

Cardiac Metabolism Imaging and Chemotherapy Cardiotoxicity

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Abstract

Cardiac oncology is a field that will become increasingly important in clinical practice as we become more effective in treating cancer and those treated with chemotherapy live longer. Potential cardiac toxicity associated with some chemotherapy treatments can cause significant morbidity. Molecular positron emission tomography (PET) to assess cardiac metabolism is a promising technology that could increase our knowledge of chemotherapy-related toxicity in the heart. We review the utility of PET cardiac imaging to evaluate the toxic effects of chemotherapy on metabolism. Free fatty acids, glucose and ketone bodies are major substrates for cardiac energy consumption, and adaptations to their use can occur under differing conditions. Cardiovascular complications of chemotherapy can include direct effects on metabolism as well as injury to myocardial tissue by effects on endothelial function, hypertension or ischemia. Even novel chemotherapies that are designed to be more specific in their actions continue to be associated with cardiotoxicity. Further study is required to understand the effects of cardiotoxicity related to chemotherapy, and to develop techniques for its detection as well as prevention. PET cardiac imaging could be used to assist in the early detection of cardiotoxicity and help guide management clinically. It may offer insights to assist in the development of novel treatments and methods for cardioprotection.

Keywords: Cardiotoxicity imaging; Chemotherapy; Cardiac metabolism; Myocardial blood flow; Cardiac PET

Introduction

Chemotherapy has been used to successfully treat or slow the progression of cancer for several decades now. Developments in tyrosine kinase inhibitors and proteasome inhibitors are also contributing to the success in the fight against cancer [1]. Whilst new therapies continue to emerge and the population lives longer as a result of improved management of cancer, the side effects of such treatments can present significant burden to the patient. Chemotherapy targets the tumor cell cycle by arresting or slowing cell division, thereby leading to apoptosis or autophagy (cell death) [2]. Toxicity associated with chemotherapy treatment can affect the hematologic, cardiac, renal, gastrointestinal and immune systems [2]. In this review we focus on the cardiotoxic effects of chemotherapy, and the potential to evaluate its impact on metabolic pathways by positron emission tomography (PET) cardiac imaging.

Cardiac molecular imaging research may provide new diagnostic tools to detect toxicity in its early stages and also to monitor the progression of disease and therapy. This could lead to a significant impact on clinical care decisions and the potential for reduction in morbidity. Improved cardiac imaging to detect chemotherapy-related toxicity could lead to a reduction in the health care cost related to complications arising from advanced disease and merits further investigation. Additionally, improved identification of the cardiotoxic effects could promote the development of novel prophylactic strategies and more efficient treatments tailored to the individual patient.

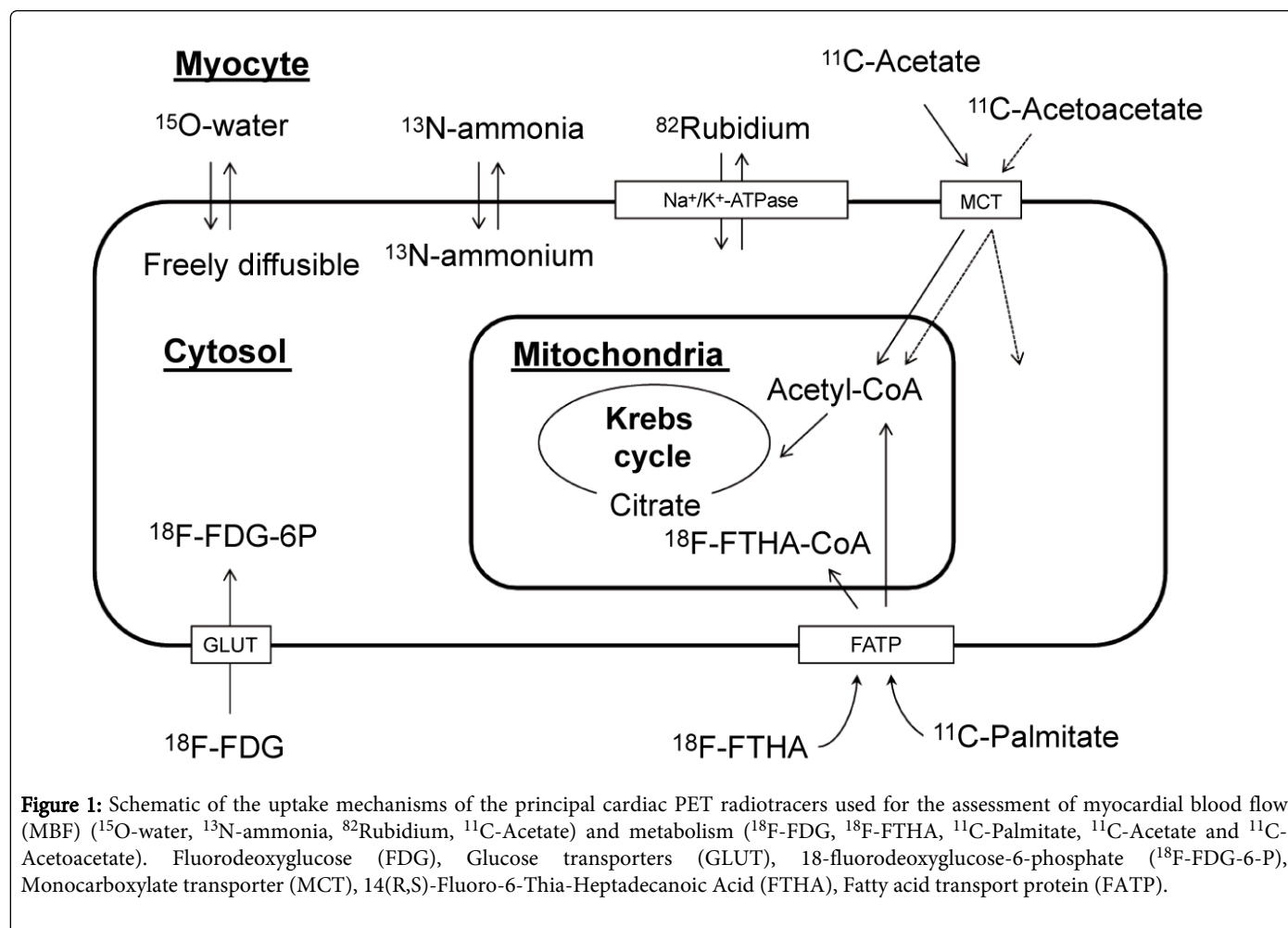
The heart utilizes several metabolic pathways in its function as a pump that drives the circulation to service the metabolic needs of all organs in the body. Cardiac nuclear imaging can study two major

