Atypical antipsychotics and oxidative cardiotoxicity: review of literature and future perspectives to prevent sudden cardiac death

Stefano D’Errico1, Raffaele La Russa23, Aniello Maiese34, Alessandro Santurro5, Matteo Scopetti5, Silvia Romano5, Martina Zanon1, Paola Frati35, Vittorio Fineschi3,5,✉

1. Department of Medical, Surgical and Health Sciences, University of Trieste, Trieste, Italy; 2. Department of Clinical and Experimental Medicine, University of Foggia, Foggia, Italy; 3. IRCSS Neuromed Mediterranean Neurological Institute, Pozzilli, Italy; 4. Department of Surgical Pathology, Medical, Molecular and Critical Area, University of Pisa, Pisa, Italy; 5. Department of Anatomical, Histological, Forensic and Orthopaedic Sciences, Sapienza University of Rome, Rome, Italy
✉Correspondence to: vittorio.fineschi@uniroma1.it
https://doi.org/10.11909/j.issn.1671-5411.2021.08.002

ABSTRACT Oxidative stress is considered the principal mediator of myocardial injury under pathological conditions. It is well known that reactive oxygen (ROS) or nitrogen species (RNS) are involved in myocardial injury and repair at the same time and that cellular damage is generally due to an unbalance between generation and elimination of the free radicals due to an inadequate mechanism of antioxidant defense or to an increase in ROS and RNS. Major adverse cardiovascular events are often associated with drugs with associated findings such as fibrosis or inflammation of the myocardium. Despite efforts in the preclinical phase of the development of drugs, cardiotoxicity still remains a great concern. Cardiac toxicity due to second-generation antipsychotics (clozapine, olanzapine, quetiapine) has been observed in preclinical studies and described in patients affected with mental disorders. A role of oxidative stress has been hypothesized but more evidence is needed to confirm a causal relationship. A better knowledge of cardiotoxicity mechanisms should address in the future to establish the right dose and length of treatment without impacting the physical health of the patients.

Second-generation (atypical) antipsychotics are used in the treatment of mental disorders (schizophrenia, bipolar disorder, major depressive disorder). Since marketed, clozapine was widely used because of its efficacy in drug-resistant schizophrenia and freedom from extrapyramidal effects. After clozapine, other antipsychotics were introduced (olanzapine, quetiapine, risperidone) with similar effects and the same safer profile and soon became the mainstay of the treatment of schizophrenia.[1] The therapeutic effect of second-generation antipsychotic agents is related to dopaminergic D2 receptor antagonism and to the blockade of serotonin receptors. Major cardiovascular adverse effects (tachycardia, bradycardia, hypertension, hypotension, syncopal episodes) and electrocardiographic abnormalities (prolonged QT interval) are reported in patients suffering from mental disorders and treated with antipsychotics.[2–8] An increased risk of sudden death has been also reported but the risk of underreporting is concrete because of the lack of a systematic post-mortem examination.[9–13] In addition, individuals with schizophrenia are known to be at greater risk of cardiac death, in part linked to inadequate lifestyles that predispose to cardiovascular disease, in part due to poor compliance with health care.[14–16] However, many typical and atypical antipsychotic drugs have been reported in the literature to significantly increase the risk of sudden cardiac death in patients with psychiatric disorders,[17] and this has led to restrictions in clinical practice or the withdrawal of these molecules from the market. A retrospective cohort study showed that the incidence of sudden cardiac death in subjects taking antipsychotics is increased (dose-related increase) compared to non-users of antipsychotics, regardless of the pharmacological class.[18] Sudden antipsychotic cardiac death appears to be
linked to arrhythmic mechanisms, dilated heart disease, and myocarditis.\cite{19-21} In the pathogenesis of the aforementioned cardiological alterations, an involvement of oxidative stress has been suggested, with an increase in reactive oxygen (ROS).\cite{22} ROS modulates multiple cellular signaling pathways in physiological conditions. However, when the production of intracellular ROS is excessive it causes damage to the molecular components of the cell, favoring the pathogenesis of various diseases with particular reference to cardiovascular ones.

Despite the large use, factors underlying cardiovascular disease in patients treated with antipsychotics are still far to be completely understood and need to be deeply studied.\cite{21-27} Growing interest is aimed at understanding the contribution of antipsychotic therapy in the genesis of cardiac toxicity in schizophrenic patients.

The purpose of the present study is to review the scientific literature on the topic and propose a possible explanation of antipsychotics-related cardiotoxicity.

**LITERATURE ANALYSIS**

Relevant scientific articles were identified from PubMed, Cochrane Central, Scopus, Web of Science, Science Direct, EMBASE up to January 2020 using the following keywords: “atypical antipsychotics”, “cardiomyopathy”, “myocarditis”, “oxidative stress”, and “sudden cardiac death”. The main keywords were individually searched in association with each of the others.

The resulting 527 references were screened to exclude duplicates, which left 67 articles for further consideration. In addition, non-English papers were excluded and the following inclusion criteria were used: original research articles, reviews and mini-reviews, documents and guidelines promulgated by scientific societies and international organizations, and book chapters.

The papers not suitable for the review were excluded, a hand search was performed through the reference lists of the included articles. These publications were carefully evaluated considering the main aims of the review. An excel data extraction form was used to extract pivotal data, including publication year, first author, types of scientific articles, the topic of the study. This evaluation left 106 scientific papers, distributed as original research articles, reviews, and mini-reviews (Figure 1). These reports were published between 1981 and 2020.

The features of included papers were described in Tables 1–3. The table was organized by dividing the main topics of the present review into three subgroups: the relationship between heart disease and use of antipsychotic drugs, the relationship between...
| Authors            | Year | Types of study designs | Methods                                                                 | Aim of the study                                                                                      |
|--------------------|------|------------------------|------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------|
| Ariyarajah, et al. | 2010 | Observational study (Case series) | Tree cases series of patients with schizophrenia, treated with clozapine | Cardiac magnetic resonance diagnostic role in clozapine-induced myocarditis                           |
| Belhani, et al.    | 2006 | Preclinical study       | Eight groups of six New-Zealand White rabbits were treated for three months: group I: controls (saline); group II: amisulpride; group III: haloperidol; group IV: levomepromazine; group V: olanzapine; group VI: risperidone; group VII: levomepromazine + haloperidol; group VIII: levomepromazine + risperidone | Evaluate the hypothesis that myocardial lesions can be induced by neuroleptic drugs                   |
| Bellissima, et al. | 2018 | Review                 | Reviewed of 359 cases of clozapine-induced myocarditis through individual case reports, case series, summated case series, spontaneous pharmacovigilance reporting and reviews | Identify and prevent myocarditis associated with clozapine therapy                                     |
| Bugg, et al.       | 2016 | Observational study (Case report) | Case report of a schizophrenic patient treated with clozapine          | Description of a case of perimyocarditis and parenchymal lung disease caused by clozapine treatment   |
| Chow, et al.       | 2014 | Observational study (Multicentre cross-sectional cohort study) | 100 schizophrenic patients treated with clozapine, without a history of cardiac pathology; 21 schizophrenic patients (controls) treated with non-clozapine antipsychotics; 20 controls without schizophrenia | Evaluation of prevalence of subclinical cardiomyopathy in the three groups analysed                   |
| Coulter, et al.    | 2001 | Observational study     | WHO database on antipsychotics side-effects                            | Examine the relation between antipsychotic drugs and myocarditis and cardiomyopathy                   |
| Curto, et al.      | 2016 | Review                 | Reviewed of 88 articles about clozapine and cardiac disease            | Analyse the correlation between clozapine and cardiac disease                                        |
| De Berardis D, et al.| 2018 | Review                 | Reviewed of 93 articles about clozapine and myocarditis                | Analyse the correlation between clozapine and myocarditis                                            |
| Fineschi, et al.   | 2004 | Observational study (Case report) | Case concerns the sudden death of a 29-year-old male during clozapine therapy | Clozapine-induced hypersensitivity myocarditis                                                        |
| Gareri, et al.     | 2008 | Review                 | Current knowledge on clozapine in elderly patients                     | Study the safety of clozapine in elderly patients                                                    |
| Hussein, et al.    | 2015 | Observational study (Case-control) | 19 patients diagnosed with schizophrenia, schizoaffective, and bipolar disorder; 10 healthy volunteers | Investigate the effect of short-term (two months) treatment with atypical antipsychotics on coronary heart disease risk factors, in psychiatric patients |
| Jerrell, et al.    | 2010 | Observational study (Retrospective cohort design) | 2,231 schizophrenic adults assemmed atypical or conventional antipsychotic medications | Examine the risk of cerebro- and cardiovascular disorders associated with antipsychotic treatment among adults with schizophrenia |
| Katta, et al.      | 2016 | Observational study (Case report) | Case concerns the sudden death of a 54-year-old male during clozapine therapy | Clozapine-induced hypersensitivity myocarditis                                                        |
| Khan, et al.       | 2017 | Observational study (Cohort study) | 503 patients with treatment-resistant schizophrenia maintained on clozapine during the study (between January 2009 and December 2015) | Incidence of sudden death and time to myocarditis in patients treated with clozapine                  |
| Kontoxangelos, et al. | 2014 | Observational study (Case report) | Case report of a schizophrenic patient treated with clozapine          | Myocarditis is a recognized complication associated with clozapine                                   |
| Korkmaz, et al.    | 2019 | Observational study (Cross sectional study) | 40 schizophrenic adults treated with antipsychotics; 40 healthy volunteers | Scrutinize the changes that occur in left ventricle in adults treated with antipsychotics             |
| Lang, et al.       | 2008 | Observational study (Case report) | Case report of a 50-year-old man treated with clozapine                | Investigate treatment options after clozapine in treatment resistant schizophrenia                   |
| Lee, et al.        | 2003 | Observational study (Case report) | Case report of a 84-year-old man treated with olanzapine               | Description of a case of cardiac side effects caused by olanzapine treatment                          |
| Mackin, et al.     | 2008 | Review                 | A revision on common side effects associated with psychotropic drugs  | Description of the common effects of psychotropic drugs on the cardiovascular system                 |
| Marti, et al.      | 2005 | Observational study (Case report) | Case report of a 29-year-old man schizophrenic patient treated with risperidone | Description of a case of sudden cardiac death attributed to risperidone therapy                      |
| Merrill, et al.    | 2005 | Review                 | Review current literature on adverse cardiac events associated with clozapine | Study the association between clozapine and adverse cardiac events                                    |
| Authors                | Year  | Types of study designs   | Methods                                                                 | Aim of the study                                                                 |
|-----------------------|-------|--------------------------|--------------------------------------------------------------------------|--------------------------------------------------------------------------------|
| Papazisis, et al.     | 2012  | Observational study (Case report) | Case report of a 60-year-old man treated with quetiapine                  | Description of a case of sudden cardiac death attributed to quetiapine therapy |
| Patel, et al.         | 2019  | Review                   | Review current literature on life-threatening cardiovascular side effects associated with clozapine, edited until 2019 | Examine the proposed aetiology, diagnostic approaches and subsequent management strategies of cardiotoxicity associated with clozapine use |
| Peters, et al.        | 2014  | Observational study (Case report) | Case report of a 62-year-old woman treated suffering from bipolar affective disorder was treated with a mixture of olanzapine, amisulpride, biperiden, benperidol and lithium carbonate | Description of a case of tako tsubo cardiomyopathy in a patient treated with a mixture of olanzapine, amisulpride, biperiden, benperidol and lithium carbonate |
| Pillinger, et al.     | 2019  | Observational study (Case-control study) | 14 schizophrenic patients treated with antipsychotic; 17 controls | Role of antipsychotics in fibro-inflammatory myocardial process that contribute to the excess cardiovascular mortality associated with schizophrenia |
| Polcwiartek, et al.  | 2015  | Review                   | Examination of preclinical, clinical, and epidemiological studies on cardiac safety of aripiprazole | Assess aripiprazole’s cardiac safety in patients at high risk for torsade |
| Polcwiartek, et al.   | 2016  | Review                   | Review current literature about cardiovascular safety of antipsychotics, edited until 2016 | Discuss side effect of antipsychotics and current guidelines regarding routine electrocardiogram monitoring |
| Ray, et al.           | 2009  | Observational study (Retrospective cohort study) | 44,218 users of typical antipsychotics; 46,089 users of atypical antipsychotics; 186,600 matched nonuser controls | Role of typical and atypical on dose-related increased risk of sudden cardiac death |
| Roden, et al.         | 2004  | Review                   | Review literature about drug-induced (including antipsychotic) prolongation of the QT interval and torsade de pointes, edited until 2004 | Summarize the current knowledge about molecular and clinical predictors of drug-induced prolongation of the QT interval and torsade de pointes, consider how new molecular predictors of a drug’s activity might be incorporated into drug-development programs and clinical practice, and suggest a general approach to drugs that are suspected of causing this problem |
| Salvo, et al.         | 2016  | Observational study (Meta-analysis) | Meta-analysis of observational studies; two cohort (740,306 person-years) and four case-control (2,557 cases; 17,670 controls) studies | Estimate the risk of sudden cardiac death or sudden unexpected death related to individual antipsychotics |
| Serrano, et al.       | 2013  | Observational study (Two cross-sectional studies) | 125 patients treated with clozapine; 59 patients with other antipsychotics; 88 patients treated with clozapine; 61 patients treated with atypical antipsychotics; 23 patients treated with typical antipsychotics; 11 patients treated with atypical and typical antipsychotics; 36 patients treated with other drugs | Examine the risk of cardiomyopathy and hyponatraemia in patients treated with antipsychotics |
| Sicouri, et al.       | 2008  | Review                   | Review current literature about arrhythmic liability of antipsychotic and antidepressant drugs capable of inducing long QT and/or Brugada syndrome phenotypes | Provide an update on the ionic and cellular mechanisms thought to be involved in, and the genetic and environmental factors that predispose to, the development of cardiac arrhythmias and sudden cardiac death among patients taking antidepressant and antipsychotic drugs that are in clinical use |
| Smolders, et al.      | 2017  | Observational study (Case report) | A case of a 37-year-old woman treated with high does of quetiapine | A case of cardiomyopathy due to quetiapine |
| Türe, et al.          | 2019  | Observational study (Case report) | A case of a 15-year-old female taking clozapine in a suicide attempt | A case of atrioventricular total cardiac block associated with the taking of clozapine in a suicide attempt |
| Unsar, et al.         | 2013  | Observational study (Case-control study) | 116 patients with schizophrenia; 88 healthy patients | Describe higher cardiovascular risk in schizophrenic patients taking antipsychotics |
| Vang, et al.          | 2016  | Observational study (Case-series) | Two schizophrenic death men under olanzapine treatment | Describe correlation between fatal eosinophilic myocarditis and olanzapine |
| Vieweg, et al.        | 2003  | Review                   | Review literature about antipsychotic drug and QT interval, edited until 2002 | Examine the risk of QT interval prolongation and antipsychotics administration |
| Wasef, et al.         | 2015  | Observational study (Case report) | A case of an 18-year-old man taking quetiapine | A case of myocarditis induced by quetiapine |
Table 2  Baseline characteristics of the studies with the correlation between use of antipsychotics and oxidative stress.

