https://doi.org/10.31925/farmacia.2025.1.2 **REVIEW**

IMPAIRMENT OF PLATELET MITOCHONDRIAL RESPIRATION: AN OVERVIEW OF ANIMAL MODELS OF DISEASE AND DRUG-INDUCED TOXICITY

BOGDAN M. LOLESCU [1,2], ADINA V. FURDUI-LINȚA [1,2,3], MARIA D. DĂNILĂ [2,3], ALEXANDRU I. BLIDIȘEL [4], CRISTINA A. DEHELEAN [5], DANINA M. MUNTEAN [2,3], RODICA LIGHEZAN [6,7]*, OCTAVIAN M. CREȚU [4]

[1]*Doctoral School Medicine, "Victor Babeș" University of Medicine and Pharmacy of Timișoara, Romania*
[2]*Centre for Translational Research and Systems Medicine, Faculty of Medicine, "Victor Babeș" University of Medicine and Pharmacy of Timișoara, Romania*
[3]*Department III – Chair of Pathophysiology, Faculty of Medicine, "Victor Babeș" University of Medicine and Pharmacy of Timișoara, Romania*
[4]*Department of Surgery I – Clinic of Surgical Semiotics & Thoracic Surgery, Centre for Hepato-Biliary-Pancreatic Surgery, Faculty of Medicine, "Victor Babeș" University of Medicine and Pharmacy of Timișoara, Romania*
[5]*Department I – Clinic of Toxicology, Drug Industry, Management & Legislation, Research Centre for Pharmaco-Toxicological Evaluation, Faculty of Pharmacy, "Victor Babeș" University of Medicine and Pharmacy of Timișoara, Romania*
[6]*Department XIII – Chair of Parasitology, Faculty of Medicine, "Victor Babeș" University of Medicine and Pharmacy of Timișoara, Romania*
[7]*Regional Blood Transfusion Centre, Timișoara, Romania*

**corresponding author: lighezan.rodica@umft.ro*

Manuscript received: October 2024

Abstract

Mitochondrial dysfunction is recognised as a central pathophysiological mechanism of most acute and chronic diseases. An emerging research field is represented by assessing the bioenergetic profile of peripheral blood cells (due to their accessibility) as a potential indicator of systemic mitochondrial dys/function. In particular, isolated platelets have been increasingly used for the *ex vivo* assessment of changes in mitochondrial respiration in animal disease models. Also, the past decades witnessed a growing interest in characterising the mechanisms underlying drug-induced mitochondrial toxicity to develop safer novel therapeutic agents, prevent their withdrawal from the market and test counteracting possibilities when mitochondrial toxicity has been demonstrated. In line with this, the modulation of platelet mitochondrial respiration by various pharmacological and toxic compounds has been increasingly tested in the field of mitochondrial toxicology.

Rezumat

Disfuncția mitocondrială este actual considerată un mecanism fiziopatologic esențial al majorității bolilor acute și cronice. Un domeniu de cercetare emergent este reprezentat de evaluarea profilului bioenergetic al elementelor figurate din sângele periferic (datorită accesibilității acestora) drept potențial indicator al (dis)funcției mitocondriale sistemice. În principal, trombocitele izolate au fost utilizate pentru evaluarea *ex vivo* a modificărilor respirației mitocondriale, pe modele de boală indusă experimental la animal. De asemenea, în ultimele decenii a existat un interes crescut pentru caracterizarea mecanismelor ce stau la baza toxicității mitocondriale induse medicamentos, în vederea dezvoltării de noi agenți terapeutici siguri, respectiv a prevenirii retragerii acestora de pe piață, precum și a testării posibilității de contracarare în cazul unei toxicități mitocondriale demonstrate. Ca atare, modularea respirației mitocondriale trombocitare de către diferiți compuși farmacologici și toxici a fost din ce în ce mai mult investigată în domeniul toxicologiei mitocondriale.

Keywords: mitochondrial respiration, platelets, animal models of disease, pharmacological modulation, drug-induced mitochondrial toxicity

Introduction

Platelets are circulating anucleated blood cells derived from the fragmentation of the megakaryocytes and have a central physiological role in haemostasis and wound healing. In the setting of disease, besides their well-known role in arterial thrombosis/ atherothrombosis when excessively activated [1, 2], they are key players in modulating inflammatory and immune responses [3] and controlling cancer progression [4], as well as metastasis [5]. Accordingly, platelets participate in immune defence both directly and indirectly, express immune receptors, interact with the immune cells by acting as antigen-presenting cells, and release pro- and anti-inflammatory mediators, immune-regulatory molecules and microparticles

that modulate the innate and adaptive immune responses [6]. Also, activated platelets may promote metastases by enabling the epithelial-to-mesenchymal transition of primary tumours, mediating tumour cell arrest, secreting growth factors that induce angiogenesis, and protecting circulating tumour cells from the attack of the immune cells [4].

Mitochondrial dysfunction has emerged as a central pathophysiological mechanism of the most prevalent chronic pathologies of the 21st century [7], *e.g.*, cardio-metabolic diseases, cancer [8], neurodegenerative pathologies [9], and ageing [10]. One aspect of mitochondrial dysfunction is represented by the disruption of cellular bioenergetics, which has been investigated at the organ level in numerous animal disease models. Oxidative phosphorylation (OXPHOS) refers to the stepwise passage of the electrons to oxygen, the final acceptor, a process coupled with ATP synthesis (collectively called mitochondrial respiration). OXPHOS involves three enzyme complexes (complex I, III and IV) classically belonging to the electron transport system (ETS) plus a Vth complex (ATP synthase), all embedded in the inner mitochondrial membrane. Complex II (succinate dehydrogenase) is a component of the Krebs cycle that works as an ETS side-branch and feeds the electrons to complex III *via* the reduced form of coenzyme Q (ubiquinol) [11]. The two main techniques used in bioenergetic research are the Oroboros high-resolution respirometry (HRR) assay and the Agilent-Seahorse extracellular flux assay (XFA).

Interference with mitochondrial respiration has been reported to occur in association with various pathologies and also with drug toxicity. In recent years, assessment of the bioenergetic profile of peripheral blood cells has emerged as an easily accessible biomarker of systemic metabolic status [12]. In particular, isolated platelets have been initially used for the *ex vivo* assessment of changes in mitochondrial respiration elicited by animal models of disease and by different drugs [13]. Despite their low number of mitochondria (5 - 8 *per* platelet), platelets have a unique metabolic plasticity, characterised by their capability to easily switch between glucose and fatty acid catabolism to support activation and, when activated, display mainly a glycolytic phenotype coupled with a minor rise in mitochondrial oxygen consumption [14].