components affecting cardiac function: perfusion (blood supplying the heart) and metabolism (the heart's consumption of energy) (Figure 1 and Table 1) [3-5]. Most chemotherapeutic agents that damage cardiac tissue affect the circulatory vessels, impairing coronary endothelial function, and can cause left ventricular dysfunction or heart failure by generation of reactive oxygen species, apoptosis and through several other inhibitory and/or compensatory mechanisms [1,6-9].

Tracer Name	Half-life (min)	Application
Clinical		
¹³ N-ammonia	9.97 min	MBF
⁸² Rubidium	1.27 min	MBF
¹⁸ F-FDG	110 min	Glucose metabolism
Research		
¹⁵ O-water	2.07min	MBF
¹⁸ F-FTHA	110min	Fatty acid metabolism
¹¹ C-Palmitate	20.4min	Fatty acid metabolism
¹¹ C-Acetate	20.4min	Oxidative metabolism
¹¹ C-Acetoacetate	20.4min	Ketone body metabolism

MBF: Myocardial Blood Flow; FDG: Fluorodeoxyglucose; FTHA: 14-(R,S)-18F-Fluoro-6-Thiaheptadecanoic Acid

Table 1: Cardiovascular PET/CT radiotracers.



Myocardial blood flow (MBF)

Myocardial perfusion imaging (MPI) can evaluate ischemic and infarcted regions of the heart by qualitatively identifying regions of reduced uptake of injected radiotracer [4]. In addition, myocardial blood flow (MBF) measurements are obtained using kinetic models which quantitatively assess the rate of the blood supply to the heart [10,11]. MBF measurements are useful as they can assess the vasodilatory capacity of coronary vessels when subjected to a pharmacological stressor.

Typically, an evaluation of coronary blood flow is performed at baseline rest, followed by a second evaluation after simulating exercise or 'stress' conditions by injecting a drug to induce coronary vasodilatation. A radiotracer is administered at both states to image blood flow and obtain the quantitative flow rates. The subsequent ratio of the MBF at stress and at baseline provides an index of the coronary flow reserve (CFR) [12].

Ideal blood flow radiotracers are able to diffuse freely across vascular and myocardial cell membranes, possess high first-pass extraction from the blood, are retained by myocardial cells, and subsequently can be rapidly and completely cleared from the blood to permit high-contrast visualization of the myocardial uptake. Intravenous radiotracers used routinely in clinical practice include

^{15}O -water, ^{13}N -ammonia and ^{82}Rb -rubidium, as they possess a number of these aforementioned properties. Pharmacological stressors including adenosine, regadenoson, dipyridamole or dobutamine can be used to induce coronary vasodilatation (Table 2) [13-15].

Adenosine and regadenoson primarily mediate vasodilatation through their effects on the coronary vascular smooth muscle (middle layer of the coronary vessel), and secondarily via effects on the endothelial layer, which interfaces with the blood [15].

Dipyridamole acts indirectly through the same adenosine pathway, by inhibiting exogenous adenosine reuptake and activating coronary adenosine receptors. Dobutamine acts as a stressor by a different mechanism, through its effects on the adrenergic receptors; it stimulates β_1 adrenergic receptors in the heart to produce positive inotropic effect, β_2 receptors in the coronary vessels to cause vasodilatation and α adrenergic receptors in the systemic arterial circulation to cause vasoconstriction. Dobutamine thus increases heart rate, myocardial contractility and oxygen demand simultaneously, and triggers coronary vasodilatation in response to the increased metabolic demand [16].