| Authors            | Year | Types of study designs | Methods                                                                 | Aim of the study                                                                                   |
|--------------------|------|------------------------|-------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------|
| Wu, et al[34]      | 2015 | Observational study    | 17,718 patients with incident ventricular arrhythmia and/or sudden cardiac death treated with antipsychotic drug (control time windows of 7, 14, and 28 days) | Describe higher ventricular arrhythmia and/or sudden cardiac death risk in schizophrenic patients taking antipsychotics |
| Zhu, et al[35]     | 2019 | Review                 | Review current literature about adverse cardiac effects associated with antipsychotics | Examine adverse cardiac effects associated with antipsychotics and suggest the application of preventive measures |
| Abdel Wahab, et al[36] | 2014 | Preclinical study      | Histological hallmarks and biochemical markers of myocarditis, proinflammatory cytokines and parameters of oxidative stress were assessed in rats treated with coadministration of captopril and clozapine | Investigate the protective effect of captopril against clozapine-induced myocarditis in rats |
| Abdel Wahab, et al[37] | 2014 | Preclinical study      | Rats treated with clozapine were assessed in echocardiographic, histopathological and immunohistochemical parameters, including markers of cardiotoxicity, oxidative stress, inflammation and apoptosis | Investigate a possible mechanisms of clozapine-induced cardiotoxicity in a rat model |
| Arumugan, et al[38] | 2012 | Preclinical study      | Rat with experimental autoimmune myocarditis and treated with edaravone were assessed on echocardiographic study and histopathological markers | Determine whether edaravone protects against cardiac remodeling in dilated cardiomyopathy |
| Arumugan, et al[39] | 2012 | Preclinical study      | Western blotting, histopathological staining and immunohistochemical analyses to measure the myocardial expressions of AMPK signaling and oxidative stress related parameters were carried out in normal and vehicle or edaravone-treated experimental autoimmune myocarditis rats, respectively | Involvement of MAPK, AMPK signaling in the progression of experimental autoimmune myocarditis and if it can be blocked by the treatment with antioxidant edaravone |
| Auger, et al[40]   | 2018 | Preclinical study      | Two groups of mice were treated once a week, for 22 weeks, with intraperitoneal injection of risperidone; two other groups received intraperitoneal injection of the vehicle of risperidone following the same schedule. Mice of one risperidone-treated groups and of one vehicle-treated groups were fed a diet with curcumin, while mice of the other two groups received the standard diet | Investigate the potential capacity of curcumin, to attenuate the risperidone-induced metabolic dysfunction |
| Breier, et al[41]  | 1994 | Observational study    | Double-blind, parallel groups design                                      | Compare the effects of clozapine and haloperidol on plasma levels of norepinephrine |
| Dogan, et al[42]   | 2018 | Observational study    | Thirteen schizophrenic patients using atypical antipsychotic drugs and 30 healthy controls | Determine the changes in oxidative status and thiol disulfide homeostasis in schizophrenic patients using atypical antipsychotic drugs |
| Elman, et al[43]   | 1999 | Observational study    | 10 schizophrenic patients treated with clozapine, 7 schizophrenic patients treated with fluphenazine; 7 schizophrenic patients treated with placebo | Compare the effects of clozapine on plasma levels of norepinephrine respect to norepinephrine levels of patients treated with fluphenazine or placebo |
| Fehsel, et al[44]  | 2005 | Observational study    | 5 Olanzapine-treated patients; 14 polymedicated schizophrenic patients; 19 healthy subjects; 8 septic shock patients | Examined cellular effects of clozapine on blood cells of treated patients with and without clozapine-induced agranulocytosis |
| Haak, et al[45]    | 2003 | Observational study    | Four cases of patients with schizophrenia, treated with clozapine | Role of clozapine in the development of inflammation |
| Heiser, et al[46]  | 2010 | Preclinical study      | Measure formation of reactive oxygen in the whole blood of rats treated with haloperidol, clozapine and olanzapine | Examine reactive oxygen formation after treatment with antipsychotics by using electron spin resonance spectroscopy and test the protective capacity of vitamin C |
| Hendouei, et al[47] | 2018 | Observational study    | 100 patients with chronic schizophrenia treated with clozapine or risperidone or perphenazine | Comparison of serum level of glutathione, protein carbonyl, lipid peroxidation, superoxide dismutase and Ferric reducing ability of plasma in the three groups analysed |
cardiovascular pathologies and oxidative stress, and the relationship between antipsychotics and cardiac oxidative stress. In each subgroup, the type of scientific article, the methods used and the purpose of the work were indicated.

Oxidative Stress and Cardiac Injury

Superoxide anion (O$_2^-$), hydroxyl radical (OH$^-$), hydrogen peroxide (H$_2$O$_2$), singlet oxygen, carbon-centered radicals, peroxynitrite (ONOO$^-$), nitric oxide (NO), and nitrogen dioxide radicals are the free radicals identified in the human heart. Under basal conditions, the production of free radicals is low. However, the maintenance of intracellular redox homeostasis is fundamental to guarantee the physiological cardiac functions (development and maturation of cardiomyocytes, the release of intracellular calcium, and the coupling of cardiac excitation/contraction). The physiological intracellular levels of ROS are maintained by an antioxidant defense system which includes superoxide dismutases, catalase, the glutathione peroxidase/reductase system, and the peroxiredoxin/thioredoxin system. When pathological conditions occur (e.g., myocardial ischemia), hyperproduction of O$_2^-$ is observed from multiple cellular sources, the cellular antioxidant defense system is depleted and the free radicals scavenging enzyme system superoxide dismutase, glutathione peroxidase, chloramphenicol acetyl-transferase is significantly reduced. ROS can cause severe oxidative damage, especially to DNA, lipids, and proteins. Lipids are the class of biological molecules most susceptible to attack by oxygen free radicals. Oxidation takes place on the fatty acids present in cell membranes or lipoproteins, producing toxic substances for cells and tissues. Furthermore, as far as proteins are concerned, these, following the oxidation of the –SH groups of some amino acids (His, Arg, Lys, Pro) and the liberation of iron by the degradation of the porphyrin rings by ROS, lose their physiological structure and therefore functionality. In DNA, the oxidative phenomena concern the purine and pyrimidine bases; one of the most studied markers of DNA damage from oxidative damage is represented by 8-OHdG. The excessive production of ROS determines the activation of cell death, with consequent apoptosis or myocardial necrosis. In the pathogenesis of cardiac
Table 3  Baseline characteristics of the studies with the correlation between oxidative stress and heart disease.