The various animal models used in preclinical research are crucial in deciphering the patho-physiological mechanisms of the diseases and also in drug development [15]. However, it has been recently reported a 92% failure rate in translating medications from animal testing to human therapies [16]. Hence, there is a surge of interest in using human samples in translational drug research.

The past decades have witnessed a growing interest in characterising the pathological mechanisms underlying drug-induced mitochondrial toxicity in human samples to prevent drug withdrawal from the market [17] and develop safer therapeutic agents, particularly in the geriatric population [18]. As such, assessment of changes in mitochondrial respiration of isolated human platelets has been increasingly used in mitochondrial toxicology.

This mini-view provides insights into the animal studies reporting the impairment of platelet mitochondrial respiration in experimental pathologies and an overview of those that used platelets for assessing drug/compound-related mitochondrial toxicity. By summarising these findings, the review aims to underscore the relevance of platelets as biomarkers and tools for investigating mitochondrial respiration dysfunction, thus opening avenues for future translational research in this area.

Data Sources

An advanced search on PubMed and Google Scholar databases was conducted, utilising the following specific terms and their combination: platelets, thrombocytes, mitochondrial respiration, mitochondrial respiratory dysfunction, animal models of disease, mitochondria drug effects, and mitochondrial toxicity. Original articles and reviews related to the topic were screened, and the relevant ones published in English up to November 2024 were included. Additionally, several papers were identified from the reference lists of the retrieved publications.

Animal studies on platelet mitochondrial respiration

Animal studies play a crucial role in biomedical research, providing tissue accessibility and pathophysiological insights that are not achievable in human studies [19]. These studies allowed researchers to dissect the complex pathomechanisms of diseases, including the impaired bioenergetics of different organs and assess correlations with the mitochondrial respiratory dys/function in peripheral blood cells, such as platelets. This section outlines the main findings related to changes in platelet mitochondrial respiration in relation to the animal gender and age and changes elicited by various acute and chronic experimental pathologies, respectively.

Influence of Age and Gender

Age- and gender-related differences in mitochondrial oxygen consumption across intact platelets and various organs were evaluated by the group of Jitka Kuncova in male and female Fischer 344 rats at 6, 12 and 24 months, respectively [20]. These authors reported that platelet respiration followed a different pattern for two respiratory parameters, with an increase in the resting respiration between 6 and 12 months in both sexes, which remained higher in 24-month-old female (but not male) rats; at variance, the maximal capacity of the electron transport system

(ETS) was significantly higher in male *vs* female at 12 months but decreased in rats of both sexes at the age of 24 months. Noteworthy, the same group reported a similar decrease in uncoupled respiration (and also in routine respiration) for the aged human platelets [21].

Animal Studies Useful for Correlations of Platelet Respiration with Respiration in Metabolically Active Organs

Whether changes in platelet mitochondrial respiration mirror bioenergetics of metabolically active organs, such as the skeletal muscle, the heart and the brain, has been interrogated by the pioneering studies performed in non-human primates by the group of Anthony Molina almost one decade ago [22, 23]. As such, in the first study, these authors investigated whether the respiratory capacity of platelets (and monocytes) correlates with the oxidative capacity of skeletal and cardiac muscles harvested from female vervet macaques (African green monkeys). They reported that the basal and maximal respiratory capacity of intact platelets were significantly correlated with the respiratory control ratio (a reliable measure of the intrinsic ETS capacity) of isolated skeletal and cardiac muscle mitochondria. Also, a strength of this study is that blood-based bioenergetic parameters were also significantly positively correlated with several measures of the oxidative capacity of permeabilised skeletal muscle fibres, e.g. platelet maximal oxygen consumption rate associated with the reserve capacity of the skeletal muscle. The authors concluded that blood cell respirometry can recapitulate skeletal and cardiac muscle mitochondrial bioenergetics, thus a potential peripheral marker of the systemic metabolic status [22]. In their second study, also performed in female non-human primates, Tyrell *et al*. investigated whether the bioenergetic profile of platelets and monocytes is related to the one of brain mitochondria. They reported that the maximal respiration of monocytes reflected the maximal respiration of mitochondria isolated from the brain's frontal cortex. Moreover, in a subset of vervets, they performed 18-fluorodeoxyglucose PET imaging to assess the brain glucose metabolism. They showed that platelets' maximal oxygen consumption rate positively correlated with glucose in multiple brain regions. The authors concluded that blood-based bioenergetic profiling is a metabolic biomarker and a potential screening tool to assess the systemic bioenergetic decline that might signal the risk for the development of neurodegenerative diseases [23].

The Swine Model of Acute Complex I Dysfunction

The group of Eskil Elmer designed a pig model of acute complex I (the largest complex of the ETS) inhibition, which is responsible for the mitochondrial dysfunction elicited by the inherited rare mitochondrial disorders [24, 25] and also by the most common chronic pathologies of our century [26, 27]. When OXPHOS is impaired, to maintain ATP production, cells shift from aerobic mitochondrial respiration to anaerobic glycolysis, and therefore, lactic acidosis is a common feature of these conditions. The authors used the acute *in vivo* rotenone infusion in anaesthetised pigs to recapitulate the metabolic crisis elicited by complex I inhibition (which triggers lactate over-production due to a shift towards non-mitochondrial metabolism, i.e. anaerobic glycolysis) and assessed mitochondrial respiration *ex vivo*, in peripheral platelets and muscle biopsies. After 3 h of rotenone infusion, they reported a trend towards inhibition of mitochondrial respiration in platelets (but not in skeletal muscle) associated with increased blood lactate concentration and glycolysis intermediates, as demonstrated by the metabolomics data. The authors suggested that the model could be further used for studies of pharmacological compounds designed to counteract acute respiratory changes due to disease- (e.g., Parkinson's) or drug-induced (e.g., metformin) complex I dysfunction [28].