Stressor Name	Mechanism	Target
Adenosine	Non-specific agonist of the Adenosine receptors	Smooth muscle vasodilatation
Dipyridamole	Increases extracellular concentration of adenosine by inhibiting reuptake	Inhibition of the phosphodiesterase
Regadenason	Specific agonist of the Adenosine A2a smooth muscle receptor	Coronary smooth vessel vasodilatation
Dobutamine	Strong β_1 agonist, moderate β_2 and mild α_1 adrenergic receptors	Increase oxygen demand, heart rate and blood pressure

Table 2: Pharmaceutical stressors to assess the coronary flow reserve (CFR).

In cardiac PET imaging, the cold-pressor test (CPT) can also be used to assess endothelial dysfunction more specifically. CPT involves the immersion of an arm or foot into ice-cold water for a period of time, resulting in increased heart rate and blood pressure due to the systemic release of norepinephrine and activation of the adrenergic receptors [17]. Alternately, a drug stressor such as norepinephrine or salbutamol, a potent β_2 adrenergic receptor agonist, could potentially be used to assess endothelial dysfunction [18,19]. Diseases such as atherosclerosis, hypertension and diabetes can also impair endothelial function. Chemotherapy can damage the vascular endothelium and induce hypertension, thus detection of toxicity may be enhanced by specific assessment of coronary endothelial function [20].

Myocardial metabolism

To understand the effects of cardiac toxicity (heart failure, hypertension, endothelium damage) by chemotherapy, we must first understand normal cardiac metabolism [21]. Local storage of adenosine triphosphate (ATP), the energy reserve, is available in the myocardium to sufficiently enable a few heart beats. For this reason the heart has evolved to be readily adaptable to utilize any number of energy substrates that may available. Principal energy sources for the heart are fatty acids, glucose, ketone bodies, lactate, pyruvate and amino acids [22]. The heart has a constant demand for energy substrates, and a third of its consumption is typically through oxidation of carbohydrates and two thirds via fatty acid β -oxidation. Oxidative phosphorylation accounts for 95% of ATP production in the mitochondria under normal conditions and this energy comes from beta-oxidation of the fatty acids and pyruvate from glycolysis.

Nearly 60-70% of ATP consumed by the myocardium is dedicated to contractile function and the remainder is used to maintain cell integrity [22]. Actomyosin ATPases are the main consumer of ATP in the heart muscle, producing adenosine diphosphate (ADP) and inorganic phosphate byproducts. This cycle is extremely well regulated, with ADP being converted back into ATP via ATP synthases. Oxygen consumption and fatty acid oxidation are generally proportional to the index of cardiac work. The heart at maximal work capacity will use 80-90% of the oxygen through oxidative phosphorylation, compared to the resting state where only 15-25% of the capacity is used. This is likely one factor that makes early detection of deficiency in ATP difficult, as the reserve is large and the heart can easily adapt. Chemotherapy induced cardiac toxicity decreases the heart's efficiency to generate ATP and leads to progressive deterioration of contractile function, generally observed as left ventricular dysfunction (measured as an impaired ejection fraction) and eventually heart failure [9].

Carbohydrates metabolism

The heart can function with 10 to 40% of its energy derived from pyruvate oxidation (glycolysis and lactate oxidation). Glycolysis converts glucose-6-phosphate to pyruvate. Pyruvate is then used in the mitochondria through the Krebs cycle under aerobic conditions. Glucose oxidation is favored under normal conditions and does not produce lactate as occurs under anaerobic conditions and ischaemic states. Phosphofructokinase-1 (PFK-1) is an important factor in the regulation of glycolysis. PFK-1 is inhibited by its products, a drop of pH or an increase in citrate. Many factors also regulate the glycolytic pathway: insulin, glucagon, epinephrine, norepinephrine and several other hormones. Oxidation of glucose in the mitochondria is facilitated by pyruvate dehydrogenase (PHD) converting pyruvate to acetyl-CoA [23]. In summary, glucose crosses the cell membrane through Glut1 and Glut4 transporters, is converted to glucose-6-phosphate and pyruvate, and then enters the mitochondria to be converted into acetyl-CoA for oxidation through the Krebs cycle in aerobic conditions (Figure 2).