| Authors            | Year | Types of study designs | Methods                                                                 | Aim of the study                                                                 |
|--------------------|------|------------------------|-------------------------------------------------------------------------|----------------------------------------------------------------------------------|
| Abu-Elsaad, et al. | 2018 | Preclinical study      | Histopathological examinations of stained heart sections on lipid profile, oxidative stress, and cardiac function biomarkers in rats received high-carbohydrate/high-fat diet concurrently with luteolin | Test luteolin protective effect against high-carbohydrate/high-fat diet-induced cardiac dysfunction in rats |
| Akao, et al.       | 2003 | Preclinical study      | Study the H2O2-induced response (loss of mitochondrial membrane potential and cell death) in cultured cardiac myocytes | Describe cardiac cellular responses after oxidant stress and identify target for intervention in the prevention of cell death |
| Brown, et al.      | 2010 | Review                 | Review of articles about cardiac mitochondria involved in the genesis of arrhythmia, edited until 2010 | Discuss the correlation between arrhythmia and cardiac mitochondrial alterations, in order to prevent arrhythmias by preserving mitochondrial membrane potential in the face of oxidative stress |
| Doroshow, et al.   | 1981 | Preclinical study      | CDF1 mice were pretreated with a pharmacologic dose of N-acetyl-L-cysteine before doxorubicin administration | Investigate the effect of N-acetylcysteine administration on the toxicity of doxorubicin |
| Esposito, et al.   | 2009 | Review                 | Review of articles about the role of nitroso radicals in circulatory shock, edited until 2009 | Analyse the involvement of oxidative and nitrosative stress in cardiovascular pathologies |
| Fineschi, et al.   | 2010 | Review                 | Review of articles about stress-induced cardiomyopathy, edited until 2010 | Examine correlation between alterations in catecholamine system functions stress-related and acute and chronic cardiovascular disorders |
| Finkel, et al.     | 1992 | Preclinical study      | Study the effects of pro-inflammatory cytokines on the contractility of mammalian heart and evaluate if the nitric oxide synthase inhibitor NG-monomethyl-L-arginine blocked these negative inotropic effects | Examine if the direct negative inotropic effect of cytokines is mediated through a myocardial nitric oxide synthase |
| Frangogiannis, et al. | 2002 | Review                 | Review of articles about processes regulating the inflammatory response following myocardial ischemia and reperfusion, edited until 2001 | Summarize current understanding of the cellular and molecular mechanisms regulating the inflammatory response following myocardial ischemia and reperfusion |
| Gong, et al.       | 2018 | Preclinical study      | Evaluation of serum glucose and insulin levels, lactate dehydrogenase and creatine kinase, the expression of pro-inflammatory cytokines and activation of nuclear factor-κB in Tmbim1 knockout mice | Examine if down regulation of Tmbim1 enhance high fat diet-induced cardiomyopathy by increasing inflammation and oxidative stress |
| Ishiyama, et al.   | 1997 | Preclinical study      | 20 rats with autoimmune cardiomydists were adserum level of ministered aminoguanidine and evaluated in serum level of creatine kinase muscle/brain, and immunohistochemical exam for inducible nitric oxide synthase and nitrotyrosine | Investigate the role of nitric oxide in the development of myocardial damage and the effects of aminoguanidine, an inhibitor of inducible nitric oxide synthase, on experimental autoimmune myocarditis |
| Javadi, et al.     | 2017 | Preclinical study      | Assess the efficacy of natural molecules (e.g. apigenin, berberine and quercetin) along with some plant extracts in experimental autoimmune myocarditis animal models | Evaluations of inflammatory and immunological mechanism and oxidative stress in experimental autoimmune myocarditis |
| Joseph, et al.     | 2016 | Preclinical study      | Evaluations of heart rhythm and calcium homeostasis in a transgenic mouse model of cardiac lipid overload, the peroxisome proliferator-activated receptor-γ | Examine the hypothesis that the increase in ventricular ectopy during cardiac lipid overload is caused by increased mitochondrial oxidative stress |
| Karagueuzian, et al. | 1982 | Review                 | Summarize current knowledge on oxidative stress-mediated arrhythmogenesis, and discuss how myocardial fibrosis promotes ventricular arrhythmias | Propose a synergic antibiotherapy, to reduce the risk of sudden cardiac death caused by arrhythmias |
| Killian, et al.    | 1999 | Preclinical study      | Identify in patients started clozapine treatment the cases of myocarditis and cardiomyopathy in Australaf from voluntary reports to the Australian Adverse Drug Reaction Committee | Investigated the cardiovascular complications for clozapine |
| Kishimoto, et al.  | 2003 | Experimental study     | Nine patients (six in myocarditis, three in acute dilated cardiomyopathy) were treated with high-dose intravenous Ig (1-2 g/kg, over two days) | Examine the effects of intravenous Ig by the analyses of inflammatory cytokines and oxidative stress |
| Koponen, et al.    | 2008 | Review                 | An update of the prevalence and mechanisms for sudden cardiac death in schizophrenia from 1966 and 2007 | Describe the sudden cardiac death related antipsychotics |
| Authors               | Year | Types of study designs     | Methods                                                                                                           | Aim of the study                                                                                           |
|----------------------|------|-----------------------------|-------------------------------------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------|
| Kresi, et al.        | 2016 | Retrospective observational study | 110 cases of sudden cardiac death in relation to a stressful event                                               | Sudden death occurred in the absence of structural heart disease may reflect the proarrhythmic potential of high catecholamines on the structurally normal heart |
| Li, et al.           | 2007 | Preclinical study           | Normal cardiomyocytes or traumatic cardiomyocytes were cultured with normal plasma or traumatic plasma and apoptosis was determined by caspase-3 activation | Study the molecular mechanisms responsible for cardiomyocyte apoptosis induced by trauma                    |
| Li, et al.           | 2013 | Preclinical study           | In a coxsackievirus B3 murine myocarditis model effects of carvedilol and metoprolol was assessed in myocardial histopathological changes, cardiac function, expression of 4-HNE, superoxide dismutase and glutathione peroxidases activities | Determine whether levels of lipid peroxides are elevated in the myocardium and whether carvedilol reduces the lipid peroxidation level and increases antioxidant enzyme activities in viral murine myocarditis model |
| Marian, et al.       | 2006 | Experimental study (Randomised controlled trial) | 42 subjects with hypertrophic cardiomyopathy, one half treated with antioxidant N-acetylcysteine and the others with placebo | Analyse the protective cardiac effect of N-acetylcysteine                                                  |
| Matsui, et al.       | 1999 | Preclinical study           | Sprague-Dawley rats were treated with doxorubicin, carvedilol, doxorubicin, +carvedilol, or atenolol +doxorubicin. Cardiac performance and myocardial lipid peroxidation were assessed | Test if carvedilol protects against doxorubicin-induced cardiomyopathy                                       |
| Melendez, et al.     | 2010 | Preclinical study           | Adult male Sprague-Dawley rats were infused with IL-6 and compared with vehicle-infused, aged-matched controls. Left ventricular function, myocardial interstitial collagen volume fraction and isolated cardiomyocyte size were assessed | Examine if elevated levels of IL-6 mediate myocardial remodeling                                           |
| Mito, et al.         | 2011 | Preclinical study           | Rats with autoimmune myocarditis were divided randomly into a treatment (curcumin) and vehicle group. Myocardial protein expression of inducible nitric oxide synthase, the catalytic subunit of nicotinamide adenine dinucleotide phosphate reduced oxidase and myocardial endoplasmic reticulum stress signaling proteins were assessed | Examine the mechanism of curcumin in experimental autoimmune myocarditis                                     |
| Miyamoto, et al.     | 2004 | Preclinical study           | Histopathology and thioredoxin expression in spontaneous myocarditis in inbred strains of mice was assessed      | Investigate the role of thioredoxin and redox-regulating system in spontaneously developed myocarditis       |
| Nimata, et al.       | 2005 | Preclinical study           | Rats with experimental autoimmune myocarditis treated with edaravone and untreated rats were compared in histological markers | Determine if the free radical scavenger edaravone protects against acute experimental autoimmune myocarditis in rats by the radical scavenging action associated with the suppression of cyotoxic myocardial injury |
| Octavia, et al.      | 2012 | Review                      | Review of articles about the role of oxidative stress in heart failure, edited until 2012                          | Discuss the importance of nicotinamide adenine dinucleotide phosphate oxidase-dependent reactive oxygen generation in the various subtypes of heart failure and its implications |
| Pacher, et al.       | 2005 | Review                      | Review of articles about the role of peroxynitrite or matrix metalloproteinases and poly (ADP-ribose) polymers in the experimental therapy of various forms of myocardial injury, edited until 2005 | Discuss the role of nitrosative stress and downstream mechanisms, including activation of matrix metalloproteinases and poly (ADP-ribos) polymers, in various forms of heart failure |
| Pacher, et al.       | 2008 | Review                      | Review of articles about the pathophysiological relevance of the peroxynitrite-poly (ADP-ribose) polymers pathway in various forms of heart injury and systemic pathology, edited until 2008 | Examine the correlations between peroxynitrite- peroxynitrite-poly (ADP-ribos) polymers pathway and oxidative DNA damage in heart injury and systemic pathology |
| Raucci, et al.       | 2019 | Review                      | Review current literature about the role of HMGB1 in heart disease                                             | Summarizes recent findings on HMGB1 biology and heart dysfunctions and discusses the therapeutic potential of modulating its expression, localization, and oxidative-dependent activities |
| Authors | Year | Types of study designs | Methods | Aim of the study |
|---------|------|------------------------|---------|------------------|
| Shimada, et al. | 2015 | Preclinical study | Rats with experimental autoimmune myocarditis were treated with N-acetylcysteine and N(G)-nitro-l-arginine methylester (inhibitor of nitric oxide)/ N(G)-nitro-d-arginine methylester (an inactive enantiomer) | Investigate the effects of N-acetylcysteine, a potent antioxidant, on experimental autoimmune myocarditis in rats |
| Shimazaki, et al. | 2010 | Preclinical study | Rats with experimental autoimmune myocarditis and treated with edaravone were studied in echocardiographic parameters and inflammation and oxidative stress parameters | Determine if edaravone ameliorate the progression of experimental autoimmune myocarditis by inhibiting oxidative and endoplasmic reticulum stress and cardiac apoptosis |
| Shinoda, et al. | 2016 | Preclinical study | Neonatal rat cardiomyocytes were treated with haloperidol, and exposed to angiotensin II. Hypertrophy, e1R expression, mitochondrial Ca (2+) transport and ATP levels were assessed | Examine if sudden cardiac failure induced by haloperidol is mediated by chronic haloperidol inhibition of cardiac e1R |
| Shin, et al. | 2002 | Review | Review literature about the role of chemokines (MCP-1, IL-8) in the pathogenesis of vascular disease, edited until 2002 | Supports the pivotal role of chemokines in the pathogenesis of vascular disease and suggest delineating the patterns of gene expression (MCP-1, IL-8) to identify molecular targets for the prevention and treatment of atherosclerosis |
| Singh, et al. | 2008 | Observational study (Randomized cross-sectional study) | Two groups of fifty schizophrenic patients treated by haloperidol and olanzapine, respectively for at least six months | Examine the correlations between serum antioxidants parameters and antipsychotics |
| Skrzypiec-Spring, et al. | 2018 | Preclinical study | Carvedilol in 3 doses (2, 10, and 30 mg/kg) was given daily to 3 study groups of rats with experimental autoimmune myocarditis | Evaluate the effects of carvedilol administration in acute myocarditis and its impact on matrix metalloproteinases’ activation |
| Sovari, et al. | 2013 | Preclinical study | Wild type mouse and ACE8/8 (an animal model of cardiac renin-angiotensin system activation) were treated with different antioxidant therapies | Determine the source of reactive oxygen and if reactive oxygen played a role in the arrhythmogenesis |
| Sugiyama, et al. | 2016 | Preclinical study | Induce oxidative stress in NOS1AP knockout mice to evaluate electrocardiographic and echocardiographic parameters | Evaluate the relationship between oxidative stress and ventricular tachyarrhythmia/heart failure in genetic variations at NOS1AP |
| Sukumaran, et al. | 2011 | Preclinical study | Experimental autoimmune myocarditis was induced in rats by immunization with porcine cardiac myosin. The rats were divided into two groups and treated with either telmisartan (10 mg/kp per day) or vehicle for 21 days and expression of inflammatory cytokines and oxidative stress markers was evaluated | Investigation of telmisartan (angiotensin II type 1 receptor antagonist) protects against experimental autoimmune myocarditis by suppression of inflammatory cytokines and oxidative stress |
| Tse, et al. | 2016 | Review | Literature review on investigation the effects of reactive oxygen on cardiac ion channel function, remodeling and arrhythmogenesis | Study the relationship between increased oxidative stress cardiovascular pathologies such as diabetes and hypertension associated with arrhythmias |
| Turillazzi, et al. | 2017 | Observational study (Case-control) | Biochemical and immunohistological markers of oxidative/nitrosative stress were evaluated in subjects who had died from high doses of cocaine, compared to the control group | Examine the hypothesis that cardiac toxicity by acute exposure to high dosage of cocaine could be mediated by unbalanced myocardial oxidative stress |
| Wu, et al. | 2018 | Preclinical study | A model of experimental autoimmune myocarditis in which Toll-like receptors 4 activation or inhibition was performed to induce chronic inflammation | Examine the role of mitochondrial dynamics in Toll-like receptors 4 activation-mediated dilated cardiomyopathy |
| Yang, et al. | 2015 | Review | Scientific evidence on the mechanisms linking metabolic derangement and cardiac excessive oxidative stress that predisposes to ventricular arrhythmias and sudden cardiac death | Report the association between cardiac oxidative stress and malignant arrhythmias providing novel therapeutics to prevent sudden cardiac death |
| Yuan, et al. | 2004 | Preclinical study | Rats with experimental autoimmune myocarditis induced by porcine myosin and treated with carvedilol, racemic carvedilol, metoprolol, or propranolol was assessed in echocardiographic study and inflammatory and oxidative stress parameters | Investigate whether carvedilol exert protective effect against experimental autoimmune myocarditis by suppression of inflammatory cytokines and its antioxidant properties |
pathologies, a fundamental role is played by mitochondrial-derived ROS, which derives from electron transfer into the mitochondrial respiratory chain complexes and from the action of mitochondrial proteins. Mitochondria are one of the main drivers of intracellular oxidant production and other relevant sources are nicotinamide adenine dinucleotide phosphate (NADPH) oxidases (NOX family of enzymes). Besides, other enzymes contribute to intracellular ROS production, including xanthine oxidase, nitric oxide synthase (NOS), cyclooxygenases, cytochrome P450 enzymes, and lipoxygenases.