The Swine Model of Cardiac Arrest

Mitochondrial function undergoes significant changes following cardiac arrest, with systemic and cerebral bioenergetics being differentially affected. In a porcine pediatric model of cardiac arrest elicited by asphyxia-related ventricular fibrillation, Ferguson *et al.* demonstrated that platelet mitochondrial respiration markedly increased four hours after the return of spontaneous circulation, and the increase was mainly due to a higher complex II (succinate)-supported respiration. More importantly, there was no evidence of mitochondrial uncoupling after cardiac arrest, indicating an increased respiration efficiency for ATP production. Interestingly, these authors also reported a negative correlation between the high platelet mitochondrial respiratory control ratio and the reduced respiratory control ratios in the cortex and hippocampus. This suggests an inverse relationship between systemic and cerebral mitochondrial function in this cardiac arrest model in piglets, which requires further investigations to elucidate cardiac arrest's effects on peripheral and brain bioenergetics [29].

The Rodent Model of Limb Fracture

The increase in platelet mitochondrial basal respiration in an acute experimental pathology was recently recapitulated in a rodent model of hindlimb fracture by the group of Matthew Kutcher. These authors reported that intact platelets isolated 24 h after the injury were elicited in Wistar rats of both sexes, which displayed a higher basal oxygen consumption vs. the sham animals. Moreover, the increased basal platelet respiration correlated with the clot strength, assessed by citrated native thromboelastography in this preclinical model. The authors emphasised the role of mitochondrial respirometry in reflecting platelet health and the utility of this model in assessing the

effects of mitochondrial-targeted drugs as potential anti-thrombotic adjuvants [30]. The translational relevance of this study was demonstrated in an elegant case-control study performed by the group of Petra Hartmann. These authors reported a significantly decreased oxidative phosphorylation in platelets isolated from patients with severe trauma, which showed a strong positive correlation with the lower maximum clot firmness; they concluded that platelet mitochondrial dysfunction likely contributes to trauma-induced coagulopathy [31].

The Chronic Obstructive Pulmonary Disease Model

Mitochondrial pathophysiology represents an emerging research field with promising therapeutic opportunities, particularly in chronic diseases. Chronic obstructive pulmonary disease (COPD) is a significant global health concern, which is unequivocally associated with mitochondrial dysfunction occurring both in the lungs and blood cells [32]. The animal models of COPD provided insights into the cumulative effects of prolonged oxidative stress and systemic inflammation on platelet mitochondrial dysfunction. In line, Bialas *et al*. aimed to evaluate mitochondrial function in platelets isolated from animals with COPD-like lung lesions induced by chronic smoke exposure. To this aim, male Dunkin Hartley guinea pigs were chronically exposed to cigarette smoke (4 h/day, 5 days/week), and platelets were isolated and analysed for mitochondrial respiration. In platelets from the smoke-exposed animals, the results (interpreted after normalisation for the protein content) indicated lower routine respiration and a significantly reduced ETS capacity (the indicator of maximal respiration), the latter being suggestive of the inner mitochondrial membrane damage and impairment of the OXPHOS efficiency. These findings suggest that chronic smoke exposure also impairs platelet mitochondrial function besides lung injury, potentially contributing to COPD patho-physiology and serving as a putative therapeutic target [33]. This is particularly important since the lungs are nowadays known as a primary site of terminal platelet production, dynamically released into the pulmonary circulation by the megakaryocytes that migrated out of the bone marrow [34].

The Streptozocin-Induced Experimental Diabetes Model

In the setting of diabetes, prolonged exposure of platelets to hyperglycaemia accelerates mitochondrial metabolism and, as a result of higher substrate oxidation, increased oxygen consumption and mitochondrial membrane hyperpolarisation occur. These observations have been reported by the group of Cezary Watala in male Sprague-Dawley rats with long-term (5 months) type 1 diabetes mellitus (T1DM) induced by a single intraperitoneal injection of streptozotocin (STZ) as compared to age-matched non-diabetic animals. Indeed, these authors reported the increase of all respiratory parameters and the mitochondrial mass (assessed as the cytochrome oxidase content) as the hallmark of accelerated mitochondrial biogenesis and a higher density of respiratory complexes proteins *per* organelle that occurred as an adaptation of the blood platelets to an augmented availability of the energy substrates. Moreover, they reported that untreated diabetes leads to an increase in platelet mitochondrial membrane potential that is strongly correlated with platelet activation [35].

More recently, the same group further investigated the causal relationship between increased mitochondrial respiratory activity and platelet activation in rats with STZ-induced T1DM after 1, 2.5 and 5 months of untreated disease. They reported a significant increase of all respiratory parameters (ROUTINE respiration, LEAK respiration and ETS capacity) after 5 months (but not at 1 and 2.5 months) of *in vivo* exposure of blood platelets to high glucose. However, the increase in platelet mitochondrial respiration occurred after the changes in platelet activation and reactivity, thus indicating that elevated mitochondrial respiration may not be the primary driver of abnormal platelet function in diabetes but rather a secondary adaptation of platelet mitochondria to the heightened availability of energy substrates. They further hypothesised that lowering OXPHOS with metformin (a complex I inhibitor) will result in a reduction in platelet activation. While metformin treatment led to a slight reduction in OXPHOS and maximal oxidative capacity in diabetic platelets, it did not interfere with platelet reactivity; notably, even though metformin decreased mitochondrial respiration, it was unable to inhibit the *in vitro* platelet activation, typically seen in diabetic conditions. These findings suggest that the beneficial cardiovascular effects of metformin in diabetic patients may not be due to its impact on platelet mitochondrial function or activation. Instead, metformin appears to stabilise mitochondrial function without reducing the hyperactivity of diabetic platelets, indicating that its protective effects might involve other mechanisms outside of platelet respiration. Overall, this elegant study highlights the complex role of platelet respiration in diabetes, where mitochondrial changes seem to be a later adaptation rather than a direct cause of abnormal platelet function [36].

More recently, the group of Mathew Neal reported an increased platelet basal respiration after 3 months of exposure to high glucose in mice with untreated STZ-induced diabetes. Acute administration of metformin in high doses (200 mg/kg twice daily *via* oral gavage for 7 days at the end of the 3 months of diabetes) decreased the susceptibility of the diabetic mice to occlusive arterial thrombosis but had no effect on platelet respiration. Interestingly, the drug elicited an unexpected increase in platelet mitochondrial maximal respiration and spare respiratory capacity in the healthy (non-diabetic) group of mice [37].