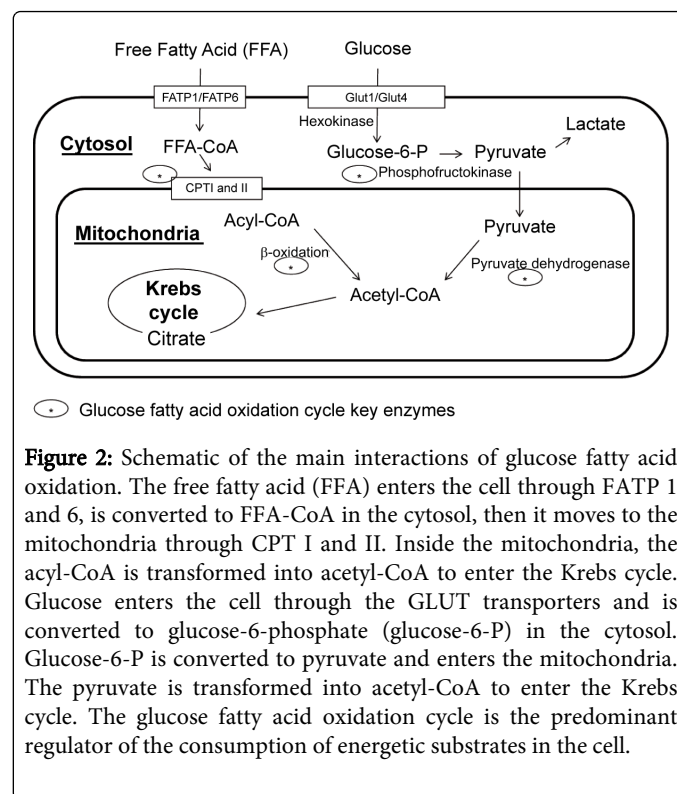


Figure 2: Schematic of the main interactions of glucose fatty acid oxidation. The free fatty acid (FFA) enters the cell through FATP 1 and 6, is converted to FFA-CoA in the cytosol, then it moves to the mitochondria through CPT I and II. Inside the mitochondria, the acyl-CoA is transformed into acetyl-CoA to enter the Krebs cycle. Glucose enters the cell through the GLUT transporters and is converted to glucose-6-phosphate (glucose-6-P) in the cytosol. Glucose-6-P is converted to pyruvate and enters the mitochondria. The pyruvate is transformed into acetyl-CoA to enter the Krebs cycle. The glucose fatty acid oxidation cycle is the predominant regulator of the consumption of energetic substrates in the cell.

The glucose analog 2-deoxy-2-(18F)fluoro-D-glucose (18F-FDG) is a PET tracer that can be used to assess glucose metabolism. 18F-FDG crosses the cell membrane in the same way as glucose and is metabolized by hexokinases into 18F-FDG-6-phosphate. When 18F-FDG is phosphorylated, it cannot move further along the glycolytic pathway. The 18F-FDG is essentially trapped in the cytosol, allowing glucose consumption to be evaluated by the rate of uptake of this radiotracer [24].

Fatty acids metabolism

The rate of transport of free fatty acids (FFA) into the myocyte is linked to the FFA concentration in the blood. FFA can diffuse freely or use facilitated transport to cross the cell membrane through the fatty

acid transporters FATP1 and FATP6. 70-90% of the FFA inside the myocyte is oxidized through the Krebs cycle, releasing CO₂ and H₂O as byproducts. Usually, FFA is bound to a plasma membrane fatty acid binding protein and requires fatty acyl-CoA synthase to activate its esterification for conversion to fatty acyl-CoA. Acyl-CoA is esterified into triglyceride or is transported into the mitochondria. Carnitine palmitoyltransferase I and II (CPT I-II) transport FFA from the cytosol into the mitochondria for conversion to acetyl-CoA by β -oxidation. If excess acetyl-CoA is produced, it can return to the cytoplasm for use in the lipogenesis pathway. The acetyl-CoA will be converted into malonyl-CoA with acetyl-CoA carboxylase (ACC). ACC and malonyl-CoA are key regulators of FFA metabolism, with inhibitory effect on CPT-I leading to an increase in lipogenesis. Oxidative metabolism is the major pathway of energy production in the normal heart and mainly consumes FFA and glucose to generate ATP [21].

The fatty acid analog 14-(R,S)-¹⁸F-fluoro-6-thiaheptadecanoic acid (¹⁸F-FTHA) is trapped inside the cell after entering and undergoing metabolic transformations in a similar fashion to ¹⁸F-FDG. ¹⁸F-FTHA is presumably sequestered into the mitochondria after the initial step of β -oxidation. This false long-chain FFA is blocked by its sulfur component, and cellular uptake represents both β -oxidation and esterification [25,26]. It is possible to use a ¹¹C-carbon radioisotope to label a tracer that follows the same pathways as carbon, which is present ubiquitously in biological molecules. However, such pathways can also create metabolites that could make the tracking process difficult to measure and interpret. ¹¹C-palmitate for example, another fatty acid radiotracer, enters into the myocyte and can proceed through either esterification or β -oxidation. To obtain a more specific assessment of FFA metabolism, a drug inhibitor can be used, such as 2-tetradecylglycidic acid, to block the CPT-I pathway, permitting better evaluation of the esterification process. This is similar to the concept of the CFR measurement at rest and stress, where β -oxidation can be evaluated with and without inhibition [27].