Several data in the literature suggest that this type of ROS is involved in the pathogenesis of numerous cardiac pathologies, such as myocardial infarction, heart failure, and diabetic cardiomyopathy. Specifically, in cardiac infarction, hypoxia induces a decrease in adenosine triphosphate (ATP) with consequent loss of depolarization of the mitochondrial membrane with activation of anaerobic glycolysis, lactate formation, and cytosolic calcium accumulation. These cellular damages are finally further implemented by the reperfusion of hypoxic cells, causing an increase in cellular necrosis. Otherwise, in heart failure, the cellular modifications related to ROS occur in a more dilated time span. The basis of these modifications is the interaction between the increase in angiotensin II and the increase in myocardial stress, with consequent cardiac remodeling, contractile dysfunction, and the onset of arrhythmias. The role played by NADPH oxidase (NOX) seems to be relevant in the failure, which can uncouple the NO synthase and promote $O_2^-$. Also, angiotensin II-mediated ROS increase activates nuclear factor kappaB induced hypertrophy pathway.

Instead, diabetic heart disease is linked to the accumulation of fatty acids in cardiomyocytes with a consequent reduction in the use of carbohydrates as an energy substrate especially during periods of high demand for ATP and, consequently, increased formation of ROS with mitochondrial damage and promotion of apoptosis of cardiomyocytes. The peroxisome proliferator-activated receptor, a nuclear hormone receptor which modulates the metabolism of glucose and fatty acids, is involved in this heart disease.

Finally, oxidative stress, in association with the increase in inflammation products, promotes endothelial dysfunction through the inactivation of NO, an important molecule with a vasodilator and anti-atherogenic role. NO inactivation is promoted by NADPH oxidase and uncoupled NOS.

In light of these data, there is growing interest in the literature about the role played by ROS in the pathogenesis of cardiovascular diseases and the possible experimental use of antioxidant molecules in the treatment of heart diseases.

### Cardiovascular Complications from Antipsychotics

Atypical antipsychotic therapy is notoriously associated with cardiovascular pathologies and an increase in sudden cardiac death. Second-generation antipsychotic drugs have a significant influence on the development of metabolic syndrome, which is known to be a risk factor for cardiovascular disease. Furthermore, a correlation is known with myocarditis, dilated heart disease, and sudden cardiac death.
Among antipsychotics, there was an increased risk of QTc prolongation for typical antipsychotics; in particular droperidol and thioridazine are associated with an increase in QTc in a dose-dependent manner. However, there are data in the literature that also describe it for atypical antipsychotics. Clinical studies have reported a clear association of the aforementioned electrocardiographic alteration and sertindole and risperidone, in a dose-dependent manner, although among the atypical antipsychotics, ziprasidone is the riskiest, so much so that it has been proposed to take an electrocardiogram at the start of therapy.

Preclinical studies have reported a dose-dependent prolongation of the QT interval with other atypical antipsychotics as well, namely clozapine and olanzapine. Finally, antipsychotic therapy, although rarely, can be associated with severe complications such as myocarditis and evolving cardiomyopathies towards decompensation. This is especially true of clozapine, an atypical antipsychotic used for resistant schizophrenia and prevention of suicide risk. In addition to myocarditis, cases of cardiomyopathy have been described, albeit more rarely. This is also more frequent with clozapine with a later onset than myocarditis (the average onset was reported in a study after 12 months) and associated with high mortality. Specifically, clozapine, in therapeutic doses, can also be associated with the occurrence of serious cardiovascular side effects, very rare but described in the literature as dilated cardiomyopathy, pericarditis and concomitant myopericarditis associated with parenchymal lung disease. Finally, complete atrioventricular blockage by ingestion of massive drug doses of clozapine has been described, which can be fatal if not promptly recognized and managed.

For this reason, the United States Food and Drug Administration has included potentially fatal events and cautious use of clozapine in patients with pre-existing cardiac diseases and with severe cardiac conditions among the adverse effects of clozapine. A retrospective study of all adverse drug reactions reported voluntarily to the Australian Adverse Drug, identified an incidence of suspected myocarditis ranging between 0.7% and 1.2% of patients treated with clozapine, with an average age of 30...
years; the disease developed on average two weeks after starting therapy with a mortality rate of 10.3%.\[31\]

Myocarditis is an uncommon, dose-independent, potentially lethal adverse effect; probably underestimated due to the often, atypical clinical manifestation and the poor compliance of these patients with medical treatment. In fact, the clinical presentation can be very variable, ranging from subclinical forms to fulminant heart failure.

Sudden death after clozapine intoxication due to eosinophilic myocarditis was first described in 1992,\[52\] but the same was also reported in subsequent several clinical cases with the use of therapeutic doses of the drug.\[53\]

Although with extremely rare incidence, cardiomyopathy has also been described with quetiapine, an atypical antipsychotic structurally similar to clozapine, suggesting a pharmacological class effect.\[54\] According to the WHO’s program for international drug monitoring, myocarditis and cardiomyopathy induced by the use of quetiapine are typically present mimicking myocardial infarction with acute ST-segment elevation on electrocardiogram. As well as for clozapine, the mechanism of quetiapine-induced cardiotoxicity is still undefined.

The use of antipsychotics is burdened by an increased risk of sudden cardiac death; in fact, in subjects taking antipsychotics, the risk of sudden cardiac death has more than doubled in consideration also of weight gain, dyslipidemia, and type 2 diabetes mellitus related to antipsychotic treatment.\[15\] To these risk factors, additional environmental cardiovascular risk factors are added, such as smoking, alcohol abuse, poor diet, a sedentary lifestyle, and stress, which are often present in individuals suffering from schizophrenia.

Sudden cardiac death in patients taking antipsychotics has a multifactorial nature, resulting from a complex interaction between the aforementioned environmental factors, any pre-existing cardiac substrates, both structural and genetic, and the pathological action of these drugs at the cardiac level. However, the underlying molecular mechanisms are still to be understood also concerning the causal weight of a possible heritable component that can make individuals taking antipsychotics more susceptible to sudden cardiac death.

OXIDATIVE STRESS IN ANTIPSYCHOTIC-RELATED CARDIOMYOPATHIES

Marketed drugs have been often associated with cardiovascular major adverse events and safety concerns due to a significantly increased risk of cardiac fibrosis, myocardial infarction, and myocardial inflammation. Despite efforts in the preclinical phase of the development of drugs, cardiotoxicity remains a great concern due to the lack of sufficient knowledge of the mechanism of cardiotoxicity. Linkage with the entity of intracellular ROS and RNS reaction has been evoked to explain drug-induced myocardial injury.\[55\] Oxidative and nitrative modifications of key mitochondrial proteins have a central role in drug-induced cardiotoxicity.\[56\] The oxidative stress resulting from increased free radical generation in cardiomyocytes produces an energetic imbalance, impairment of mitochondrial function, activation of stress-related signaling pathways, p53 accumulation, and cellular loss.\[57\] By the way, it has been supposed that administration of antioxidants (vitamin C and vitamin E, carvedilol, L-carnitine, N-acetylcysteine, coenzyme Q10, dexrazoxane) is associated with a decrease in ROS-induced cardiomyocytes damage and can play a role in reducing drug-induced cardiotoxicity.\[58,59\]

An analysis of the literature highlights the need to acquire more data on the pathogenesis of heart disease induced by atypical antipsychotics through post-mortem studies. Conversely, numerous preclinical studies on animal models have been carried out to understand the pathogenesis of heart disease associated with individual antipsychotics. To clarify the role played by oxidative stress in the origin of pathologies affecting the heart induced by atypical antipsychotic drugs, it appears useful to analyze the cardiac pictures related to them individually (Figure 2).

Myocarditis

Myocarditis is mainly described in patients taking clozapine, although it has also been reported in association with the use of other second-generation antipsychotics.

