage of chromosomal DNA into fragments which are then packaged into apoptotic bodies.[39] These apoptotic bodies are then identified and removed by phagocytes, resulting in an important lack of inflammation around the cell. Although they can function separately, the extrinsic and intrinsic pathways overlap, as p53 can upregulate some pro-apoptotic receptors in the extrinsic pathway and caspase-8 can upregulate the Bax protein and trigger the intrinsic pathway.[41]

Autophagy

Apoptosis and autophagy are characterised by different mechanisms, apoptosis being defined by cell shrinkage and blebbing, with little change to organelles and then phagocytosis without an inflammatory response. In contrast, autophagy involves the formation of an acidic vesicular organelle

in the cytoplasm.[38] It appears that autophagy can be either cytoprotective or cytotoxic, as it helps cells survive under stress but also leads to what is termed type II programmed cell death.[49] It is a natural biological process involved in maintaining homeostasis and atrophy, commonly involved in the turnover of long-lived proteins and organelles, and removing dysfunctional organelles and protein aggregates. Due to autophagy's ability to eliminate abnormal proteins, failure of autophagy has been implicated in neurodegenerative diseases, including Huntington's disease as it has a role in the clearance of toxic mutant huntingtin protein and preventing disease progression.[38,50,51] Autophagy also has a role in embryonic growth and development; it appears to be crucial in the protein degradation process used in the oocyte to embryo conversion, and also later on in neurodevelopment. The process is upregulated in times of stress in cells, such as starvation, oxidative stress or hormonal imbalance, and serves as a survival mechanism for the cells.[38] During autophagy, cells convert to catabolic metabolism in which macromolecules and organelles are degraded and recycled for energy and nutrients by being sent to the lysosome. There is a family of genes called AuTophaGy-related (Atg) genes that regulate autophagy through interaction with protein complexes formed during the autophagy pathway. The activation or inhibition of autophagy is dependent on mammalian target for rapamycin (mTOR), as the pathway is initiated when there is an increase in the adenosine monophosphate/adenosine triphosphate (AMP/ATP) ratio due to a low-energy state in the cell (Figure 2). This then activates AMPK, leading to the inhibition of mTOR, which is responsible for the phosphorylation of Atg13 and Unc-like-51 kinase 1 (Ulk1). During starvation, the dissociation of mTOR from the Atg13-FIP200-Ulk1 complex results in the dephosphorylation of Atg13 and Ulk1. This activates Ulk1, which then phosphorylates Atg13 and focal adhesion kinase family interacting protein of 200 kDa (FIP200), resulting in the formation of the pre-autophagosomal membrane.[51,52] Vacuolar-sorting protein 34 (Vps34), also known as class III phosphoinositide 3-kinase (PI3K) is involved in another pathway of autophagy regulation as it controls the maturation of the autophagosome. Vps34 is activated by forming a protein complex with beclin-1, Vps15, and is enhanced by Bax interacting factor-1 (Bif-1) and ultraviolet radiation resistance-associated gene (UVRAG). Vps34 then interacts with Atg14, forming the autophagosome (Figure 2).[52]

Beclin-1 has been shown to be essential in initiating the autophagic process, its interaction with Vps34 being a key step in the process. The binding of anti-apoptotic Bcl-2 with beclin-1 has an important regulatory role in whether a cell undergoes apoptosis or autophagy. Bcl-2 has been shown to mediate autophagy by interfering with the activity of beclin-1 by inhibiting the formation of its complex with Vps34. This suggests that the interaction between Bcl-2 and beclin-1 is the primary regulatory mechanism of autophagy, and that anything affecting this interaction or the expression of these proteins therefore has a role in regulating autophagy.[40,47,53] JNK has been shown to regulate autophagy as the activation of JNK causes the dissociation of Bcl-2 from beclin-1, stopping Bcl-2's inhibition of beclin-1 and allowing the autophagic pathway to continue.[47,51,54] It has been suggested that a low level of autophagy promotes cell survival by preventing apoptosis and cell death, while significant upregulation of autophagy leads to programmed cell death as excessive degradation of proteins and organelles disrupts energy homeostasis.[40,49] The presence of caspases is also an important factor in determining the type of cell death, as an inhibition of these enzymes can lead to a switch from apoptosis to autophagy.[39] Doxorubicin may induce autophagy through the oxidative stress resulting from ROS production in the mitochondria. This occurs in complex I in the electron transport train, causing mitochondrial dysfunction and a disruption in energy production. Autophagy is then initiated by calmodulin-dependent kinase and AMPK, which are activated by ROS damage to calcium-handling proteins and an increase in calcium.[55] Doxorubicin may also promote autophagy in response to the activation of poly (ADP-ribose) polymerase-1 (PARP-1), leading to the inhibition of mTOR, during periods of cell stress due to lack of nutrients.[9,52,56]

Senescence

Senescence is a state in which a cell can no longer divide, but remains metabolically active. There are three types of senescence; this includes replicative senescence which involves growth arrest due to telomere shortening after a predetermined amount of cell divisions in non-transformed cells. Oncogene-induced senescence occurs in the presence of oncogenes as an attempt to prevent or postpone a cells transformation.[57] Accelerated senescence can arrest cell growth by inhibiting the self-renewal capability of cells, and it primarily occurs after ionising radiation or drug therapy that causes damage to DNA. Premature and accelerated senescence has been shown to occur in cells exposed to chemotherapeutic agents and is thought to be a part of the immune system's tumour regression activity.[36] The induction of accelerated senescence has similarities to apoptosis and other pathways that inhibit cell growth. As shown in Figure 3, it involves the induction of the p53 protein, which upregulates the cdk p21 protein and downregulates cdc2/cdk1, although this may not be the only cause. This pathway has been shown to occur even without the p53 protein, indicating that p21 and cell-division cycle 2 (cdc2) have vital roles in inducing senescence, and that p53