The glucose fatty acid oxidation cycle (Randle cycle) balances the cardiac consumption of energetic substrates (Figure 2) [28,29]. Glucose fatty acid oxidation is controlled by hormones, enzyme inhibition or activation, and reversible enzyme phosphorylation. The key enzymes are: malonyl-CoA, phosphofructokinase (PFK), acetyl-CoA carboxylase (ACC), citrate and AMP-activated protein kinase (AMPK). There are several mechanisms to decrease FFA oxidation: increasing the malonyl-CoA concentration to inhibit β -oxidation, increasing citrate in the cytosol to activate the acetyl-CoA carboxylase, or inhibiting the β -oxidation pathway and rerouting the FFA to esterification using drugs like trimetazidine. Several mechanisms could be used to decrease glucose oxidation. From the mitochondria, an increase of acetyl-CoA or NADH to inhibit PDH will decrease the glucose oxidation reducing the conversion of pyruvate into acetyl-CoA. From the cytosol, the glucose-6-P could be rerouted to glycogen formation if there is an increase of citrate to inhibit the PFK, an enzyme of the glucose oxidation pathway. The pharmaceutical used, such as dichloroacetate, could also reduce glucose oxidation by inhibiting the deactivation of the PDH. Finally, AMPK is another key enzyme that stimulates both glucose and FFA oxidation, inactivating ACC and increasing the malonyl-CoA concentration [29].

Ketone bodies metabolism

The heart uses ketone bodies in proportion to their concentration in the blood. Under normal conditions the blood has low ketone body

concentration. During certain physiologic conditions such as intense and prolonged exercise, fasting, fever, certain disease processes and when glucose supply is limited, the level of ketone bodies in the blood can rise substantially [30,31]. The heart's consumption of FFA and ketone bodies (e.g. acetoacetate and 3-hydroxybutyrate) consequently rises under these conditions also, becoming the main energetic substrate for the heart. Ketone bodies, and the small molecule acetate, are preferred over the classic substrates when they are present in the blood.

Ketone bodies and acetate both use the same monocarboxylic acid transporters (MCT) to enter the cytosol of myocytes. Acetate needs to be in the mitochondria before being converted into acetyl-CoA. Acetoacetate in the cytosol can be transformed into acetoacetyl-CoA and has the potential to proceed directly into lipogenesis. Acetoacetate can also be transformed in the mitochondria into acetoacetyl-CoA, and enter into the Krebs cycle for energy production. Acetate is used almost exclusively as an energy substrate [32]. Acetoacetate is used where needed, including in lipogenesis or to repair damage to the mitochondrial and cell membranes, or as an energy substrate if there is no structural abnormality in the cell. Pyruvate from glucose, acyl-CoA from FFA, acetate and acetoacetate all are converted in the mitochondria to acetyl-CoA to enter the Krebs cycle. These energy substrates undergo oxidative phosphorylation to produce ATP needed for contractility and respond to the energy demand related to the cardiac work [30,33].

Cardiac oxidative metabolism has been intensively studied with ¹¹C-acetate; washout of the radiotracer from the myocytes in the form of CO₂ after utilization in the Krebs cycle represents an index of myocardial oxygen consumption [34]. Although primarily a metabolic tracer, acetate possesses good first-pass extraction and its uptake phase thus reflects an index of MBF [35]. Therefore, ¹¹C-acetate efficiently allows simultaneous observation of both blood flow and mitochondrial oxygen metabolism in the myocyte [36]. On the other hand, the ketone body ¹¹C-acetoacetate is an emerging cardiac radiotracer [37]. It has been studied in fasting mode in small animals compared to controls, and appears to have slower clearance from the myocyte compared to ¹¹C-acetate. When correction for the presence of ketones in the blood was applied however, an increase in the ¹¹C-acetoacetate consumption rate was observed [38]. In another study, cardiac ¹¹C-acetoacetate demonstrated different uptake and washout patterns in the early stages of heart failure, secondary to doxorubicin toxicity, as compared to controls [36].

Cardiac metabolic imaging may be used to assess the interaction between glucose and FFA metabolism. All of the aforementioned radiotracers have the potential to evaluate cardiac disease, including left ventricular dysfunction or damage to the endothelium [39]. Cardiac toxicity alters metabolism and cell integrity, and further investigation is required to develop early detection methods and improve management of chemotherapy-related side effects.

Cardiomyopathy

Heart failure

A number of chemotherapy treatments have severe cardiac side effects and toxicity, mainly manifesting as symptoms of heart failure. The anthracycline family, proteasome inhibitors, antimicrotubule agents, alkylating agents and tyrosine kinase inhibitors will indirectly (though effects of hypertension or endothelium damage) or directly

(by apoptosis and autophagy) affect the heart tissue and can lead to clinical heart failure. Dilated cardiomyopathy is the most common form of heart failure due to chemotherapeutic toxicity [40,41]. Heart failure is a syndrome characterized by a structural or functional impairment that affects the filling of the heart (diastolic dysfunction) or contractile ejection of the blood (systolic dysfunction) [42].

When the heart is unable to pump enough blood to meet the body's requirements, it tries to adapt by a processes of structural 'remodeling' that generally leads to dilatation of the ventricular cavity. Heart failure is also associated with neuro-hormonal adaptation; there is increased sympathetic tone and elevated norepinephrine levels that increase heart rate and contractility in an attempt to improve cardiac output, as well as rise in blood pressure via activation of the renin-angiotensin system. Heart failure progression is associated with a process of desensitization; the catecholamine response is gradually blunted by mediation of G-protein-coupled receptor kinases (GRKs) and β -arrestin [43]. Desensitization of GRKs affects control of vascular tone (vasodilatation and vasoconstriction). Hypertension increases the afterload on the heart, and contributes to heart failure by causing a rise in intra-ventricular pressure, which can lead to diastolic dysfunction. Progression of heart failure is associated with cardiac remodeling and a change in the efficiency of oxygen consumption. An increased catecholamine level and activation affects the heart and produces several metabolic responses mimicking stress conditions, which is detrimental in the long term [44]. Medical imaging has been used extensively to diagnose heart failure and assess cardiac function. Cardiac structure and function can be assessed by a number of modalities including nuclear medicine (PET and SPECT), echocardiography, MRI, cardiac CT and invasive coronary catheterization [39,45].