Clear evidence about dose-related clozapine cardiotoxicity is still far to be demonstrated. The mechanism by which clozapine induces cardiotoxicity remains unclear but numerous hypotheses have been
An immunoglobulin E-mediated hypersensitivity (type I allergy) has been proposed for the first time by Killian, et al.\[60\] as a mechanism of cardiac toxicity in clozapine-treated patients. The results of necropsy showed mainly lymphocytic and mixed infiltrates with myocytolysis, consistent with an acute pharmacological reaction.\[61\] Then, eosinophilic myocarditis was described in the sudden death of patients treated with clozapine, and a hypereosinophilic action of clozapine was proposed as the main mechanism of cardiotoxicity.\[13,20,62,63\] Finally, a role of pro-inflammatory cytokines, catecholamines, and oxidative stress was proposed.\[64–66\] Bioactivation of clozapine in the myocardial tissues is associated with the generation of a chemically reactive nitronium ion metabolite which stimulates cellular injury, lipid peroxidation, and formation of free radical. When bound with a protein of the myocardium, this nitronium ion produces an antigenic complex that stimulates an immune response, and activates macrophages releasing proinflammatory cytokines.\[67,68\]

To support the importance of oxidative stress in clozapine-associated myocarditis, there is a study conducted by Abdel-Wahab, et al.\[69\] This study aimed to study the protective effect of captopril against clozapine-induced myocarditis in rats and the possible mechanisms underlying this effect. Administration of the angiotensin converting enzyme inhibitor reduced the histological signs and biochemical markers (creatine phosphokinase–MP isoenzyme and lactate dehydrogenase) of myocarditis, as well as reduced the oxidative stress parameters (NO and DNA degradation products) in a dose-dependent manner, suggesting how the cardioprotective effect exerted by captopril, in the presence of clozapine-induced myocarditis, is also mediated by the reduction of oxidative stress.

A clinical study reported that the erythrocyte concentration of selenium, an antioxidant molecule, was reduced in schizophrenic patients taking clozapine compared to those who did not take it and compared to patients with mood disorders and healthy controls. This evidence suggests that reduced antioxidant activity may contribute to the development of cardiological complications related to clozapine, including myocarditis.\[70\]

High levels of tumor necrosis factor-alpha (TNF-α) in association with an increase in catecholamines have also been described in antipsychotic-related myocarditis and dose dependence has been demonstrated. TNF-α hyperproduction is related to the attraction of leukocytes and the generation of further free radicals and cytotoxic proteins.\[71–76\] High levels of myeloperoxidase were observed in experimental studies with clozapine-treated animals and were in-
dicated as a sensitive marker of neutrophil migration and myocardial injury. Histopathological examination of the heart of mice treated with clozapine showed a significant dose-related increase in myocardial inflammation. The latter correlated with plasma catecholamine and TNF-α levels. Furthermore, this effect was significantly reduced in mice treated with propranolol, compared to controls, suggesting a potential immune-mediated mechanism of action of clozapine.

A preclinical study was conducted to investigate the influence of clozapine, ziprasidone, and sertindole on rat heart morphology and determine whether redox status plays a role in the development of cardiac histopathological changes induced by antipsychotic. The results showed that all three drugs induced cardiac histopathological changes related to a picture of toxic myocarditis. In particular, clozapine increased the activity of superoxide dismutase 1 while ziprasidone reduced the activity of glutathione reductase. Sertindole did not exert any marked effect on the function of antioxidant enzymes in the heart even though myocardial degeneration was observed. In conclusion, treatment with clozapine or ziprasidone induced pathophysiological changes in the rat heart, which seemed to be associated with disturbances in antioxidant capacity. Finally, an antioxidant molecule (edaravone) is effective in reducing myocardial damage in animal models of acute autoimmune myocarditis in association with a reduced state of oxidative stress.

Cardiomyopathy and Cardiac Injury

Heart disease is another adverse effect associated with antipsychotics, although the pathophysiological mechanisms underlying the processes of myocardial remodeling in association with a pro-arrhythmic condition with increased risk of sudden death are not clear in this context too.

A preclinical study supports the importance of mitochondrial oxidative stress in the presence of heart disease characterized by an increase in angiotensin II levels, proposing the use of mitochondrial antioxidants in the prevention of evolving arrhythmias towards sudden cardiac death in the presence of the renin-angiotensin axis. An increasing amount of data in the literature indicate that cardiac mitochondria are involved in the genesis of arrhythmia. In fact, the energetic state of the mitochondrial network can alter the potassium flows through the ATP-sensitive potassium channels (essential for cell survival in the face of oxidative stress), creating the electrical conditions to favor fatal arrhythmias. For this reason, the cardiac mitochondrial network has emerged as a key target for strategies that seek to reduce arrhythmias through the maintenance of mitochondrial integrity in the face of metabolic stress, preserving the integrity of the membrane potential.

Preclinical data also clearly show that edaravone, a new free radical scavenger, improves the evolution of dilated cardiomyopathy and cardiac remodeling by modulating oxidative stress.

Cardiomyopathy related to taking atypical antipsychotics can, at least in part, be due to cardiomyocyte apoptosis, known to be important in the pathogenesis of patients with severe heart failure from various etiologies. Experimental studies on mice proposed a programmed cell necrotic death (necroptosis) as a possible explanation for the cardiotoxicity of quetiapine. Myocytes treated with quetiapine showed disruption of the cellular membrane, mitochondria swelling with reticulum expansion and generation of oxygen reactive species. These effects are reversed by the administration of necrostatin-1, a TNF inhibitor, suggesting a close correlation between inflammation, oxidative stress, and cell death.

Treatment with clozapine has been also associated with an increased cardiac level of total nitrite, a stable end product, an indirect marker of NO. Increased formation of NO produced by inducible NOS has a negative inotropic effect and contributes to the progression of myocardial damage potentially leading to cardiac fibrosis. Besides, hyperexpression of peroxynitrite in cardiac samples was observed at an immunohistochemical study which was involved in direct oxidative damage to lipids, proteins, cardiac cells, and myocytes and in the nitration of tyrosine residues of pro-apoptotic proteins in cardiomyocytes. In fact, increased NO formation is associated with lipid peroxidation, inactivation of enzymes, and depletion of reduced glutathione and it confirmed that antipsychotic cardiotoxicity is related to increased oxidative stress and weakened antioxidant defense.
Metabolic Syndrome

The use of atypical antipsychotics is associated with the development of the metabolic syndrome. It has been proposed that the metabolic alterations induced by these molecules and the increase in arrhythmic risk related to lipid accumulation in cardiomyocytes involve oxidative stress. Cardiac lipid accumulation gives rise to non-ischemic cardiomyopathy called lipotoxic cardiomyopathy.

A preclinical study used a transgenic mouse model of cardiac lipid overload with cardiac overexpression specific to the peroxisome proliferator-activated receptor gamma, a paradigm of metabolic syndrome in humans. Lipotoxic cardiomyocytes have been shown to exhibit increased ROS production and impaired calcium homeostasis with increased ventricular ectopy. This arrhythmic effect is improved by the inhibition of mitochondrial ROS through the use of a mitochondrial antioxidant molecule suggesting a potential role of mitochondrial antioxidants in the prevention of arrhythmia and sudden cardiac death in obesity and diabetes mellitus.

The role of metabolic stress concerning oxidative stress in the development of these cardiomyopathies is also highlighted in the preclinical study conducted by Gong, et al. Here it was shown that the knockout mice of BAX transmembrane inhibitor motif-containing 1, a suppressor of BAX-mediated cell death and associated with the regulation of ROS, present promotion of metabolic disorders and cardiac dysfunctions induced by the diet rich in fats with increased proinflammatory cytokines and oxidative damage.

In the light of these data, a clear relationship seems to emerge between metabolic/inflammatory alterations and oxidative stress, key elements for the development of structural and arrhythmogenic heart diseases that can lead to fulminant fatal events.

Sudden Cardiac Death

As for sudden cardiac death linked to antipsychotics, this is probably caused by an arrhythmic mechanism, but other mechanisms may be involved, including autonomic effects, inhibition of other ion channels or other acute cardiotoxicities, such as myocarditis associated with the use of clozapine.

In the literature, numerous data are supporting the involvement of oxidative stress in pathological conditions involved in the genesis of sudden cardiac death.

Ventricular arrhythmia is the main cause of sudden cardiac death. A relationship between metabolic disorder and excessive oxidative stress has been proposed with cardiac ion channel dysfunction, which predisposes to ventricular arrhythmias and sudden cardiac death.

ROS production has been hypothesized to play a significant role in arrhythmic substrate production. In particular, an abnormal mitochondrial function can lead to an altered function or expression of the cardiac ion channels responsible for the generation of the cardiac action potential with the possibility of generating re-entry phenomena implicated in the development of malignant arrhythmias.

It is also known that an altered redox state is implicated in cardiovascular pathologies such as atherosclerosis, diabetes mellitus and myocardial ischemia, all conditions that can create a structural substrate for increasing sudden cardiac death. In particular, in myocardial ischemia and heart failure, the efficiency of the mitochondrial electron transport chain is compromised with a consequent increase in ROS production. The aforementioned pathological conditions are notoriously arrhythmogenic and predispose to sudden cardiac death and this could be linked to an altered intracellular electrical and ionic hemostasis consequent to the increase in ROS production.

To support the importance of oxidative stress in the genesis of heart disease associated with sudden cardiac death, some data are emerging from preclinical studies. Doxorubicin (molecule that increases oxidative stress) injection knockout mice for the neuronal nitric oxide synthase-1 adaptive (NOS1AP) protein compared to wild types showed significantly higher mortality, QTc prolongation, and reduced contractile function, as well as the development of spontaneous ventricular tachyarrhythmias. The administration of the antioxidant N-acetyl-L-cysteine significantly reduced the mortality of knockout NOS1AP mice and prevented the prolongation of the QT interval and the reduction of systolic function, suggesting a fundamental role of oxidative stress in the
development of ventricular tachyarrhythmias and heart failure, which can cause sudden cardiac death.\textsuperscript{[102]}

**POST-MORTEM STUDIES**

Several post-mortem studies have been carried out on subjects who used antipsychotics in life to highlight a correlation between the increase in mortality from cardiovascular causes observed on schizophrenics and the use of antipsychotics, typical and atypical (Table 4).

As can be seen in Table 4, epidemiological/observational studies and a cohort study have been carried out to assess cardiovascular risk in subjects taking antipsychotics with a descriptive methodology.\textsuperscript{[8,18,103–107]} In fact, databases were analyzed from hospitals, mainly psychiatric, or from national registers, where the causes of death (cardiac and non-cardiac) were reported in the appropriate death certificates.