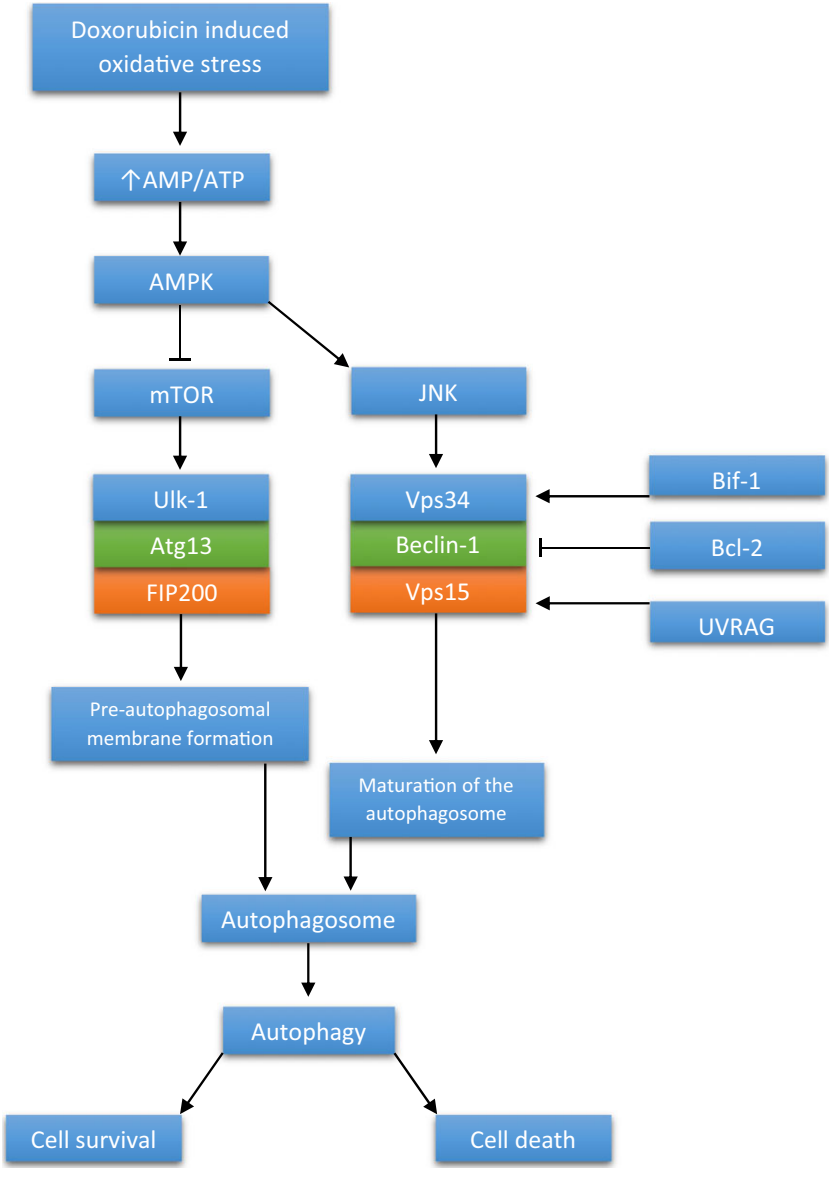

Figure 2 Doxorubicin-mediated induction of autophagy. Doxorubicin produces ROS which disrupt mitochondrial function and energy production. This causes AMPK to be upregulated, inhibiting mTOR and upregulating JNK. The inhibition of mTOR results in the activation of Ulk-1 and dephosphorylation of Atg13 and FIP200. This leads to the formation of the pre-autophagosomal membrane. The upregulation of JNK causes Bcl-2 to dissociate from beclin-1, allowing the complexation of Vps34, beclin-1 and Vps15, a complex responsible for the maturation of the autophagosome. Once the autophagosome is formed, the cell can either survive or die depending on the level of stress in the cell. AMP, adenosine monophosphate; AMPK, AMP-activated protein kinase; Atg, AuTophaGy-related; ATP, adenosine triphosphate; Bcl-2, B-cell leukaemia/lymphoma 2; Bif-1, Bax interacting factor-1; FIP200, focal adhesion kinase family interacting protein of 200kDa; JNK, c-Jun N-terminal kinase; mTOR, mammalian target for rapamycin; Ulk-1, Unc-like-51 kinase 1; UVRAG, ultraviolet radiation resistance-associated gene; and Vps, vacuolar-sorting protein.

is not a critical element.[36] Other studies have alternatively demonstrated that p53 is required for senescence, and that p53's presence determines which cell biological event will proceed, suggesting that a loss of p53 leads to apoptosis in preference over senescence.[58,59] Doxorubicin has been shown to upregulate p53, which can trigger senescence; however, accelerated senescence is not likely to contribute greatly to doxorubicin's activity.[42,60] It may have a role in stopping growth in cells that avoided death by primary mechanisms, and therefore may be important in cancer recurrence. Senescence appears to be an alternative pathway used by cells in which apoptosis is inhibited, as studies have shown cells to switch from apoptosis to senescence, arresting cell growth.[61,62] This has been shown to be