Ischemia

Ischemia is caused by an insufficient blood supply to the heart. Ischemia, in general terms, can result in both reversible and irreversible myocardial injury. The functional and structural adaptation by the heart in an attempt to maintain output can result in paradoxical strain and further decline in cardiac function. Oxidative metabolism can be reduced in favor of an anaerobic process to generate energy to survive in the short term. While this alternative-anaerobic glycolysis-is definitely less efficient, it is easy to produce ATP [46]. Chemotherapy, particularly agents such as alkylating agents, antimicrotubule and tyrosine kinase inhibitors are associated with ischemia. Chemotherapy can cause ischemia by a number of mechanisms, including coronary artery vasospasm, direct injury of vessel endothelium leading to plaque formation, and endothelial dysfunction in the microcirculation [47].

Endothelial dysfunction

A principal function of endothelium is auto-regulation of vessel size to maintain a constant blood pressure and blood flow to meet the changing metabolic demands of the myocardium [48]. Acting via several signaling mechanisms, the vascular endothelium communicates with the smooth muscle cells to control the vessel diameter. Physico-chemical and neuro-hormonal stimuli are the two main mechanisms regulating vasodilatation and vasoconstriction [48]. Neuro-hormonal stimulation (through action of norepinephrine, acetylcholine, histamine, substance P and others) generally increases intracellular Ca^{2+} in vascular endothelial cells and activates release of several endothelium-derived relaxing factors (EDRF), including nitric

oxide (NO), prostacyclin and endothelium-derived hyperpolarizing factor (EDHF). NO is produced locally in the vessel wall by endothelial nitric oxide synthase (eNOS), and can rapidly diffuse across the vessel wall to activate relaxation of the vessel, mediated by its action on receptors in the vascular smooth muscle cells (VSMC).

G-protein-coupled cell surface receptors (GPCRs) have been identified as being important in the control of vascular flow [49]. The GPCRs involved in regulating vascular tone are on the endothelium layer and the VSMC. The associated receptor ligands induce vasodilatation including the neuro-hormones mentioned earlier or adenosine and dopamine. Alternatively, they can induce vasoconstriction with angiotensin II or endothelin on specific cell surfaces, or both depending on the size of the artery and its location [48,49]. The presence of reactive oxygen species (ROS), such as superoxide anions, can cause dysfunction of the NO signaling pathway, and their byproducts, such as peroxynitrite, can directly damage the vessel wall [50]. Endothelial dysfunction (ED) is a vascular disease where normal auto-regulation is not properly maintained. Decreased NO synthesis and an increase in the rate of its degradation results in reduced bioavailability, which is an important early manifestation of ED. Furthermore, the GPCRs can reduce eNOS function, increase its expression level leading to endothelial dysfunction, and introduce a hypertensive profile which may contribute to heart failure progression [51,52]. Chemotherapy can also cause an inflammatory response, and depending on the intensity and duration of this stress, preconditioning may contribute to dysfunction of coronary endothelium [53]. GPCR expression induced by chemotherapy or cardiac disease may cause hypertension by desensitization of the β -adrenergic receptor [52]. Early assessment of endothelial function is important as ED can trigger or accelerate the progression of cardiotoxicity.

Chemotherapy

Anthracyclines

Doxorubicin is an anthracycline that has been widely associated with cardiotoxicity [41,54]. Anthracyclines are used in the treatment of many malignancies, including solid tumors such as breast, ovarian, and lung cancer, as well as leukemias and lymphomas. Doxorubicin is often a component of chemotherapy regimens, and maintaining a cumulative dose under 400 mg/m² can significantly reduce the risk of toxicity to the heart. Whilst side effects of anthracyclines on other tissues are known and have established prophylactic measures in routine clinical use, there does not appear to be adequate treatment available for cardiotoxicity without reducing the anti-tumor effect of the therapy. Anthracycline associated cardiotoxicity has been observed to have acute, subacute, chronic and late phases, however the mechanism of its toxicity has not been entirely elucidated. Morphologic cellular characteristics of cardiotoxicity include loss of myofibrils, dilation of the sarcoplasmic reticulum, increased lysosome size and cytoplasmic vacuolation of the mitochondria [55].