However, in the literature, there are few systematic necropsy studies aimed at analyzing this aspect. The post-mortem investigation described by Kelly, \textit{et al.}\textsuperscript{[104]} analyzed a sample of sixty-two deceased subjects treated at life with atypical antipsychotics (clozapine and risperidone), highlighting that 11\% of the patients treated with clozapine and 7\% of those treated with risperidone had died of cardiovascular causes. Also, three cases of cardiomyopathy were described in the group of subjects taking clozapine and two cases in those taking risperidone. Besides, three cases of myocarditis have been described only in subjects taking clozapine.\textsuperscript{[104]} A post-mortem diagnosis of myocarditis has also been described by Vang, \textit{et al.}\textsuperscript{[8]} This study included two case reports relating to the death of two young men, both who took olanzapine at therapeutic doses, who died suddenly. In both cases, an autopsy, histological and toxicological tests were carried out, which made it possible to make diagnoses of death of eosinophilic myocarditis. Another epidemiological study carried out using a database on autopsy data of all deaths occurring over a 26-year period (1984–2009) in adults receiving care in one large psychiatric hospital in New York, revealed the presence of twenty-two deaths from cardiovascular disease on one hundred cases of sudden death. The twenty-two deaths from cardiovascular death were divided as follows: fifteen deaths due to acute coronary syndrome, two deaths due to heart failure, two deaths due to aortic dissection, two deaths due to myocarditis, and one death due to commotio cordis.\textsuperscript{[103]} Also, Ifteni, \textit{et al.}\textsuperscript{[106]} used a register of autopsy data from Maryland hospital databases from 1989 to 2013. This study included a cohort of 7,198 schizophrenic patients hospitalized in psychiatric facilities. Of these, fifty-seven patients died of sudden death during hospitalization and fifty-one patients underwent autopsy. On autopsy, 62.8\% of deaths were attributed to cardiovascular causes, such as myocardial infarction (52.9\%), myocarditis (5.9\%), and dilated cardiomyopathy.

However, it should be noted that no studies have analyzed the correlation between antipsychotic-induced cardiac death and oxidative stress in humans. In this regard, numerous preclinical studies previously exposed have highlighted the link between cardiac damage induced by oxidative stress/inflammation and antipsychotics. Concerning this, numerous molecules have been analyzed at the murine level; the alterations highlighted in the various studies conducted included an increase in TNF-\alpha, an increase in catecholaminergic tone, an increase in the ROS and nitrate pathways, the presence of DNA degradation products, and lipid peroxidation, as well as alteration of endogenous antioxidant systems.

Specifically, animal models of myocarditis associated with the use of clozapine have shown an increase in TNF-\alpha and free radicals, effects that were reduced with the use of propranolol, suggesting that the increase in catecholaminergic tone could mediate the inflammatory increase and oxidative stress in this pathology.\textsuperscript{[64]} The scarcity of post-mortem studies suggests the need for research in this sense, to be able to highlight whether oxidative stress can at least partially mediate cardiotoxicity due to antipsychotics also in human subjects. In this sense, the increase in TNF-\alpha can be assessed in humans through immunohistochemistry investigations on cardiac histological sections previously treated with anti-TNF-\alpha.\textsuperscript{[57]} Furthermore, an index of cardiac damage from oxidative stress is represented by the increase in the levels of malondialdehyde, an indicator of lipid peroxidation, and the reduced glutathione/oxidized glutathione ratio, both
Table 4  Post-mortem studies carried out on subjects who used antipsychotics in life to highlight a correlation between the increase in mortality from cardiovascular causes observed on schizophrenics and the use of antipsychotics, typical and atypical.

| Authors | Year | Type of article | Case | Drug | Death diagnosis | Investigations |
|---------|------|-----------------|------|------|-----------------|---------------|
| Kelly, et al. | 2009 | Descriptive epidemiological study | People with schizophrenia who had received clozapine (n = 62) or risperidone (n = 42). Use of a database on autopsy data about cardiac disease | Clozapina; risperidone | 7 of the 62 (11%) in the clozapine treated group died of cardiovascular disease; 3 of the 42 (7%) in the risperidone group died of cardiovascular disease; 3 of the 62 (5%) patients died of cardiomypathy in the clozapine group; 2 of the 42 (5%) patients of risperidone groups died of cardiomypathy; 3 of the 62 (5%) died of myocarditis in the clozapine group | Autopsy; histological examination |
| Kelly, et al. | 2010 | Descriptive epidemiological study | 136 deaths; use of an administrative database of schizophrenia patients treated in Maryland, con clozapine (n = 1,084) con risperidone (n = 602) between 1994 and 2000 | Clozapina; risperidone (Control) | 43 cardiovascular disease | Revision of an administrative database; identifications of cardiovascular disease in death certificates; no autopsy were done; no histological examination |
| Vang, et al. | 2016 | Case reports | 2 cases: a 39-year-old man with known substance abuse and schizophrenia; 36-year-old man diagnosed with schizophrenia, found dead unexpectedly | Case 1: olanzapine 40 mg/day; pregabalin; venlafaxine; oxazepam Case 2: olanzapine 20 mg/day +5 mg; aripiprazole 30 mg/day; mirtazapine 30 mg/day | Eosinophilic myocarditis | Autopsy; histological examination; toxicological investigation |
| Manu, et al. | 2011 | Descriptive epidemiological study | 100 cases of sudden death, utilizo di un database on autopsy data of all deaths occurring over a 26-year period (1984–2009) in adults receiving care in one large psychiatric hospital in New York | First and second generation; antipsychotics; antidepressants; mood stabilizers; benzodiazepine; methadone; psychostimulants | Cardiovascular diseases (22); acute coronary syndrome (15); heart failure (2); aortic dissection (2); myocarditis (2); commotio cordis (1); upper airway obstruction (5); pulmonary emboli (4); thrombotic strokes (3) | Autopsy; histological examination |
| Ifteni, et al. | 2014 | Descriptive epidemiological study, autopsy data | cohort of 7,189 schizophrenia patients admitted to a free-standing, psychiatric teaching hospital from 1989 to 2013 | Antipsychotics | 57 patients died suddenly and unexpectedly; 51 autopsies performed. Causes of sudden death were most commonly cardiovascular disorders (62.8%), including myocardial infarction (52.9%), pneumonia (11.8%), airway obstruction (7.8%), myocarditis (5.9%), and dilated cardiomypathy, hemopericardium, pulmonary embolus, hemorrhagic stroke and brain tumor (2.0% each) | Autopsy findings using data extracted from their medical records and the post-mortem examination report |
| Ray, et al. | 2009 | Retrospective cohort | 1,870 sudden cardiac deaths. Cohort: 44,218 and 46,089 baseline users of single typical and atypical drugs; and 186,600 matched nonuser controls. Study data were obtained from computerized files of Tennessee Medicaid from 1990 to 2005 | Antipsychotics | 1,870 sudden cardiac deaths | Revision of a database (computerized files of Tennessee Medicaid) |
| Jones, et al. | 2013 | Descriptive epidemiological study | General Practice Research Database was used to identify cohorts of antipsychotic users and nonusers with psychiatric illness (patients registered in database from January 1995 to January 2011) | 183,392 antipsychotic users (20,954 olanzapine users) and 193,920 psychiatric nonusers | Cardiac mortality (number of events): nonuser psychiatric (1,289). User psychiatric: atypical (1,200); typical (1,180); olanzapine (206) | Evaluate cardiac mortality, including coronary heart disease, and life-threatening ventricular arrhythmias using death certificate data and records from their medical registry of hospital admission |

[http://www.jgc301.com; jgc@jgc301.com](http://www.jgc301.com; jgc@jgc301.com)
of which can be assessed in humans through biochemical analyzes, respectively of chromatographic and spectrophotometric type.\textsuperscript{[108,109]}

In addition to this, to highlight the presence of a correlation between oxidative stress and heart disease induced by antipsychotics, it would be useful to carry out immunohistochemical investigations aimed at assessing the presence of oxidative stress markers, such as inducible NOS, 8-OHdG, NOX2, and finally the pathways of apoptotic death by TUNEL assay.\textsuperscript{[57]}

CONCLUSIONS

Cardiotoxicity of atypical antipsychotics represents a major concern in clinical practice due to its large use in mental disorders. Major cardiovascular adverse events and sudden unexpected deaths are reported, but the mechanism of myocardial injury is not completely known. An increase in myocardial oxidative stress and inflammatory cytokines may play an important role in cellular death and DNA damage. Post-mortem examination is still crucial for research and must be seen as an opportunity for early detection of patients at risk for an unexpected death, and discovery of preventive measures.\textsuperscript{[110,111]}

Post-mortem studies carried out show increased cardiac death in subjects taking antipsychotics compared to the general population, reporting cases of myocarditis, cardiomyopathy, and sudden cardiac death. However, the pathogenesis of cardiac toxicity induced by these molecules is still unclear. A potential role of oxidative stress in cardiotoxicity caused by antipsychotics has been proposed in numerous preclinical studies, but post-mortem data from subjects taking atypical antipsychotics during life is extremely lacking. It would be desirable to carry out biochemical and immunohistochemical investigations on cardiac tissue of subjects who died of cardiac death related to antipsychotics, to verify whether oxidative stress can at least partially mediate the cardiotoxicity associated with the use of these molecules. If the mechanism of cardiac toxicity of antipsychotics is clarified in the future clinicians will be able to better establish the dose and length of treatment improving mental disease without impacting the physical health of the patients reducing mortality.\textsuperscript{[112-114]}

ACKNOWLEDGMENTS

All authors had no conflicts of interest to disclose.

REFERENCES

[1] Divac N, Prostan M, Jakovcevski I, et al. Second-generation antipsychotics and extrapyramidal adverse effects. Biomed Res Int 2014; 2014: 656370.
[2] Curto M, Girardi N, Lionetto L, et al. Systematic review of clozapine cardiotoxicity. Curr Psychiatry Rep 2016; 18: 68.
[3] Lee TW, Tsai SJ, Hwang JP. Severe cardiovascular side effects of olanzapine in elderly patient: case report. Int J Psychiatry Med 2003; 33: 399–401.
[4] Coulter DM, Bate A, Meyboom RH, et al. Antipsychotic drugs and heart muscle disorder in international pharmacovigilance: data miming study. BMJ 2001; 322: 1207–1209.
[5] Li KJ, Greenstein AP, Delisi LE. Sudden death in schizophrenia. Curr Opin Psychiatry 2018; 31: 169–175.
[6] Abdel-Wahab BA, Abdalla ME, El-khawanki MM. Does clozapine induce myocarditis, myocardial oxidative stress and DNA damage in rats? Egypt J Forensic Sci 2014; 4: 75–82.
[7] Haas SJ, Hill R, Krum H, et al. Clozapine-associated myocarditis: a review of 116 cases of suspected myocarditis associated with the use of clozapine in Australia during 1993–2003. Drug Saf 2007; 30: 47–57.
[8] Vang T, Rosenzweig M, Bruhn CH, et al. Eosinophilic myocarditis during treatment with olanzapine-report of two possible cases. BMC Psychiatry 2016; 16: 70.
[9] Papazisis G, Mastrogianni O, Chatzinikolaou F, et al. Sudden cardiac death due to quetiapine overdose. Psychiatry Clin Neurosci 2012; 66: 535.
[10] Wu CS, Tsai YT, Tsai HJ. Antipsychotic drugs and the risk of ventricular arrhythmia and/or sudden cardiac death: a nation-wide case-crossover study. J Am Heart Assoc 2015; 4: e001568.
[11] Pillinger T, Oimo EF, de Marvao A, et al. Cardiac structure and function in patients with schizophrenia taking antipsychotic drugs: an MRI study. Transl Psychiatry 2019; 9: 163.
[12] Khan AA, Ashraf A, Baker D, et al. Clozapine and incidence of myocarditis and sudden death: long-term Australian experience. Int J Cardiol 2017; 238: 136–139.
[13] Fineschi V, Neri M, Riezzo I, et al. Sudden cardiac death due to hypersensitivity myocarditis during clozapine treatment. Int J Legal Med 2004; 118: 307–309.
[14] Barber S, Thornicroft G. Reducing the mortality gap in people with severe mental disorders: the role of lifestyle psychosocial interventions. Front Psychiatry 2018; 9: 463.
[15] Engl J, Laimer M, Fleischhacker WW, et al. To: Mackin P, Watkinson HM, Young AH (2005) Prevalence of obesity, glucose homeostasis disorders and metabolic syndrome in psychiatric patients taking typical or atypical antipsychotic drugs: a cross-sectional study. Diabetologia 2005; 48: 1430–1431.
[16] Kritharides L, Chow V, Lambert TJ, et al. Cardiovascular disease in patients with schizophrenia. Med J
[17] Salvo F, Pariente A, Shakir S, et al. Sudden cardiac and sudden unexpected death related to antipsychotics: a meta-analysis of observational studies. Clin Pharmacol Ther 2016; 99: 306–314.