Examples of Knock-Out Animal Models Relevant for Platelet Function

In the setting of diabetes, hyperglycaemia and insulin resistance are responsible for the platelet glycolytic phenotype that supports platelet activation and favours a prothrombotic state [38]. In an elegant study, the group of Dale Abel investigated whether hyperglycaemia impacts on mitochondrial metabolism, platelet activation and prothrombotic risk in the murine model of T1DM induced by STZ. They demonstrated that glucose uptake and glycolysis were increased in platelets isolated from diabetic mice and correlated with an increased expression of the glucose transporter GLUT3 protein. Furthermore, these platelets exhibited increased *ex vivo* activation, and the diabetic mice were prone to *in vivo* thrombosis when subjected to collagen/epinephrine-induced pulmonary embolism. These authors generated a platelet-specific double-knockout (DKO) mouse for both glucose transporters GLUT1 and GLUT3 (thus preventing glucose uptake by the platelets) and further showed that hyperglycaemia-related increase in platelet function/activation and also thrombosis were abolished when glucose utilisation was blocked in the DKO animals. They speculated that inhibition of platelet glucose metabolism in diabetic patients could alleviate platelet activation, thus reducing the increased risk of thrombosis [39].

Mitochondria are dynamic organelles that are interconnected in networks regulated by a balance between two major processes: fusion and fission. Mitofusins 1 and 2 are proteins associated with the outer mitochondrial membrane that promote mitochondrial fusion in order to preserve mitochondrial integrity and function. Jacob *et al*. investigated, in a multicentric study, the role of *mitofusin-2 (Mfn2)* in regulating platelet function. To this aim, these authors generated a *Mfn2* conditional knockout mouse model with megakaryocyte/platelet deletion of *Mfn2* (*Mfn2-/-*). They reported that Mfn2 contributes to platelet lifespan and regulates mitochondrial function in platelets. Platelet counts and platelet life span were reduced in *Mfn2-/-* mice, while platelet complex I activity was decreased (possibly due to an assembly defect). The absence of *Mfn2* decreased platelet mitochondrial respiration but did not interfere with glycolysis. Platelets isolated from *Mfn2−/−* mice had lower basal and maximal oxygen consumption rates and lower ATP-linked respiration than *Mfn2+/+* platelets when unstimulated. Stimulation with thrombin increased the oxygen consumption rate in *Mfn2+/+* platelets, while in *Mfn2−/−* platelets were significantly blunted. Also, maximal and ATP-linked respiration remained significantly reduced in *Mfn2−/− vs Mfn2+/+* platelets after thrombin stimulation. These findings uncover a role for Mfn2 in shaping platelet function and number, which might be relevant for the genome-wide association studies that have reported the occurrence of variants of *Mfn2* in relation to a decreased platelet count [40].

All these experimental data provided the scientific and methodological background that paved the way towards the characterisation of mitochondrial respiration of human blood cells in healthy and non-healthy populations, in line with the concept of translational research in the field of bioenergetic health [41].

More recent, circulating platelets have been increasingly used as a tool for integrating bioenergetics and metabolomic studies for the analysis of mitochondrial function, including susceptibility to toxicity in the emerging field of precision medicine [42].

Moreover, the evolving high-throughput techniques for drug safety screening have increasingly used isolated mitochondria, various cell lines and, more recently, human blood cells [43], and engineered tissues [44], approaches that ultimately reduced the number of laboratory animals used in experimental studies, leading to improved animal welfare and decreased costs [45]. In Table I, there are presented a summary of experimental studies on platelet mitochondrial respiration.

Assessing mitochondrial toxicity in platelets

In the past two decades, drug safety has become a priority of the pharmaceutical industry [46-48], as witnessed by the literature abounding with reviews covering the topic of drug-induced mitochondrial toxicity [18, 49-54]. Drug-induced mitochondrial toxicity has been experimentally linked to organ damage in several organs, such as the liver, skeletal muscles, kidneys, pancreatic beta cells, central nervous system, and the heart. Among the several classes of drugs that have been studied in various cell lines and isolated mitochondria, the following have been reported to elicit various degrees of mitochondrial toxicity in the above-mentioned organs: cholesterol-lowering drugs (*e.g.*, statins), anti-diabetic drugs (*e.g.*, biguanides, thiazolidinediones), non-steroidal anti-inflammatory drugs (*e.g.*, acetaminophen, naproxen), some antibiotics (*e.g.*, macrolides), anti-depressants (*e.g.*, serotonin receptor antagonists and reuptake inhibitors), anti-cancer drugs (*e.g.*, anthracyclines), and diuretics (*e.g.*, furosemide). Important, within each class of these drugs, there are compounds that displayed more severe mitochondrial toxicity as compared to others; one classical example belonging to the antidiabetics class is phenformin, which was discontinued due to the high risk of lactic acidosis, while metformin has remained for more than half a century the cornerstone therapy for type 2 diabetes [18].

Table I

Summary of Animal Studies on Platelet Mitochondrial Respiration

Animal	Physiological/ Pathophysiological model	Assay	Relevant findings	Ref.
Ageing male and female Fischer 344 rats (assessed at 6, 12 and 24 months)	Age and Gender	HRR	Increased routine respiration between the ages of 6 and 12 months in both sexes remained higher in 24-month-old female rats; Increased capacity of the ETS in males than females at the age of 12 months	[20]
Pigs	Mitochondrial complex I inhibition through rotenone infusion	HRR	The trend towards mitochondrial respiration inhibition	[28]
Piglets	Asphyxia-associated ventricular fibrillation cardiac arrest	HRR	Increased maximal oxidative phosphorylation ($OXPHOS_{CI+CII}$); Increased maximal respiratory capacity (ETS_{CI+CII}); Increased succinate-supported respiration through Complex II ($OXPHOS_{CII}$ and ETS_{CII})	[29]
Wistar rats of both sexes	Bilateral hindlimb orthopaedic injury	HRR	Increased basal oxygen consumption	[30]
Male Dunkin Hartley guinea pigs	Chronic smoke exposure resulting in COPD-like lung lesions	HRR	Significantly decreased ETS capacity and slightly lower routine respiration	[33]
Male Sprague-Dawley rats	Streptozocin-induced diabetes	HRR	Increased values of all respiratory parameters	[35]
Male Sprague-Dawley rats	Streptozocin-induced diabetes	HRR	Significant increase of all respiratory parameters (ROUTINE respiration, LEAK respiration and ETS capacity) at 5 months	[36]
Mice	Streptozocin-induced diabetes	XFA	Increased platelet basal respiration after 3 months	[37]
Mfn2 conditional knockout mouse model with platelet-specific deletion		XFA	Decreased basal and maximal oxygen consumption rate and lower ATP-linked respiration	[40]

Abbreviations: ATP, adenosine triphosphate; CI, complex I; CII, complex II; ETS, electron transport system; HRR, high-resolution respirometry; OXPHOS, oxidative phosphorylation; XFA, extracellular flux assay.