Doxorubicin is an important topic of research, both for the mechanism of its antitumor action and its cardiotoxicity. Doxorubicin interacts in tumour cells with topoisomerase II and the DNA to inhibit mitotic activity, leading to apoptosis. The rate of cell division in the heart is low, and this action on tumor cells is unlikely to play a role in the myocyte. It is hypothesized that doxorubicin and its metabolites lead to damage of myocyte integrity through the generation of ROS, such as the superoxide radical ($\cdot O_2^-$), hydroxyl radical ($\cdot OH$) and

hydrogen peroxide (H₂O₂) [55]. Although the predominant cardiotoxic mechanism of doxorubicin is thought to act through ROS generation, its damage to the heart may also be induced by other biologic pathways, including alteration of the DNA and sarcomere integrity [56].

The heart does not have a high concentration of protectors against ROS, such as catalase, superoxide dismutase (SOD) or glutathione peroxidase. Epirubicin, another anthracycline, has also been identified as cardiotoxic due to ROS generation [57]. To compensate, aconitase, designed to convert citrate to isocitrate in the Krebs cycle, loses an iron and sulfur molecule, and this enzyme is converted to an iron regulatory protein I (IRP-I), which controls iron uptake and helps protect against ROS in the heart. The enzyme IRP-I seems to be part of the cell defense against the ROS (oxidative stress); breaking the equilibrium between aconitase and the IRP-I appears to be harmful to the integrity of the cell. Doxorubicin attacks mitochondrial function where the release of cytochrome C in the cytosol is associated with a decrease of ATP production. Dysfunction of the mitochondria affects oxidative metabolism, altering myocyte-energy generation [58].

Alkylating agents

Cyclophosphamide and ifosfamide can induce left ventricular dysfunction within a few days of initiation of treatment, and the risk appears to be dose dependent [59,60]. Alkylating agents are commonly used as a part of regimens such as R-CHOP (cyclophosphamide, doxorubicin, vincristine, rituximab and prednisolone) used to treat large B-cell lymphoma. Metabolites of alkylating agents interact on DNA and RNA to inhibit DNA synthesis. In a similar fashion to anthracycline, they can damage the mitochondrial membrane and impair oxidative phosphorylation by oxidative stress [59]. ROS production appears to be less important in causing cardiotoxicity by alkylating agents compared to anthracyclines, and even when the effects are severe they are often reversible.

Antimicrotubule agents

Docetaxel is associated with cardiotoxicity, but to a lesser degree than the anthracyclines and alkylating agents. Docetaxel is used for adjuvant therapy in breast cancer. It arrests cell division by inducing dysfunction of microtubules, thereby promoting polymerization of tubulin and at the same time inhibiting its depolymerization [60,61]. Interaction of Docetaxel with the microtubules may induce contractile dysfunction of the left ventricle; however, further study is still required to better delineate the mechanism of its toxicity.

Antibody-based and small molecule tyrosine kinase inhibitors

The tyrosine kinase inhibitor family primarily targets growth factor receptors, such as epidermal growth factor (EGF), platelet-derived growth factor (PDGF) and vascular endothelial growth factors (VEGF). The mechanism of action targeting the tumor is the same as that which induces hypertension via vessel and heart toxicity. The VEGF inhibitors, whether monoclonal antibodies or small molecules, bind VEGF receptors and block their signaling. This action will induce a reduction of NO generation, causing an imbalance of vascular tone and leading to systemic hypertension [62]. The VEGF inhibitors can reduce bioavailability of NO, reduce eNOS expression, decrease the prostacyclin signaling and increase the release of endothelin-1 [62]. An example of a monoclonal antibody-based tyrosine kinase inhibitor is

trastuzumab, which is used in breast cancer patients who over-express the human epidermal growth receptor 2 (HER2) [63]. Few studies are available that assess the mechanism of its cardiotoxicity. It is hypothesized that interference in the survival pathways of HER4 and HER2 leads to increased oxidative stress and ROS production, which in turn induces apoptosis and the byproduct, nitric oxide, involved in endothelial function [64,65]. Furthermore, trastuzumab inhibits HER2, which is responsible for the heart survival pathways, by increasing cellular transcription factors, NO production and ROS inhibition [65]. Another example is sunitinib, a small molecule tyrosine kinase inhibitor that targets multiple tyrosine kinase receptors, including PDGF and VEGF. It reduces tumour vascularization and the rate of cell division. Cardiotoxicity in these agents seems to be associated with their effect on coronary vessel pericytes, leading to microvascular disease and endothelial dysfunction [66]. Fewer than 10 tyrosine kinase inhibitors are clinically approved in the USA, and these emerging chemotherapies have arisen as a result of increased understanding of tumor biology and its mechanisms of progression. Integrins, such as $\alpha v \beta 3$ which could activate VEGFR, is another targeted step in the biologic pathway of angiogenesis and this is also part of the emerging chemotherapy arsenal [67].

Proteasome inhibitor

Bortezomib inhibits the 26S proteasome of the intracellular multi-enzyme complex. The proteasome degrades proteins and its inhibition results in apoptosis of the tumor cell. The proteasome inhibitor effect on myocytes and smooth muscle cells seems to deteriorate their normal function. Bortezomib treatment has been associated with heart failure symptoms [68]. Additionally, non-anthracycline agents, such as antimetabolites, like capecitabine and fluorouracil, have been associated with ischemia, but the mechanism of their toxicity is poorly defined [60].