[18] Ray WA, Chung CP, Murray KT, et al. Atypical antipsychotic drugs and the risk of sudden cardiac death. N Engl J Med 2009; 360: 225–235.

[19] Lynghe TH, Nielsen TS, Gregers Winkel B, et al. Sudden cardiac death caused by myocarditis in persons aged 1–49 years: a nationwide study of 14, 294 deaths in Denmark. Forensic Sci Res 2019; 4: 247–256.

[20] De Berardinis D, Rapini G, Olivieri L, et al. Safety of antipsychotics for the treatment of schizophrenia: a focus on the adverse effects of clozapine. Ther Adv Drug Saf 2018; 9: 237–256.

[21] Arias LHM, Fadrique RS, García SP, et al. Antipsychotics and cardiovascular risk: a case/non-case study. Psychiatry Res 2018; 270: 341–347.

[22] Xu T, Ding W, Ji X, et al. Oxidative stress in cell death and cardiovascular diseases. Oxid Med Cell Longev 2019; 2019: 9030563.

[23] Korkmaz S, Korkmaz H, Özer Ö, et al. Assessment of left ventricular systolic and diastolic functions in schizophrenia patients. Psychiatry Res 2016; 240: 346–351.

[24] Chow V, Yeoh T, Ng AC, et al. Asymptomatic left ventricular dysfunction with long-term clozapine treatment for schizophrenia: a multicentre cross-sectional cohort study. Open Heart 2014; 1: e000030.

[25] Curto M, Comparelli A, Ciavarella CM, et al. Impairment of left ventricular function early in treatment with clozapine: a preliminary study. Int Clin Psychopharmacol 2015; 30: 282–289.

[26] Unsal C, Oran M, Tureli HO, et al. Detection of subclinical atherosclerosis and diastolic dysfunction in patients with schizophrenia. Neuropsychiatr Dis Treat 2013; 9: 1531–1537.

[27] Li X, Peng Z, Zhou Y, et al. Quetiapine induces myocardial necrotic cell death through bidirectional regulation of cannabinoid receptors. Toxicol Lett 2019; 313: 77–90.

[28] Peoples JN, Saraf A, Ghazal N, et al. Mitochondrial dysfunction and oxidative stress in heart disease. Exp Mol Med 2019; 51: 1–13.

[29] Collin F. Chemical basis of reactive oxygen species reactivity and involvement in neurodegenerative diseases. Int J Mol Sci 2019; 20: 2407.

[30] Nikolaidis MG, Kyparos A, Spanou C, et al. Redox biology of exercise: an integrative and comparative consideration of some overlooked issues. J Exp Biol 2012; 215: 1615–1625.

[31] Valavanidis A, Vlachogianni T, Fiotakis C. 8-hydroxy-2’-deoxyguanosine (8-OHdG): a critical biomarker of oxidative stress and carcinogenesis. J Environ Sci Health C Environ Carcinog Ecotoxicol Rev 2009; 27: 120–139.

[32] Xu T, Ding W, Ji X, et al. Oxidative stress in cell death and cardiovascular diseases. Oxid Med Cell Longev 2019; 2019: 9030563.

[33] Murphy E, Steenbergen C. Mechanisms underlying acute protection from cardiac ischemia-reperfusion injury. Physiol Rev 2008; 88: 581–609.

[34] Zhang M, Prosser BL, Bamboye MA, et al. Contractile function during angiotensin-II activation: increased NOX2 activity modulates cardiac calcium handling via phospholamban phosphorylation. J Am Coll Cardiol 2015; 66: 261–272.

[35] Zhang M, Brewer AC, Schröder K, et al. NADPH oxidase 4 mediates protection against chronic load-induced stress in mouse hearts by enhancing angiogenesis. Proc Natl Acad Sci USA 2010; 107: 18121–18126.

[36] Duncan JG. Mitochondrial dysfunction in diabetic cardiomyopathy. Biochim Biophys Acta 2011; 1813: 1351–1359.

[37] Senoner T, Dichtl W. Oxidative stress in cardiovascular diseases: still a therapeutic target? Nutrients 2019; 11: 2090.

[38] Reilly JG, Ayis SA, Ferrier IN, et al. QTc-interval abnormalities and psychotropic drug therapy in psychiatric patients. Lancet 2000; 355: 1048-1052.

[39] Zemva A, Zemva Z. Ventricular ectopic activity, left ventricular mass, hyperinsulinemia, and intracellular magnesium in normotensive patients with obesity. Angiology 2000; 51: 101–106.

[40] Smith RC, Leucht S, Davis JM. Maximizing response to first-line antipsychotics in schizophrenia: a review focused on finding from meta-analysis. Psychopharmacology (Berl) 2019; 236: 545-559.

[41] Howell S, Yarowova E, Khwanda A, et al. Cardiovascular effects of psychotic illnesses and antipsychotic therapy. Heart 2019; 105: 1852–1859.

[42] Mackin P. Cardiac side effects of psychiatric drugs. Hum Psychopharmacol 2008; 23: S3-S14.

[43] Jensen KG, Juul K, Fink-Jensen A, et al. Corrected QT changes during antipsychotic treatment of children and adolescents: a systematic review and meta-analysis of clinical trials. J Am Acad Child Adolesc Psychiatry 2015; 54: 25–36.

[44] Lin YL, Wu YC, Tsai CF. Electrocardiographic monitoring for QT prolongation in patients treated with ziprasidone—a claims database approach. Pharmacoepidemiol Drug Saf 2009; 18: 842–847.

[45] Coulther DM, Bate A, Meyboom RH, et al. Antipsychotic drugs and heart muscle disorder in international pharmacovigilance: data mining study. BMJ 2001; 322: 1207–1209.

[46] Kerwin RW, Bolonna AA. Is clozapine antisuicidal? Expert Rev Neurother 2004; 4: 187–190.

[47] Patel RK, Moore AM, Piper S, et al. Clozapine and cardiotoxicity: a guide for psychiatrists written by cardiologists. Psychiatry Res 2019; 282: 112491.

[48] Merrill DB, Dec GW, Goff DC. Adverse cardiac effects of psychiatric drugs and heart muscle disorder in international pharmacovigilance: data mining study. BMJ 2001; 322: 1207–1209.

[49] Kerwin RW, Bolonna AA. Is clozapine antisuicidal? Expert Rev Neurother 2004; 4: 187–190.

[50] Patel RK, Moore AM, Piper S, et al. Clozapine and cardiotoxicity: a guide for psychiatrists written by cardiologists. Psychiatry Res 2019; 282: 112491.
during 1993–2003. Drug Saf 2007; 30: 47–57.

Meeker JE, Herrmann PW, Som CW, et al. Clozapine tissue concentrations following an apparent suicidal overdose of Clozaril. J Anal Toxicol 1992; 16: 54–56.

Bandelow B, Degner D, Kreusch U, et al. Myocarditis under therapy with clozapine. Schizophr Res 1995; 17: 293–294.

Wassif N, Khan N, Munir S. Quetiapine-induced myocarditis presenting as acute STEMI. BMJ Case Rep 2015; 2015: bcr2014207151.

Varga ZV, Ferdinandy P, Llaudet L, et al. Drug-induced mithochondrial dysfunction and cardiotoxicity. Am J Physiol Heart Circ Physiol 2015; 309: H1453–H1467.

Cerretani D, Fineschi V, Bello S, et al. Role of oxidative stress in cocaine-induced cardiotoxicity and cocaine-related death. Curr Med Chem 2012; 19: 5619–5623.

Turillazzi E, Cerretani D, Cantatore S, et al. Myocardial oxidative damage is induced by cardiac Fas-dependent and mitochondria-dependent apoptotic pathways in human cocaine-related overdose. Sci Rep 2017; 7: 44262.

Matsu H, Morishima I, Numaguchi Y, et al. Protective effects of carvedilol against doxorubicin induced cardiomyopathy in rats. Life Sci 1999; 65: 1265–1274.

Doroshov JH, Locker GY, Iffirm I, et al. Prevention of doxorubicin cardiac toxicity in the mouse by N-acetylcysteine. J Clin Invest 1981; 68: 1053–1064.

Kilian JG, Kerr K, Lawrence C, et al. Myocarditis and cardiomyopathy associated with clozapine. Lancet 1999; 354: 1841–1845.

Hägg S, Spigset O, Bate A, et al. Myocarditis related to clozapine treatment. J Clin Psychopharmacol 2001; 21: 382–388.

Patel RK, Moore AM, Piper S, et al. Clozapine and cardiotoxicity: a guide for psychiatrists written by cardiologists. Psychiatry Res 2019; 282: 112491.

Christoffersen RK, Vestergård LD, Hømmark L, et al. [Eosinophilic myocarditis and sudden unexpected death in a younger patient treated with antipsychotics]. Ugeskr Laeger 2011; 173: 2799–2800. [In Danish].

Wang JF, Min JY, Hampton TG, et al. Clozapine-induced myocarditis: role of catecholamines in a murine model. Eur J Pharmacol 2008; 592: 123–127.

Breier A, Buchanan RW, Waltrip RW 2nd, et al. The effect of clozapine on plasma norepinephrine: relationship to clinical efficacy. Neuropsychopharmacology 1994; 10: 1–7.

Elman I, Goldstein DS, Eisenhofer G, et al. Mechanism of peripheral noradrenergic stimulation by clozapine. Neuropsychopharmacology 1999; 20: 29–34.

Nikolić-Kokić A, Tatalović N, Nestorov I, et al. Clozapine, ziprasidone, and serindole-induced morphological changes in the rat heart and their relationship to antioxidant enzymes function. J Toxicol Environ Health A 2018; 81: 844–853.