Drugs can inhibit mitochondrial function *via* direct and indirect mechanisms. Direct mechanisms include inhibition of ETS protein complexes (including the ATP synthase), uncoupling the ETS from ATP synthase (and thus decreasing ATP generation), inhibition of enzymes of the citric acid cycle, inhibition of the mitochondrial transcription and/or translation of complexes/enzymes, inhibition of various mitochondrial transporters or channels. Indirect mechanisms include the damage of all mitochondrial components, e.g., ETS complexes, metabolic enzymes, channels/transporters, and mitochondrial DNA *via* increased ROS production and/or decreased endogenous antioxidant systems. The plethora of studies that have reported drug-related mitochondrial toxicity in the above-mentioned organs and in various experimental settings have been thoroughly reviewed in references [18, 55-57].

Platelets have also been, more recently, used to assess the toxicity of various drugs and compounds. As such, the group of Eskil Elmer first characterized the mitochondrial toxicity elicited by acute *in vitro* exposure of human platelets (and also of human primary hepatocytes) to a high concentration of acetaminophen. Piel *et al*. reported the inhibition of complex I- (but not of complex II) supported mitochondrial respiration, which was rescued by NV241, a cell-permeable succinate prodrug [58]. Cell-permeable succinate prodrugs are compounds that, once delivered to the intracellular space, readily diffuse through the mitochondrial membrane (independent of active transporters) and release succinate, the complex II substrate, thus supporting ATP synthesis *via* the OXPHOS. More recently, our group reported that acetaminophen and ibuprofen elicited a concentration-dependent decrease in oxygen consumption in isolated intact and permeabilised human platelets by inhibiting complex I-linked respiration. Betiu *et al*. also found that ibuprofen significantly decreased the maximal ETS capacity even at the lowest concentration applied [59].

In a collaborative work with the group of Eskil Elmer, we have also assessed the *in vitro* concentration-dependent effects of three statins on platelet mitochondrial respiration and reported that simvastatin and atorvastatin (but surprisingly, not cerivastatin, withdrawn from the market due to muscle toxicity) directly inhibited complex I-supported respiration; in the highest concentration tested, simvastatin also inhibited complex II-linked respiration. Also,

atorvastatin and cerivastatin (but not simvastatin) increased the non-ATP-generating respiration, thus limiting the OXPHOS coupling efficiency. Avram *et al*. further reported that NV118, a cell-permeable succinate prodrug, bypassed the statin-induced mitochondrial dysfunction in atorvastatin/ cerivastatin-exposed platelets and recovered the coupled respiration [60]. Of note, NV118 has been previously reported to bypass the complex I-inhibition caused by acute exposure of human platelets to high doses of metformin and increased mitochondrial respiration (that was linked to phosphorylation by ATP-synthase) [61].

More recently, Bețiu *et al*. reported that amiodarone and its metabolite, desethylamiodarone (DEA), dose-dependently impaired mitochondrial respiration in both intact and permeabilised platelets *via* the inhibition of both complex I and II-supported respiration. This inhibitory effect was recapitulated in human mononuclear cells, where amiodarone also elicited a severe concentration-dependent ATP depletion (that cannot be solely explained by the inhibition of mitochondrial respiration). Furthermore, we showed that NV118, the cell-permeable succinate prodrug, counteracted the drug-induced acute mitochondrial respiratory dysfunction [62].

Last but not least, platelets can be used to dissect the mechanisms of mitochondrial toxicity elicited by various toxic compounds. As such, Janowska *et al*. assessed the effects on mitochondrial respiration of N-succinimidyl N-methylcarbamate (NSNM), a surrogate for carbamate insecticide analogue, by measuring the cellular oxygen consumption in intact and permeabilised human platelets. In intact cells, lactate production was also measured after acute exposure to NV118 or its vehicle (DMSO) in order to pharmacologically overcome the acute bioenergetic failure. The authors reported a concentration-dependent decrease in mitochondrial oxygen consumption linked to complex I (but not to complex II). Lactate production was increased in intact platelets due to the compensatory increase in anaerobic glycolysis. Acute incubation, with the cell-permeable succinate prodrug, restored the NSNM-related decrease in mitochondrial respiration and normalised lactate production, highlighting the potential of targeting mitochondria in treating carbamate-induced toxicity [63]. Table II provides a summary of studies investigating mitochondrial toxicity in platelets.

Table II

Summary of Studies Reporting Mitochondrial Toxicity in Platelets

Drug/Compound	Assay	Relevant findings	Ref.
Acetaminophen	XFA	Inhibition of complex I (but not of complex II) - supported respiration	[58]
Acetaminophen	HRR	Decreased oxygen consumption in both intact and permeabilised platelets, mainly by inhibiting CI-supported active respiration	[59]
Ibuprofen	HRR	Decreased oxygen consumption in both intact and permeabilised platelets, mainly by inhibiting CI-supported active respiration Decreased maximal ETS capacity	[59]
Statins	HRR	Inhibited complex I-supported respiration (simvastatin and atorvastatin) Inhibited complex II-linked respiration (simvastatin in the highest concentration tested) Increased non-ATP generating respiration, limiting the OXPHOS coupling efficiency (atorvastatin and cerivastatin)	[60]
Amiodarone and DEA	HRR	Dose-dependently impaired mitochondrial respiration in intact and permeabilised platelets *via* the inhibition of both complex I and II-supported respiration	[62]
NSNM	HRR	Concentration-dependent decrease in mitochondrial oxygen consumption linked to complex I (but not to complex II)	[63]
CORM-A1	XFA	Inhibited mitochondrial respiration	[65]

Abbreviations: CORM, carbon monoxide-releasing molecule; DEA, desethylamiodarone; ETS, electron transport system; HRR, high-resolution respirometry; NSNM, N-succinimidyl N-methylcarbamate; OXPHOS, oxidative phosphorylation; XFA, extracellular flux assay

Carbon monoxide (CO), a by-product of heme catabolism, when released in high amounts, has been reported to elicit mitochondrial toxicity *via* binding to the ETS complex IV (cytochrome c oxidase) with subsequent inhibition of mitochondrial respiration. However, low concentrations of endogenous or exogenous CO exert pleiotropic cytoprotective effects, an observation that led to the development of carbon monoxide-releasing molecules (CORMs), prodrugs able to deliver CO in a controlled manner [64]. Kaczara *et al*. performed a comprehensive study aimed at unveiling the effects of a carbon monoxide-releasing molecule, CORM-A1, on human platelet bioenergetics and aggregation. They reported that the anti-aggregation effect of CO is mediated by inhibiting both ATP-generating processes available in platelets, the mitochondrial respiration (at the level of cytochrome c oxidase), and glycolysis (*via* the depletion of cytosolic NAD+) [65].