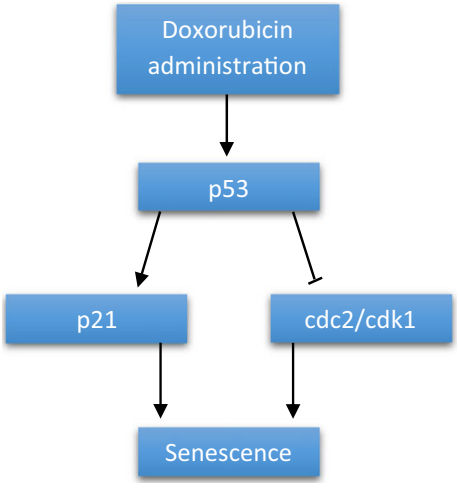

Figure 3 Doxorubicin's induction of premature and accelerated senescence. Doxorubicin initiates the upregulation of p53, which then upregulates p21 and inhibits the cdc2/cdk1 ratio. This induces senescence and therefore cell growth arrest. cdc2, cell-division cycle 2 and cdk, cyclin-dependent kinase.

insignificant compared with the induction of autophagy in cells unable to undergo apoptosis, and most likely represents a secondary downstream response when both are inhibited.[36]

Necrosis

Another cause of cell death not induced by apoptosis is necrosis, which has been considered to be a passive process that results from a reduction in ATP to levels that makes cell survival impossible, leading to bioenergetic catastrophe. This is thought to be caused by either physical or toxic damage, suggesting that doxorubicin's cytotoxic actions, including damage to DNA and oxidative stress, may lead to programmed necrosis.[39,63] This includes when the apoptotic pathway is impaired, which can occur when there is a deficiency of p53 and/or Bcl-2 family proteins, most notably Bax. This allows doxorubicin to remain active independent of p53 and Bax.[39,63] As shown in Figure 4, doxorubicin-induced ROS production may be the cause of this programmed necrosis as it increases mitochondrial calcium concentrations, which leads to cyclophilin D (Cyp D)-dependent mitochondrial permeability transition (MPT) pore opening and mitochondrial swelling, resulting in a decrease in ATP.[64] Doxorubicin has also been shown to damage mitochondrial DNA leading to dysfunction of the mitochondria and therefore respiration. This causes a depletion in ATP, triggering necrosis.[42,65,66] Unlike apoptosis, necrosis induces an inflammatory response around the cell in vivo due to the release of cell contents and proinflammatory factors. There is also no condensation of the chromatin and fragmentation of DNA as there is in apoptosis.[39] Programmed necrosis gives DNA-damaged proliferating cells another means of death when apoptosis is not possible. It is thought to be induced by PARP-1, the DNA repair protein, in response to DNA damage.[64] This is the same protein that can trigger autophagy, indicating that PARP-1 has a significant role in a cell's response to injury or stress.[9] By intercalating and damaging DNA, doxoru-

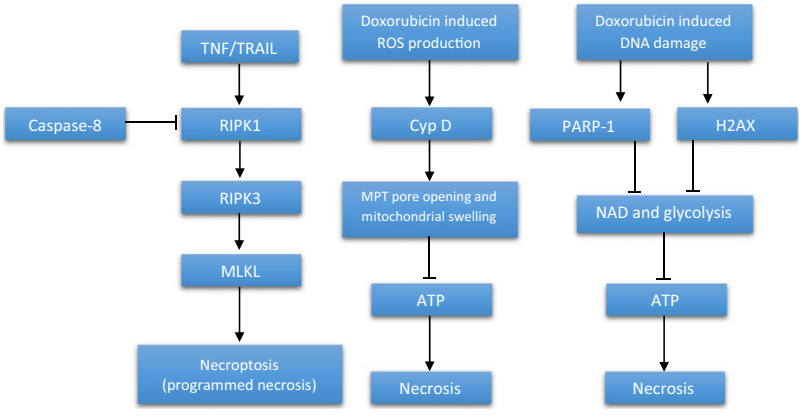

Figure 4 Doxorubicin-mediated induction of necrosis. There are three pathways of programmed necrosis. The necroptosis pathway is initiated by the activation of TNF or TRAIL and inhibition of caspase-8. RIPK1 activates RIPK3, leading to the activation of MLKL and necroptosis. Doxorubicin can produce ROS, causing oxidative damage to the mitochondria. This activates Cyp D and induces MPT pore opening and mitochondrial swelling, decreasing ATP levels, leading to necrotic cell death. Doxorubicin can also initiate necrosis through damaging cellular DNA; this upregulates PARP-1 and H2AX, which decreases in NAD levels and glycolysis. This results in a decrease in ATP and therefore necrosis. ATP, adenosine triphosphate; Cyp D, cyclophilin D; H2AX, H2A histone family member X; MLKL, mixed-lineage kinase domain-like protein; MPT, mitochondrial permeability transition; NAD, nicotinamide adenine dinucleotide; PARP-1, poly (ADP-ribose) polymerase-1; RIPK, receptor-interacting serine/threonine protein kinase; ROS, reactive oxygen species; TNF, tumour necrosis factor; and TRAIL, TNF-related apoptosis-inducing ligand.

bicin activates PARP-1 and H2A histone family member X (H2AX), causing the cell to undergo programmed necrosis (Figure 4).[63,67,68] Programmed necrosis due to PARP-1 activation has only been found in cells actively proliferating due to the inhibition of nicotinamide adenine dinucleotide (NAD) and therefore glycolysis by PARP-1, giving rise to selectivity for cells reliant on glycolysis for ATP production.[39] Vegetative non-cancerous cells are subjected to DNA repair rather than cell death as they are able to use oxidative phosphorylation and the catabolism of amino acids and lipids in the mitochondria, producing a sufficient amount of energy to repair DNA. Proliferating cells, such as tumour cells, are more dependent on glycolysis and ATP production because they use their amino acids and lipids for protein and membrane synthesis. As many tumours arise from mutations that inhibit apoptosis and/or allow cells to continue growing past normal growth cycle checkpoints, this alternative pathway could explain how chemotherapeutic drugs such as doxorubicin still induce cell death when other pathways are blocked.[39] Another pathway of programmed necrosis is initiated by the activation of TNF or TRAIL and inhibition of caspase-8. The activation of RIPK1 in turn activates RIPK3, leading to the upregulation of MLKL and necroptosis. Doxorubicin's role in this pathway is unclear.[64,68]