Cardiac imaging investigation of chemotherapeutic cardiotoxicity

In summary, cardiac PET imaging has an increasing potential to be a major player in the next generation of cardio-oncologic investigation via assessment of blood flow and metabolism, to evaluate the potential cardiotoxic effects of cancer treatment. In basic science, small animal experiments *in-vivo*, *ex-vivo* and isolated perfused heart studies can be used to more easily evaluate a specific effect or biologic pathway. Cardiac imaging is translatable from animal models to humans, as the same radiotracer and pharmacological stressor could be used to assess MBF and heart metabolism. As an example, a mouse model of breast cancer metastasis could be treated with chemotherapy and its heart cardiotoxicity be evaluated with non-invasive imaging prior to human use. The toxic effects of chemotherapeutic agents generally lead to heart failure, as ROS production and the associated increase in oxidative stress induce endothelial dysfunction and mitochondrial disturbance. As the mitochondria are the major intracellular source of ROS production, further investigation of strategies to protect the mitochondria from oxidative stress is required [69]. The monitoring of anthracycline cardiotoxicity is performed by evaluating cardiac function during and after treatment and is traditionally performed with echocardiography or radionuclide ventriculography. In cardiac PET imaging, to assess coronary vasodilatation, blood perfusion radiotracers can be used to assess the MBF reserve when they are used in conjunction with appropriate pharmacological stressors. Maximal vasodilatation is reached with pharmaceuticals that preferentially

affect the smooth muscle. If a study is focused primarily on endothelial function, a pharmacological stressor targeting β_2 adrenergic receptors may be useful.

The glucose analog ^{18}F -FDG can be used in conjunction with a PDH inhibitor to assess cardiac metabolism by measuring glycolytic activity. The fatty acid tracers (^{18}F -FTHA and ^{11}C -palmitate) can be used in combination with statin, malonyl or ACC inhibitors to assess esterification and β -oxidation effects. Oxidative metabolism may also be assessed with the use of the small molecule ^{11}C -acetate, a component of the Krebs cycle [70]. Finally, ^{11}C -acetoacetate assessing the oxidative metabolism and esterification may be a superior tracer as it seems to also be involved in mitochondrial membrane repair. ^{11}C -acetoacetate has the potential to be used in the diagnosis of early stage heart failure and to detect chemotherapeutic alterations in cardiac metabolism [37].

Assessment of cardiotoxicity related to alkylating agents should utilize MBF vasodilatation. Cyclophosphamide and ifosfamide primarily cause injury to the endothelium. The antibody-based tyrosine kinase inhibitors target growth factors in tumors, but VEGF receptors in vessels and the heart can lead to hypertension and heart failure [60]. The small molecule tyrosine kinase inhibitors are multi-target growth factor inhibitors and their toxicity seems more related to pericytes leading to endothelial dysfunction [60]. Unresolved endothelial dysfunction can lead to eventual progression of heart failure.

While cardiotoxicity affects many physiological processes other than perfusion and metabolism only, in this review the focus was on the assessment of these two major factors with cardiac PET imaging. There are several other imaging modalities that could be used to improve the diagnosis of the heart integrity that are not covered in detail in this review, and are briefly described below.

Using novel echocardiography indices assessing cardiac compliance (tissue Doppler imaging), abnormal diastolic wall motion has been observed following anthracycline therapy; measurement of the deformation and the deformation rate are complementary to the typical evaluation of heart function evaluation [71]. Contrast echocardiography can be used to enhance evaluation of cardiac function. Micro-bubble contrast agents administered intravenously may improve the result of stress echocardiography by better delineating the endocardial border, and could lead to more reliable functional assessments for cardiotoxicity [72].

Cardiac MRI is considered as a gold-standard in cardiac function evaluation. Certain imaging pulse sequences in MRI could be used to evaluate cardiotoxicity, including T2 weighting and enhancement with gadolinium contrast to assess myocardial inflammation, or iron oxide combined with a recombinant human annexin to visualize apoptosis.

In conventional SPECT imaging, apoptosis imaging with Tc-99m-annexinV may become a promising technique for evaluation and follow-up of chemotherapy-induced cardiotoxicity. Additionally, techniques to radiolabel some drug therapies, such as ^{111}In -trastuzumab or ^{123}I -doxorubicin, have been used to investigate their bio distribution, retention time and biologic pathway. Furthermore, as altered adrenergic receptor expression and reduction of efficacy is observed in heart failure progression, the clearance of ^{123}I -MIBG could be used to evaluate heart failure via the sympathetic neuronal activity deregulation. ^{111}In -antimyosin may also be used to show myocyte damage and necrosis. SPECT and PET have many available cardiac radiotracers, and multi-modality cardiac imaging could be

advantageous for assessing the multi-factorial etiology of cardiotoxicity with chemotherapy, as well as improving treatment and cardiac protection mechanisms [73].

In conclusion, there are a number of potential radiotracers available to investigate the toxic effects of chemotherapy on the heart using PET imaging. Each may have a specific role to play given the varied mechanism by which myocardial toxicity is induced in the process of treating malignancy. Further investigations are required to assess early markers of cardiotoxicity and to develop appropriate clinical measures to address them.

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