We have to acknowledge the limitation to extrapolating the results of the *in vitro* assays of cytotoxicity to the complex situation of the ageing population with multimorbidities and polypharmacy, plus persistent environmental exposure to various pollutants. However, combining novel high-throughput methods of assessing mitochondrial toxicity in human samples with epidemiologic studies will clearly accelerate in the future our understanding of this field [66, 67].

Conclusions

In summary, the assessment of platelet bioenergetics/ mitochondrial respiration has emerged as a minimally invasive method that offers valuable insights into the alterations of the systemic metabolic status from acute and chronic diseases while also highlighting potential therapeutic avenues for intervention. Animal models have significantly contributed to understanding the cellular and molecular mechanisms underpinning mitochondrial dysfunction, paving the way for human studies to further elucidate the disorder-related disruption in platelet bioenergetics. Last but not least, platelets can be used in studies assessing mitochondrial toxicity elicited by drugs or various chemical compounds, as well as for evaluating the capability of mitochondria-targeted compounds to counteract it.

Conflict of interest

The authors declare no conflict of interest.

References

1. Montague SJ, Lim YJ, Lee WM, Gardiner EE, Imaging platelet processes and function - Current and emerging approaches for imaging *in vitro* and *in vivo*. *Front Immunol.*, 2020; 11: 78.
2. Asada Y, Yamashita A, Sato Y, Hatakeyama K, Pathophysiology of atherothrombosis: Mechanisms of thrombus formation on disrupted atherosclerotic plaques. *Pathol Int.*, 2020; 70(6): 309-322.
3. Scherlinger M, Richez C, Tsokos GC, Boilard E, Blanco P, The role of platelets in immune-mediated inflammatory diseases. *Nat Rev Immunol.*, 2023; 23(8): 495-510.
4. Zhou L, Zhang Z, Tian Y, Li Z, Liu Z, Zhu S, The critical role of platelets in cancer progression and metastasis. *Eur J Med Res.*, 2023; 28(1): 385.
5. Garcia-Leon MJ, Liboni C, Mittelheisser V, Bochler L, Follain G, Mouriaux C, Busnelli I, Larnicol A, Colin F, Peralta M, Osmani N, Gensbittel V, Bourdon C, Samaniego R, Pichot A, Paul N, Molitor A, Carapito R, Jandrot-Perrus M, Lefebvre O, Mangin PH, Goetz JG, Platelets favour the outgrowth of established metastases. *Nat Commun.*, 2024; 15(1): 3297.
6. Maouia A, Rebetz J, Kapur R, Semple JW, The immune nature of platelets revisited. *Transfus Med Rev.*, 2020; 34(4): 209-220.
7. San-Millán I, The key role of mitochondrial function in health and disease. *Antioxidants*, 2023; 12(4): 782.
8. Rocca C, Soda T, De Francesco EM, Fiorillo M, Moccia F, Viglietto G, Angelone T, Amodio N, Mitochondrial dysfunction at the crossroad of cardiovascular diseases and cancer. *J Transl Med.*, 2023; 21(1): 635.
9. Macdonald R, Barnes K, Hastings C, Mortiboys H, Mitochondrial abnormalities in Parkinson's disease and Alzheimer's disease: Can mitochondria be targeted therapeutically? *Biochem Soc Trans.*, 2018; 46(4): 891-909.
10. Harrington JS, Ryter SW, Plataki M, Price DR, Choi AMK, Mitochondria in health, disease, and aging. *Physiol Rev.*, 2023; 103(4): 2349-2422.
11. Jacobs HT, A century of mitochondrial research, 1922-2022. *Enzymes*, 2023; 54: 37-70.
12. Kramer PA, Ravi S, Chacko B, Johnson MS, Darley-Usmar VM, A review of the mitochondrial and glycolytic metabolism in human platelets and leukocytes: Implications for their use as bioenergetic biomarkers. *Redox Biol.*, 2014; 2: 206-210.
13. George MJ, Bynum J, Nair P, Cap AP, Wade CE, Cox CS Jr, Gill BS, Platelet biomechanics, platelet bioenergetics, and applications to clinical practice and translational research. *Platelets*, 2018; 29(5): 431-439.
14. Aibibula M, Naseem KM, Sturmey RG, Glucose metabolism and metabolic flexibility in blood platelets. *J Thromb Haemost.*, 2018; 16(11): 2300-2314.
15. Mukherjee P, Roy S, Ghosh D, Nandi SK, Role of animal models in biomedical research: A review. *Lab Anim Res.*, 2022; 38(1): 18.
16. Marshall LJ, Bailey J, Cassotta M, Herrmann K, Pistollato F, Poor translatability of biomedical research using animals—A narrative review. *Altern Lab Anim.*, 2023; 51(2): 102-135.
17. Tang X, Wang Z, Hu S, Zhou B, Assessing drug-induced mitochondrial toxicity in cardiomyocytes: Implications for preclinical cardiac safety evaluation. *Pharmaceutics*, 2022; 14(7): 1313.
18. Will Y, Shields JE, Wallace KB, Drug-induced mitochondrial toxicity in the geriatric population: Challenges and future directions. *Biology (Basel)*, 2019; 8(2): 32..
19. Domínguez-Oliva A, Hernández-Ávalos I, Martínez-Burnes J, Olmos-Hernández A, Verduzco-Mendoza A, Mota-Rojas D, The importance of animal models in biomedical research: Current insights and applications. *Animals (Basel)*, 2023; 13(7): 1223.
20. Jedlička J, Tůma Z, Razak K, Kunc R, Kala A, Proskauer Pena S, Lerchner T, Ježek K, Kuncová J, Impact of aging on mitochondrial respiration in various organs. *Physiol Res.*, 2022; 71(S2): S227-S236.
21. Jedlička J, Kunc R, Kuncová J, Mitochondrial respiration of human platelets in young adult and advanced age - Seahorse or O2k? *Physiol Res.*, 2021; 70(S3): S369-S379.
22. Tyrrell DJ, Bharadwaj MS, Jorgensen MJ, Register TC, Molina AJ, Blood cell respirometry is associated with skeletal and cardiac muscle bioenergetics:

Implications for a minimally invasive biomarker of mitochondrial health. *Redox Biol.*, 2016; 10: 65-77.
23. Tyrrell DJ, Bharadwaj MS, Jorgensen MJ, Register TC, Shively C, Andrews RN, Neth B, Keene CD, Mintz A, Craft S, Molina AJA, Blood-based bioenergetic profiling reflects differences in brain bioenergetics and metabolism. *Oxid Med Cell Longev.*, 2017; 2017: 7317251.
24. Distelmaier F, Koopman WJH, van den Heuvel LP, Rodenburg RJ, Mayatepek E, Willems PHGM, Smeitink JAM, Mitochondrial complex I deficiency: From organelle dysfunction to clinical disease. *Brain.*, 2009; 132(4): 833-842.
25. Rodenburg RJ, Mitochondrial complex I-linked disease. *Biochim Biophys Acta.*, 2016; 1857(7): 938-945.
26. Henrich MT, Oertel WH, Surmeier DJ, Geibl FF, Mitochondrial dysfunction in Parkinson's disease – A key disease hallmark with therapeutic potential. *Mol Neurodegener.*, 2023; 18(1): 83.
27. Forte M, Palmerio S, Bianchi F, Volpe M, Rubattu S, Mitochondrial complex I deficiency and cardiovascular diseases: Current evidence and future directions. *J Mol Med (Berl).*, 2019; 97(5): 579-591.
28. Karlsson M, Ehinger JK, Piel S, Sjövall F, Henriksnäs J, Höglund U, Hansson MJ, Elmér E, Changes in energy metabolism due to acute rotenone-induced mitochondrial complex I dysfunction - An *in vivo* large animal model. *Mitochondrion.*, 2016; 31: 56-62.
29. Ferguson MA, Sutton RM, Karlsson M, Sjövall F, Becker LB, Berg RA, Margulies SS, Kilbaugh TJ, Increased platelet mitochondrial respiration after cardiac arrest and resuscitation as a potential peripheral biosignature of cerebral bioenergetic dysfunction. *J Bioenerg Biomembr.*, 2016; 48(3): 269-279.
30. Littlejohn JB, Grenn EE, Carter KT, Palei AC, Spradley FT, Hosler JP, Hoang NH, Edwards KS, Kutcher ME, Increased platelet mitochondrial function correlates with clot strength in a rodent fracture model. *J Trauma Acute Care Surg.*, 2024; 96(3): 378-385.
31. Sándor L, Donka T, Baráth B, Jávor P, Jász DK, Perényi D, Babik B, Varga E, Török L, Hartmann P, Mitochondrial dysfunction in platelets from severe trauma patients - A prospective case-control study. *Injury.*, 2024; 55(Suppl 3): 111481.
32. Riou M, Alfatni A, Charles AL, Andrès E, Pistea C, Charloux A, Geny B, New insights into the implication of mitochondrial dysfunction in tissue, peripheral blood mononuclear cells, and platelets during lung diseases. *J Clin Med.*, 2020; 9(5): 1253.
33. Bialas AJ, Siewiera K, Watala C, Rybicka A, Grobelski B, Kosmider L, Kurek J, Milkowska-Dymanowska J, Piotrowski WJ, Gorski P, Mitochondrial functioning abnormalities observed in blood platelets of chronic smoke-exposed guinea pigs - A pilot study. *Int J Chron Obstruct Pulmon Dis.*, 2018; 13: 3707-3717.
34. Lefrançais E, Ortiz-Muñoz G, Caudrillier A, Mallavia B, Liu F, Sayah DM, Thornton EE, Headley MB, David T, Coughlin SR, Krummel MF, Leavitt AD, Passegué E, Looney MR, The lung is a site of platelet biogenesis and a reservoir for haematopoietic progenitors. *Nature*, 2017; 544(7648): 105-109.
35. Siewiera K, Kassassir H, Talar M, Wieteska L, Watala C, Higher mitochondrial potential and elevated mitochondrial respiration are associated with excessive activation of blood platelets in diabetic rats. *Life Sci.*, 2016; 148: 293-304.
36. Siewiera K, Labieniec-Watala M, Kassassir H, Wolska N, Polak D, Watala C, Potential role of mitochondria as modulators of blood platelet activation and reactivity in diabetes and effect of metformin on blood platelet bioenergetics and platelet activation. *Int J Mol Sci.*, 2022; 23(7): 3666.
37. Alvidrez RIM, Annarapu GK, Srinivasan AJ, Liu Z, Yazdani HO, Nolfi-Donegan D, Simmons RL, Shiva S, Neal MD, High dose of metformin decreases susceptibility to occlusive arterial thrombosis in diabetic mice. *J Pharm Pharmacol Res.*, 2023; 7(4): 192-202.
38. Li X, Weber NC, Cohn DM, Hollmann MW, DeVries JH, Hermanides J, Preckel B, Effects of hyperglycemia and diabetes mellitus on coagulation and hemostasis. *J Clin Med.*, 2021; 10(11): 2419.
39. Fidler TP, Marti A, Gerth K, Middleton EA, Campbell RA, Rondina MT, Weyrich AS, Abel ED, Glucose metabolism is required for platelet hyperactivation in a murine model of type 1 diabetes. *Diabetes*, 2019; 68(5): 932-938.
40. Jacob S, Kosaka Y, Bhatlekar S, Denorme F, Benzon H, Moody A, Moody V, Tugolukova EA, Hull G, Kishimoto N, Manne BK, Guo L, Souvenir R, Seliger BJ, Eustes AS, Hoerger K, Tolley ND, Fatahian AN, Boudina S, Christiani DC, Wei Y, Ju C, Campbell RA, Rondina MT, Abel ED, Bray PF, Weyrich AS, Rowley JW, Mitofusin-2 regulates platelet mitochondria and function. *Circ Res.*, 2024; 134(2): 143-161.
41. Braganza A, Annarapu GK, Shiva S, Blood-based bioenergetics: An emerging translational and clinical tool. *Mol Aspects Med.*, 2020; 71: 100835.
42. Chacko BK, Smith MR, Johnson MS, Benavides G, Culp ML, Pilli J, Shiva S, Uppal K, Go YM, Jones DP, Darley-Usmar VM, Mitochondria in precision medicine; linking bioenergetics and metabolomics in platelets. *Redox Biol.*, 2019; 22: 101165.
43. Hubens WHG, Vallbona-Garcia A, de Coo IFM, van Tienen FHJ, Webers CAB, Smeets HJM, Gorgels T, Blood biomarkers for assessment of mitochondrial dysfunction: An expert review. *Mitochondrion*, 2022; 62: 187-204.
44. Davis BN, Santoso JW, Walker MJ, Cheng CS, Koves TR, Kraus WE, Truskey GA, Human, tissue-engineered, skeletal muscle myobundles to measure oxygen uptake and assess mitochondrial toxicity. *Tissue Eng Part C Methods.*, 2017; 23(4): 189-199.
45. Pereira SP, Pereira GC, Moreno AJ, Oliveira PJ, Can drug safety be predicted and animal experiments reduced by using isolated mitochondrial fractions? *Altern Lab Anim.*, 2009; 37(4): 355-365.
46. Nadanaciva S, Will Y, Investigating mitochondrial dysfunction to increase drug safety in the pharmaceutical industry. *Curr Drug Targets.*, 2011; 12(6): 774-782.