Considering the various mechanisms of action shown by doxorubicin, it is possible that the targets of this drug vary between different tumours, and as such, the importance of each target and mechanism changes for each individual case.[23] There has been evidence of this in a study using leukaemia cells from numerous patients in which the different cells, even when obtained from the same patient, responded differently despite the uptake and retention of the drug remaining the same for the different cell populations.[15] This may be due to the numerous gene alterations that often occur in cancer, meaning that chemotherapeutic agents have an altered sensitivity and activity in different cancer cells.[9] This may result in a preference for one of the doxorubicin's mechanisms of action over another depending on the cell, for example topoisomerase II inhibition being the primary mechanism in some cells, and DNA intercalation being the primary mechanism of activity in other cells.[15] Doxorubicin's concentration also appears to be a factor in deciding which of the drug's actions act as the predominant cause of cell death or cell growth arrest. Further knowledge as to what concentration is most effective in killing various cell types may allow not only more effective treatment but also a reduction in toxicity.[9,69]

Cell metabolism

The mitochondrion is the centre of a cells' metabolism. It is responsible for most of the energy production required by the cell, as well as regulating various other cellular pathways such as apoptosis, metabolite synthesis, redox potential maintenance and ion concentrations, in particular calcium ion homeostasis. Dysfunction of the mitochondria usually has dire consequences for a cell.[70] It is commonly a result of cell stress, such as oxidative damage from ROS or energy depletion, or can be due to the activation of intracellular messengers such as cytochrome *c* which leads to apoptosis. The result of mitochondrial dysfunction in these circumstances is typically cell death, giving it a role in the pathogenesis of many diseases and ageing. The mitochondria is therefore the organelle, where it is determined whether a cell will die or survive in response to cell damage or stress.[70] Therefore, it has a central role in the survival of a cell.

The mitochondria have two membranes separating the organelle into four compartments, the outer membrane, intermembrane space, inner membrane and matrix. The inner membrane is folded into cristae and is home to the respiratory complexes of the electron transport chain and ATP synthase. This makes the inner membrane the location at which the rate of cell metabolism is controlled.[71] Although the nuclear genome is responsible for the production of a majority of the mitochondria's proteins which are then transferred to the mitochondria, the mitochondria has its own genome that is crucial for respiration. The mitochondrial DNA (mtDNA) genome comprises 37 genes, 13 of which encode proteins used in the formation of complexes I, III, IV and V. Only complex II is formed by proteins encoded by nuclear DNA.[72] Mitochondrial transcription is regulated by the peroxisome proliferator-activated receptor gamma coactivator 1 (PGC-1) family of co-activators that respond to an alteration in a cell's nutritional status, often due to a change in NAD^+/NADH and AMP/ATP ratios. These changes are monitored by sirtuin-1 (SIRT1) and AMPK, respectively.[73]

To produce energy in the form of ATP, electrons are transferred from oxidative substrates such as pyruvate, glutamate and succinate to oxygen in a series of redox reactions, producing water. During this process, protons are pumped from the mitochondrial matrix across the inner membrane and through respiratory complexes I, III and IV.[72,74] To produce the energy to pump protons through the complexes, NADH donates two electrons to complex I (NADH dehydrogenase), which then flow into complex II. They then move on to ubiquinone (coenzyme Q), which forms ubisemiquinone and then ubiquinol. The electrons are then transferred from ubiquinol to complex III, from there they move on to cytochrome *c*. The electrons then move on to complex IV and are used to produce water from hydrogen and oxygen. As protons are pumped through complexes, a capacitance is formed across the inner membrane. The potential energy from the electro-

chemical gradient is stored as ΔP. The protons move down their electrochemical gradient back into the matrix and ΔP is used to convert adenosine diphosphate (ADP) into ATP via complex V, also known as ATP synthase.[72,74]

Doxorubicin's effects on cell metabolism

Administration of doxorubicin has been shown to cause dysfunction of the mitochondria's respiratory function through the production of ROS, and the subsequent damage to cells can lead to apoptotic cell death. Doxorubicin impacts respiration in cardiomyocytes through more than one mechanism. It has been shown to inhibit NADH and succinate oxidase in both in-vitro and in-vivo studies and may also reduce antioxidant capacity in the mitochondria.[66] Doxorubicin also converts to a semiquinone-free radical by redox cycling at complex I of the electron transport chain in the mitochondria.[66,75] This results in the formation of the superoxide anion-free radical, and this anion, or its reduction products, is detoxified by glutathione. This causes glutathione to be oxidised to form glutathione disulfide and a reduction in glutathione levels. Without glutathione, the critical protein thiol groups that regulate the MPT are oxidised, leading to membrane depolarisation and the release of solutes such as calcium from the matrix. This impairs the oxidative phosphorylation process and there is a subsequent depletion of ATP due to the inhibition of ATP synthesis. This depletion in ATP is considered to be responsible for the resulting cell death by necrosis.[66,75]

Doxorubicin also interferes with calcium homeostasis, which can lead to increased permeability of the MPT and the opening of the MPT pores. MPT pores are comprised of the outer membrane voltage-dependent anion channel (VDAC), the inner membrane adenosine nucleotide translocase (ANT) and matrix chaperone cyclophilin D.[66,75] Doxorubicin can interfere with calcium homeostasis by modifying the calcium-conducting ANT, impairing the mitochondria's ability to obtain calcium from the cytoplasm and increasing susceptibility to calcium-induced depolarisation of the mitochondria and permeability of the MPT. Increased susceptibility to MPT pore opening can result from oxidative stress or calcium and phosphate overload, as this causes a loss of membrane potential, mitochondrial swelling and rupture of the outer mitochondrial membrane. Opening of the MPT pores is related to apoptosis due to the release of pro-apoptotic proteins including cytochrome *c*, second mitochondria-derived activator of caspase/direct inhibitor of apoptosis-binding protein with low pI (Smac/DIABLO) and the apoptosis-inducing factor (AIF).[66,75] The ROS formed by doxorubicin have been found to oxidise cardiolipin, a unique phospholipid found in the inner mitochondrial membrane. The oxidation of cardiolipin appears to be required for the increased permeability of the mitochondrial membrane and the release of pro-apoptotic proteins. It has also been found that inhibition of cardiolipin peroxidation can prevent apoptosis. This indicates that cardiolipin may be important in the regulation of apoptosis.[76] Oxidative damage resulting in doxorubicin administration has been shown to have a significant effect on pro- and anti-apoptotic proteins. Silymarin, a hepatoprotective antioxidant, has been shown to reduce doxorubicin-associated hepatotoxicity, likely due to the inhibition of apoptotic cell death by the upregulation of anti-apoptotic B-cell lymphoma-extra-large (Bcl-xl) protein.[77] Doxorubicin has been shown to downregulate Bcl-xl and Bcl-2, leading to the release of cytochrome *c* from the mitochondria and increased p53 and PARP levels. There is also an associated upregulation of the pro-apoptotic Apaf-1, caspase-3 and Bax proteins.[78] This has therefore demonstrated that the mitochondrial dysfunction and subsequent cell death caused by doxorubicin is related to ROS production and the regulation of pro- and anti-apoptotic proteins.[77]

Due to the fact that there is increased sensitivity to MPT pore opening in heart mitochondria, it is thought that mitochondrial dysfunction is the primary cause of doxorubicin's cardiotoxicity.[1,42] A structural analogue of doxorubicin called 5-iminodaunorubicin helps to confirm this hypothesis, as it is not cardiotoxic, and nor does it cause the formation of free radicals.[79] While calcium induces the MPT, cyclosporin A, sulfhydryl-reducing agents and adenine nucleotides are inhibitors of the MPT.[66] Doxorubicin's effect on calcium homeostasis is further evidenced by the cardioprotection demonstrated during the co-administration of cyclosporin A, as cyclosporin A blocks the MPT and prevents calcium release.[80] The cardiotoxic effect appears to occur over time as treatment with doxorubicin has been shown to decrease the ability of cardiac mitochondria to accumulate and retain calcium. The severity of this effect increases with cumulative dosing of doxorubicin and is not improved by treatment-free recovery periods.[66,79]

Other studies have found that doxorubicin's cardiotoxicity may be due to the accumulation of iron in the mitochondria through interference with iron homeostasis. Administration of doxorubicin increases iron levels in the mitochondria by inhibiting iron regulatory proteins 1 and 2 (IRP1 and IRP2).[81] These proteins bind to iron response elements in untranslated regions of target mRNA and are either stabilised or degraded, respectively. A decrease in the expression of transferrin receptor 1 (TfR1), which is necessary for iron uptake into the cell, was seen in conjunction with IRP1/2 inhibition as these proteins are positive regulators of the receptor.[81] There was also an increase in ferroportin 1, an iron exporter, which is negatively regulated by

Table 1 Effects of doxorubicin on the mitochondria and cell metabolism

Effect of doxorubicin on cell metabolism	Reference
Doxorubicin produces ROS and causes oxidative damage to the mitochondria, leading to: • Decreased glutathione levels • Mitochondrial membrane depolarisation • Calcium release into the matrix and MPT pore opening • Impairment of ATP synthesis and decreased ATP levels	[75]
Doxorubicin can cause iron to accumulate in the mitochondria which leads to higher ROS levels and increased toxicity	[81]
Doxorubicin's cardiotoxic effect appears to be proportional to increased length of cumulative dosing with doxorubicin as it decreases the ability of cardiac mitochondria to accumulate and retain calcium	[79]
A structural analogue of doxorubicin that does not produce free radicals is also not cardiotoxic	
Doxorubicin interferes with calcium homeostasis, leading to: • MPT pore opening and cytochrome *c* release which is associated with the induction of apoptosis • Loss of mitochondrial membrane potential, mitochondrial swelling and outer membrane rupture	[66]

IRP1/2. There are also studies demonstrating that the overexpression of ATP-binding cassette subfamily B member 8 (ABCB8), a gene responsible for controlling mitochondrial iron export, can reverse doxorubicin toxicity. In addition to this, doxorubicin has been shown to downregulate ABCB8 expression, which contributes to the drug's cytotoxicity.[81] These findings suggest that the increase in mitochondrial iron levels due to doxorubicin is the result of a reduction in iron export, not an increase in import. Iron chelators such as dexrazoxane have also been found to reduce doxorubicin toxicity in cardiomyocytes by decreasing iron levels and ROS in the mitochondria, further indicating that iron accumulation may be responsible for doxorubicin's cardiotoxicity.[81]