47. Rana P, Aleo MD, Wen X, Kogut S, Hepatotoxicity reports in the FDA adverse event reporting system database: A comparison of drugs that cause injury via mitochondrial or other mechanisms. *Acta Pharm Sin B.*, 2021; 11(12): 3857-3868.
48. Will Y, Dykens J, Mitochondrial toxicity assessment in industry—a decade of technology development and insight. *Expert Opin Drug Metab Toxicol.*, 2014; 10(8): 1061-1067.
49. Pereira CV, Moreira AC, Pereira SP, Machado NG, Carvalho FS, Sardão VA, Oliveira PJ, Investigating drug-induced mitochondrial toxicity: A biosensor to increase drug safety? *Curr Drug Saf.*, 2009; 4(1): 34-54.
50. Wallace KB, Multiple targets for drug-induced mitochondrial toxicity. *Curr Med Chem.*, 2015; 22(20): 2488-2492.
51. Nadanaciva S, Will Y, New insights in drug-induced mitochondrial toxicity. *Curr Pharm Des.*, 2011; 17(20): 2100-2112.
52. Wills LP, The use of high-throughput screening techniques to evaluate mitochondrial toxicity. *Toxicology.*, 2017; 391: 34-41.
53. Lin YT, Lin KH, Huang CJ, Wei AC, MitoTox: A comprehensive mitochondrial toxicity database. *BMC Bioinformatics.*, 2021; 22(10): 369.
54. Dykens JA, Will Y, The significance of mitochondrial toxicity testing in drug development. *Drug Discov Today.*, 2007; 12(17-18): 777-785.
55. Olszewska A, Szewczyk A, Mitochondria as a pharmacological target: Magnum overview. *IUBMB Life.*, 2013; 65(3): 273-281.
56. Kuretu A, Arineitwe C, Mothibe M, Ngubane P, Khathi A, Sibiya N, Drug-induced mitochondrial toxicity: Risks of developing glucose handling impairments. *Front Endocrinol (Lausanne).*, 2023; 14: 1123928.
57. Bețiu AM, Noveanu L, Hâncu IM, Lascu A, Petrescu L, Maack C, Elmér E, Muntean DM, Mitochondrial effects of common cardiovascular medications: The good, the bad, and the mixed. *Int J Mol Sci.*, 2022; 23(21): 13653.
58. Piel S, Chamkha I, Dehlin AK, Ehinger JK, Sjövall F, Elmér E, Hansson MJ, Cell-permeable succinate prodrugs rescue mitochondrial respiration in cellular models of acute acetaminophen overdose. *PLoS One.*, 2020; 15(4): e0231173.
59. Bețiu AM, Lighezan R, Avram VF, Muntean DM, Elmér E, Petrescu L, Dose-dependent effects of acetaminophen and ibuprofen on mitochondrial respiration of human platelets. *Mol Cell Biochem.*, 2024; 479(6): 1501-1512.
60. Avram VF, Chamkha I, Åsander-Frostner E, Ehinger JK, Timar RZ, Hansson MJ, Muntean DM, Elmér E, Cell-permeable succinate rescues mitochondrial respiration in cellular models of statin toxicity. *Int J Mol Sci.*, 2021; 22(1): 424.
61. Piel S, Ehinger JK, Chamkha I, Frostner E, Sjövall F, Elmér E, Hansson MJ, Bioenergetic bypass using cell-permeable succinate, but not methylene blue, attenuates metformin-induced lactate production. *Intensive Care Med Exp.*, 2018; 6(1): 22.
62. Bețiu AM, Chamkha I, Gustafsson E, Meijer E, Avram VF, Åsander Frostner E, Ehinger JK, Petrescu L, Muntean DM, Elmér E, Cell-permeable succinate rescues mitochondrial respiration in cellular models of amiodarone toxicity. *Int J Mol Sci.*, 2021; 22(21): 11786.
63. Janowska JI, Piel S, Saliba N, Kim CD, Jang DH, Karlsson M, Kilbaugh TJ, Ehinger JK, Mitochondrial respiratory chain complex I dysfunction induced by *N-methyl carbamate* ex vivo can be alleviated with a cell-permeable succinate prodrug. *Toxicol In Vitro*, 2020; 65: 104794.
64. Cardoso-Pires C, Vieira HLA, Carbon monoxide and mitochondria: Cell energy and fate control. *Biochim Biophys Acta Mol Basis Dis.*, 2024; 1870(7): 167446.
65. Kaczara P, Sitek B, Przyborowski K, Kurpinska A, Kus K, Stojak M, Chlopicki S, Antiplatelet effect of carbon monoxide is mediated by NAD(+) and ATP depletion. *Arterioscler Thromb Vasc Biol.*, 2020; 40(10): 2376-2390.
66. Dascalu AM, Ilie MI, Serban D, Bratu DG, Smarandache AM, Trotea T, Dumitrescu D, Tudor C, Stana D, Costea DO, Tribus LC, Bobirca FT, Faur M, Comandasu M, Vancea G, Polypharmacy in geriatric patients undergoing surgery - strategies to reduce the risk of iatrogenic events. *Farmacia*, 2023; 71(3): 463-470.
67. Meyer JN, Hartman JH, Mello DF, Mitochondrial toxicity. *Toxicol Sci.*, 2018; 162(1): 15-23.