Conclusion

Doxorubicin has been used to treat a wide spectrum of cancers for many years. Despite being highly effective, its use has been limited due to the significant toxicity than occurs during and after treatment.[1] Cardiotoxicity in particular has been a major dose-limiting adverse effect and can occur both during and years after doxorubicin administration.[9] Doxorubicin has multiple mechanisms of action; it intercalates DNA and DNA polymerase and inhibits topoisomerase II, resulting in DNA synthesis inhibition.[11,13,23] Doxorubicin is also reduced to form free radicals, producing ROS in the process and inducing oxidative damage to cellular DNA and the mitochondria.[21] This oxidative damage is also responsible for lipid peroxidation in cells.[34] In recent years, it has been discovered that these mechanisms of action can induce various cell biological events including apoptosis, autophagy, senescence and necrosis. These cell biological events are responsible for doxorubicin-induced cell death and cell growth arrest.[9,42] As the mechanisms surrounding these cell biological events predominantly involve the function and dysfunction of the mitochondria and cell energy levels (Table 1), how doxorubicin influences cell metabolism and energy is important in further understanding doxorubicin's activity and toxicity.[66,75] It also appears that the concentration of doxorubicin is an important factor in deciding which cell biological event will eventuate after doxorubicin treatment.[49] Investigating this may lead to the optimisation of clinical doxorubicin concentrations to improve and maintain drug efficacy and selectivity while minimising toxicity.[39]

Declarations

Conflict of interest

The Authors declare that they have no conflicts of interest to disclose.

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References

1. Minotti G *et al.* Anthracyclines: molecular advances and pharmacologic developments in antitumor activity and cardiotoxicity. *Pharmacol Rev* 2004; 56: 185–229.
2. Keizer HG *et al.* Doxorubicin (adriamycin): a critical review of free radical-dependent mechanisms of cytotoxicity. *Pharmacol Ther* 1990; 47: 219–231.
3. Ruggiero A *et al.* Myocardial performance index and biochemical markers for early detection of doxorubicin-induced cardiotoxicity in children with acute lymphoblastic leukaemia. *Int J Clin Oncol* 2013; 18: 927–933.
4. Batist G *et al.* Myocet (liposome-encapsulated doxorubicin citrate): a new approach in breast cancer ther-

apy. *Exp Opin Pharmacother* 2002; 3: 1739–1751.
5. Marina NM *et al.* Dose escalation and pharmacokinetics of pegylated liposomal doxorubicin (Doxil) in children with solid tumors: a pediatric oncology group study. *Clin Cancer Res* 2002; 8: 413.
6. Guo B *et al.* Individualized liposomal doxorubicin-based treatment in elderly patients with non-Hodgkin's lymphoma. *Onkologie* 2011; 34: 1087–1093.
7. Weekes CD *et al.* Hodgkin's disease in the elderly: improved treatment outcome with a doxorubicin-containing regimen. *J Clin Oncol* 2002; 20: 184–188.
8. Carvalho C *et al.* Doxorubicin: the good, the bad and the ugly effect. *Curr Med Chem* 2009; 16: 3267–3285.
9. Tacar O *et al.* Doxorubicin: an update on anticancer molecular action, toxicity and novel drug delivery systems. *J Pharm Pharmacol* 2013; 65: 157–170.
10. Chatterjee K *et al.* Doxorubicin cardiomyopathy. *Cardiology* 2010; 115: 155–162.
11. Gewirtz DA. A critical evaluation of the mechanisms of action proposed for the antitumor effects of the anthracycline antibiotics adriamycin and daunorubicin. *Biochem Pharmacol* 1999; 57: 727–741.
12. Tanaka M, Yoshida S. Mechanism of the inhibition of calf thymus DNA polymerases alpha and beta by daunomycin and adriamycin. *J Biochem* 1980; 87: 911–918.
13. Zunino F *et al.* The inhibition in vitro of DNA polymerase and RNA polymerases by daunomycin and adriamycin. *Biochem Pharmacol* 1975; 24: 309–311.
14. Chen N-T *et al.* Probing the dynamics of doxorubicin-DNA intercalation during the initial activation of apoptosis by fluorescence lifetime imaging microscopy (FLIM). *PLoS One* 2012; 7: e44947.
15. Aubel-Sadron G, Londos-Gagliardi D. Daunorubicin and doxorubicin, anthracycline antibiotics, a physicochemical and biological review. *Biochimie* 1984; 66: 333–352.
16. Fornari FA Jr *et al.* Growth arrest and non-apoptotic cell death associated with the suppression of c-myc expression in MCF-7 breast tumor cells following acute exposure to doxorubicin. *Biochem Pharmacol* 1996; 51: 931–940.
17. Dano K *et al.* Inhibition of DNA and RNA synthesis by daunorubicin in sensitive and resistant Ehrlich ascites tumor cells in vitro. *Cancer Res* 1972; 32: 1307–1314.
18. Wang JJ *et al.* Comparative biochemical studies of adriamycin and daunomycin in leukemic cells. *Cancer Res* 1972; 32: 511–515.
19. Kim SH, Kim JH. Lethal effect of adriamycin on the division cycle of HeLa cells. *Cancer Res* 1972; 32: 323–325.
20. Liu Y, Kulesz-Martin M. p53 protein at the hub of cellular DNA damage response pathways through sequence-specific and non-sequence-specific DNA binding. *Carcinogenesis* 2001; 22: 851–860.
21. Mizutani H *et al.* Mechanism of apoptosis induced by doxorubicin through the generation of hydrogen peroxide. *Life Sci* 2005; 76: 1439–1453.
22. Nitiss JL. Investigating the biological functions of DNA topoisomerases in eukaryotic cells. *Biochem Biophys Acta* 1998; 1400: 63–81.
23. Hurley LH. DNA and its associated processes as targets for cancer therapy. *Nat Rev Cancer* 2002; 2: 188–200.
24. Tewey KM *et al.* Adriamycin-induced DNA damage mediated by mammalian DNA topoisomerase II. *Science* 1984; 226: 466–468.
25. Saijo M *et al.* Growth state and cell cycle dependent phosphorylation of DNA topoisomerase II in Swiss 3T3 cells. *Biochemistry* 1992; 31: 359–363.
26. Burden DA, Osheroff N. Mechanism of action of eukaryotic topoisomerase II and drugs targeted to the enzyme. *Biochem Biophys Acta* 1998; 1400: 139–154.
27. Deffie AM *et al.* Direct correlation between DNA topoisomerase II activity and cytotoxicity in adriamycin-sensitive and -resistant P388 leukemia cell lines. *Cancer Res* 1989; 49: 58–62.
28. Son YS *et al.* Reduced activity of topoisomerase II in an Adriamycin-resistant human stomach-adenocarcinoma cell line. *Cancer Chemother Pharmacol* 1998; 41: 353–360.
29. Capranico G *et al.* Markedly reduced levels of anthracycline-induced DNA strand breaks in resistant P388 leukemia cells and isolated nuclei. *Cancer Res* 1987; 47: 3752–3756.
30. Bates DA, Winterbourn CC. Deoxyribose breakdown by the adriamycin semiquinone and H2O2: evidence for hydroxyl radical participation. *FEBS Lett* 1982; 145: 137–142.
31. Kharasch ED, Novak RF. Inhibitory effects of anthracenedione antineoplastic agents on hepatic and cardiac lipid peroxidation. *J Pharmacol Exp Ther* 1983; 226: 500–506.
32. Griffin-Green EA *et al.* Adriamycin-induced lipid peroxidation in mitochondria and microsomes. *Biochem Pharmacol* 1988; 37: 3071–3077.
33. Fumiyasu F *et al.* Evaluation of adriamycin-induced lipid peroxidation. *Biochem Pharmacol* 1992; 44: 755–760.
34. Hrelia S *et al.* Doxorubicin induces early lipid peroxidation associated with changes in glucose transport in cultured cardiomyocytes. *Biochim Biophys Acta* 2002; 1567: 150–156.
35. Thandavarayan R *et al.* Schisandrin B prevents doxorubicin induced cardiac dysfunction by modulation of DNA damage, oxidative stress and inflammation through inhibition of MAPK/p53 signaling. *PLoS One* 2015; 10: e0119214.
36. Di X *et al.* Apoptosis, autophagy, accelerated senescence and reactive oxygen in the response of human breast tumor cells to adriamycin. *Biochem Pharmacol* 2009; 77: 1139–1150.
37. Skladanowski A, Konopa J. Adriamycin and daunomycin induce programmed cell death (apoptosis) in tumour cells. *Biochem Pharmacol* 1993; 46: 375–382.
38. Lockshin RA, Zakeri Z. Apoptosis, autophagy, and more. *Int J Biochem Cell B* 2004; 36: 2405–2419.
39. Edinger AL, Thompson CB. Death by design: apoptosis, necrosis and

autophagy. *Curr Opin Cell Biol* 2004; 16: 663–669.
40. Hsieh Y-C *et al.* When apoptosis meets autophagy: deciding cell fate after trauma and sepsis. *Trends Mol Med* 2009; 15: 129–138.
41. Avi A. Directing cancer cells to self-destruct with pro-apoptotic receptor agonists. *Nat Rev Drug Discov* 2008; 7: 1001–1012.
42. Zhang Y-W *et al.* Cardiomyocyte death in doxorubicin-induced cardiotoxicity. *Arch Immunol Ther Exp (Warsz)* 2009; 57: 435–445.
43. Niu J *et al.* Cardiac-targeted expression of soluble fas attenuates doxorubicin-induced cardiotoxicity in mice. *J Pharmacol Exp Ther* 2009; 328: 740–748.
44. Kalivendi SV *et al.* Doxorubicin activates nuclear factor of activated T-lymphocytes and Fas ligand transcription: role of mitochondrial reactive oxygen species and calcium. *Biochem J* 2005; 389: 527.
45. Kim D-S *et al.* Plantainoside D protects adriamycin-induced apoptosis in H9c2 cardiac muscle cells via the inhibition of ROS generation and NF-κB activation. *Life Sci* 2007; 80: 314–323.
46. Wang S *et al.* Activation of nuclear factor-kappaB during doxorubicin-induced apoptosis in endothelial cells and myocytes is pro-apoptotic: the role of hydrogen peroxide. *Biochem J* 2002; 367: 729–740.
47. Zhou Y-Y *et al.* MAPK/JNK signaling: a potential autophagy regulation pathway. *Biosci Rep* 2015; 35: e00199.
48. Chen MB *et al.* Activation of AMP-activated protein kinase contributes to doxorubicin-induced cell death and apoptosis in cultured myocardial H9c2 cells. *Cell Biochem Biophys* 2011; 60: 311–322.
49. Dong Z *et al.* Promotion of autophagy and inhibition of apoptosis by low concentrations of cadmium in vascular endothelial cells. *Toxicol In Vitro* 2009; 23: 105–110.
50. Qin Z-H *et al.* Autophagy regulates the processing of amino terminal huntingtin fragments. *Hum Mol Genet* 2003; 12: 3231–3244.
51. Martinet W *et al.* Autophagy in disease: a double-edged sword with therapeutic potential. *Clin Sci* 2009;116:697–712.
52. Piotr C *et al.* Autophagy in DNA damage response. *Int J Mol Sci* 2015; 16: 2641–2662.
53. Pattingre S *et al.* Bcl-2 antiapoptotic proteins inhibit beclin 1-dependent autophagy. *Cell* 2005; 122: 927–939.
54. Guanghong J *et al.* Insulin-like growth factor-1 and TNF-α regulate autophagy through c-jun N-terminal kinase and Akt pathways in human atherosclerotic vascular smooth cells. *Immunol Cell Biol* 2006; 84: 448–454.
55. Smuder AJ *et al.* Exercise protects against doxorubicin-induced markers of autophagy signaling in skeletal muscle. *J Appl Physiol* 2011; 111: 1190–1198.
56. Rodriguez-Vargas J *et al.* ROS-induced DNA damage and PARP-1 are required for optimal induction of starvation-induced autophagy. *Cell Res* 2012; 22: 1181–1198.
57. Goehe RW *et al.* The autophagy-senescence connection in chemotherapy: must tumor cells (self) eat before they sleep? *J Pharmacol Exp Ther* 2012; 343: 763–768.
58. Elmore LW *et al.* Adriamycin-induced senescence in breast tumor cells involves functional p53 and telomere dysfunction. *J Biol Chem* 2002; 277: 35509–35515.
59. Gewirtz DA *et al.* Accelerated senescence: an emerging role in tumor cell response to chemotherapy and radiation. *Biochem Pharmacol* 2008; 76: 947–957.
60. Maejima Y *et al.* Induction of premature senescence in cardiomyocytes by doxorubicin as a novel mechanism of myocardial damage. *Aging Cell* 2008; 7: 125–136.
61. Abdelhadi R *et al.* Caspase inhibition switches doxorubicin-induced apoptosis to senescence. *Oncogene* 2003; 22: 2805–2811.
62. Schmitt CA *et al.* A senescence program controlled by p53 and p16 INK4a contributes to the outcome of cancer therapy. *Cell* 2002; 109: 335–346.
63. Zong W-X *et al.* Alkylating DNA damage stimulates a regulated form of necrotic cell death. *Genes Dev* 2004; 18: 1272–1282.
64. Feoktistova M, Leverkus M. Programmed necrosis and necroptosis signalling. *FEBS J* 2015; 282: 19–31.
65. Lebrecht D, Walker U. Role of mtDNA lesions in anthracycline cardiotoxicity. *Cardiovasc Toxicol* 2007; 7: 108–113.
66. Wallace K. Adriamycin-induced interference with cardiac mitochondrial calcium homeostasis. *Cardiovasc Toxicol* 2007; 7: 101–107.
67. Wang H *et al.* Effect of adriamycin on BRCA1 and PARP-1 expression in MCF-7 breast cancer cells. *Int J Clin Exp Pathol* 2014; 7: 5909–5915.
68. Hyeon-Jun S *et al.* Doxorubicin-induced necrosis is mediated by poly-(ADP-ribose) polymerase 1 (PARP1) but is independent of p53. *Sci Rep* 2015; 5: 15798.
69. Jackson TL. Intracellular accumulation and mechanism of action of doxorubicin in a spatio-temporal tumor model. *J Theor Biol* 2003; 220: 201–213.
70. Kuznetsov A, Margreiter R. Heterogeneity of mitochondria and mitochondrial function within cells as another Level of mitochondrial complexity. *Int J Mol Sci* 2009; 10: 1911–1929.
71. McBride HM *et al.* Mitochondria: more than just a powerhouse. *Curr Biol* 2006; 16: R551–R560.
72. Chan DC. Mitochondria: dynamic organelles in disease, aging, and development. *Cell* 2006; 125: 1241–1252.
73. Friedman JR, Nunnari J. Mitochondrial form and function. *Nature* 2014; 505: 335–343.
74. Wallace DC. A mitochondrial paradigm of metabolic and degenerative diseases, aging, and cancer: a dawn for evolutionary medicine. *FASEB J* 2006; 20: A1474–A1474.
75. Ascensão A *et al.* Exercise as a beneficial adjunct therapy during Doxorubicin treatment – role of mitochondria in cardioprotection. *Int J Cardiol* 2012; 156: 4–10.
76. Wang ZC *et al.* Mitochondria-derived reactive oxygen species play an impor-

tant role in doxorubicin-induced platelet apoptosis. *Int J Mol Sci* 2015; 16: 11087–11100.
77. Patel N *et al.* Silymarin modulates doxorubicin-induced oxidative stress, Bcl-xL and p53 expression while preventing apoptotic and necrotic cell death in the liver. *Toxicol Appl Pharmacol* 2010; 245: 143–152.
78. Lahoti TS *et al.* Doxorubicin-induced in vivo nephrotoxicity involves oxidative stress-mediated multiple pro- and anti-apoptotic signaling pathways. *Curr Neurovasc Res* 2012; 9: 282–295.
79. Solem LE *et al.* Disruption of mitochondrial calcium homeostasis following chronic doxorubicin administration. *Toxicol Appl Pharmacol* 1994; 129: 214–222.
80. Al-Nasser IA. In vivo prevention of adriamycin cardiotoxicity by cyclosporin A or FK506. *Toxicology* 1998; 131: 175–181.
81. Ichikawa Y *et al.* Cardiotoxicity of doxorubicin is mediated through mitochondrial iron accumulation. *J Clin Invest* 2014; 124: 617–630.