Pirmohamed M, Williams D, Madden S, et al. Metabolism and bioactivation of clozapine by human liver in vitro. J Pharmacol Exp Ther 1995; 272: 984–990.

Abdel-Wahab BA, Metwally ME, El-khawanki MM, et al. Protective effect of captopril against clozapine-induced myocarditis in rats: role of oxidative stress, proinflammatory cytokines and DNA damage. Chem Biol Interact 2014; 216: 43–52.

Vaddadi KS, Soosai E, Vaddadi G. Low blood selenium concentrations in schizophrenic patients on clozapine. Br J Clin Pharmacol 2003; 55: 307–309.

Heiser P, Sommer O, Schmidt AJ, et al. Effects of antipsychotics and vitamin C on the formation of reactive oxygen species. J Psychopharmacol 2010; 24: 1499–1504.

Williams DP, O’Donnell CJ, Maggs JL, et al. Bioactivation of clozapine by murine cardiac tissue in vivo and in vitro. Chem Res Toxicol 2013; 16: 1359–1364.

Pollmächer T, Hinze-Selch D, Mullington J. Effects of clozapine on plasma cytokine and soluble cytokine receptor levels. J Clin Psychopharmacol 1996; 16: 403–409.

Abdel-Wahab BA, Metwally ME. Clozapine-induced cardiotoxicity: role of oxidative stress, tumour necrosis factor Alpha and NF-κB. Cardiovasc Toxicol 2015; 15: 355–365.

Shin WS, Szuba A, Rockson SG. The role of chemokines in human cardiovascular pathology: enhanced biological insights. Atherosclerosis 2002; 160: 91–102.

Haack MJ, Bak ML, Beurskens R, et al. Toxic rise of clozapine plasma concentrations in relation to inflammation. Eur Neuropsychopharmacol 2003; 13: 381–385.

Nimata M, Okabe TA, Hattori M, et al. MCI-186 (edaravone), a novel free radical scavenger, protects against acute autoimmune myocarditis in rats. Am J Physiol Heart Circ Physiol 2005; 289: H1251–H1258.

Sovari AA, Rutledge CA, Jeong EM, et al. Mitochondria oxidative stress, connexin43 remodeling, and sudden arrhythmic death. Circ Arrhythm Electrophysiol 2013; 6: 623–631.

Arumugam S, Thanadavaryan RA, Veeradevu PT, et al. Beneficial effects of edaravone, a novel antioxidiant, in rats with dilated cardiomyopathy. J Cell Mol Med 2012; 16: 2176–2185.

Watanabe N, Nakagawa S, Fukunaga T, et al. Acute necrotizing eosinophilic myocarditis successfully treated by high dose methylprednisolone. Jpn Circ J 2001; 65: 923–926.

Xie X, Zhao Y, Ma CY, et al. Dimethyl fumarate induces necroptosis in colon cancer cells through GSH depletion/ROS increase/MAPKs activation pathway. Br J Pharmacol 2015; 172: 3929–3943.

Frangogiannis NG, Smith CW, Entman ML. The inflammatory response in myocardial infarction. Cardiovasc Res 2002; 53: 31–47.

Ishiyama S, Hiroe M, Nishikawa T, et al. Nitric oxide contributes to the progression of myocardial damage in experimental autoimmune myocarditis in rats. Circulation 1997; 95: 489–496.

Hogan K, Ahmed O, Markos F. N-desmethylclozapine treatment of M1 receptor agonist enhances nitric oxide’s cardiac vgal facilitation in the isolated innervated rat right atrium. Auton Neurosci 2007; 137: 51–55.

Esposito E, Cuzzocrea S. Role of nitroso radicals as drug targets in circulatory shock. Br J Pharmacol 2009; 157: 494–508.

Finkel MS, Oddis CV, Jacob TD, et al. Negative inotropic effects of cytokines on the heart mediated by nitric oxide. Science 1992; 257: 387–389.
induced by neuroleptic drugs in the rabbit. *Exp Toxicol Pathol* 2006; 57: 207–212.

[88] Yan H, Li WL, Xu JJ, *et al.* D2 dopamine receptor antagonist raclopride induces non-canonical autophagy in cardiac myocytes. *J Cell Biochem* 2013; 114: 103–110.

[89] Shinoda Y, Tagashira H, Bhuiyan MS, *et al.* Haloperidol aggravates transverse aortic constriction-induced heart failure via mitochondrial dysfunction. *J Pharmacol Sci* 2016; 131: 172–183.

[90] Roden DM. Drug-induced prolongation of the QT interval. *N Engl J Med* 2004; 350: 1013–1022.

[91] Meléndez GC, McLarty JL, Levick SP, *et al.* Interleukin-6 mediates myocardial fibrosis, concentric hypertrophy, and diastolic dysfunction in rats. *Hypertension* 2010; 56: 225–231.

[92] Johnson TL, Tulis DA, Keeler BE, *et al.* The dopamine D3 receptor knockout mouse mimics aging-related changes in autonomic function and cardiac fibrosis. *PLoS One* 2013; 8: e74416.

[93] Pacher P, Schulz R, Liaudet L, *et al.* Nitrosative stress and pharmacological modulation of heart failure. *Trends Pharmacol Sci* 2005; 26: 302–310.

[94] Li S, Jiao X, Tao L, *et al.* Tumor necrosis factor-alpha in mechanic trauma plasma mediates cardiomyocyte apoptosis. *Am J Heart Circ Physiol* 2007; 293: H1847–H1852.

[95] Heiser P, Sommer O, Schmidt AJ, *et al.* Effects of antipsychotics and vitamin C on the formation of reactive oxygen species. *JP3ychopharmacol* 2010; 24: 1499–1504.

[96] Duncan JG. Mitochondrial dysfunction in diabetic cardiomyopathy. *Biochim Biophys Acta* 2011; 1813: 1351–1359.

[97] Joseph LC, Subramaniam P, Radicz C, *et al.* Mitochondrial oxidative stress during cardiac lipid overload causes intracellular calcium leak and arrhythmia. *Heart Rhythm* 2016; 13: 1699–1706.

[98] Gong F, Gu J, Wang H. Up regulated Tmbim1 activation promotes high fat diet (HFD)-induced cardiomyopathy by enhancement of inflammation and oxidative stress. *Biochim Biophys Res Commun* 2018; 504: 797–804.

[99] Tse G, Yan BP, Chan YW, *et al.* Reactive oxygen species, endoplasmic reticulum stress and mitochondrial dysfunction: the link with cardiac arrhythmogenesis. *Front Physiol* 2016; 7: 313.

[100] Bornfeldt KE, Tabas I. Insulin resistance, hyperglycemia, and atherosclerosis. *Cell Metab* 2011; 14: 575–585.

[101] Tompkins AJ, Burwell LS, Diggeress SB, *et al.* Mitochondrial dysfunction in cardiac ischemia-reperfusion injury: ROS from complex I, without inhibition. *Biochim Biophys Acta* 2006; 1762: 223–231.

[102] Sugiyama K, Sasano T, Kurokawa J, *et al.* Oxidative stress induced ventricular arrhythmia and impairment of cardiac function in NOS1AP deleted mice. *Int Heart J* 2016; 57: 341–349.

[103] Manu P, Kane JM, Correll CU. Sudden deaths in psychiatric patients. *J Clin Psychiatry* 2011; 72: 936–941.

[104] Kelly DL, Wehring HJ, Linthicum J, *et al.* Cardiac-related findings at autopsy in people with severe mental illness treated with clozapine or risperidone. *Schizophr Res* 2009; 107: 134–138.

[105] Kelly DL, McMahon RP, Liu F, *et al.* Cardiovascular disease mortality in patients with chronic schizophrenia treated with clozapine: a retrospective cohort study. *J Clin Psychiatry* 2010; 71: 304–311.

[106] Ifeni P, Correll CU, Burtea V, *et al.* Sudden unexpected death in schizophrenia: autopsy findings in psychiatric inpatients. *Schizophr Res* 2014; 155: 72–76.

[107] Jones ME, Campbell G, Patel D, *et al.* Risk of mortality (including sudden cardiac death) and major cardiovascular events in users of olanzapine and other antipsychotics: a study with the general practice research database. *Cardiovasc Psychiatry Neurol* 2013; 2013: 647476.

[108] Tietze F. Enzymic method for quantitative determination of nanogram amounts of total and oxidized glutathione: applications to mammalian blood and other tissues. *Anal Biochem* 1969; 27: 502–522.

[109] Shara MA, Dickson PH, Bagchi D, *et al.* Excretion of formaldehyde, malondialdehyde, acetaldehyde and acetone in the urine of rats in response to 2, 3, 7, 8- tetrachlorodibenz-p-dioxin, paraxat, endrin and carbon tetrachloride. *J Chromatogr* 1992; 576: 221–233.

[110] Santurro A, Vullo AM, Borro M, *et al.* Personalized medicine applied to forensic sciences: new advances and perspectives for a tailored forensic approach. *Curr Pharm Biotechnol* 2017; 18: 263–273.

[111] La Russa R, Catalano C, Di Sanzo M, *et al.* Postmortem computed tomography angiography (PMCTA) and traditional autopsy in cases of sudden cardiac death due to coronary artery disease: a systematic review and meta-analysis. *Radiol Med* 2019; 124: 109–117.

[112] Di Sanzo M, Cipolloni L, Borro M, *et al.* Clinical applications of personalized medicine: a new paradigm and challenge. *Curr Pharm Biotechnol* 2017; 18: 194–203.

[113] La Russa R, Fineschi V, Di Sanzo M, *et al.* Personalized medicine and adverse drug reactions: the experience of an Italian teaching hospital. *Curr Pharm Biotechnol* 2017; 18: 274–281.

[114] Borro M, Gentile G, Cipolloni L, *et al.* Personalised healthcare: the DiMA clinical model. *Curr Pharm Biotechnol* 2017; 18: 242–252.

[115] Aiyararajah V, Shaikh N, Garber PJ, *et al.* Cardiovascular magnetic resonance in mild to moderate clozapine-induced myocarditis: is there a role in the absence of electrocardiographic and echocardiographic abnormalities? *J Magn Reson Imaging* 2010; 31: 1473–1476.

[116] Bellissima BL, Tingle MD, Covic C, *et al.* A systematic review of clozapine-induced myocarditis. *Int J Cardiol* 2018; 259: 121–129.

[117] Gareri P, De Fazio P, Russo E, *et al.* The safety of clozapine in the elderly. *Expert Opin Drug Saf* 2008; 7: 525–538.

[118] Hussein O, Izikson L, Bathish Y, *et al.* Anti-atherogenic properties of high-density lipoproteins in psychiatric patients before and after two months of atypical antipsychotic therapy. *J Psychopharmacol* 2015; 29: 1262–1270.

[119] Jerrell JM, McIntyre RS, Tripathi A. Incidence and costs of cardiometabolic conditions in patients with schizophrenia treated with antipsychotic medications. *Clin Schizophr Relat Psychoses* 2010; 4: 161–168.
Katta N, Balla S, Aggarwal K. Clozapine-induced hypersensitivity myocarditis presenting as sudden cardiac death. *Autops Case Rep* 2016; 6: 9–13.

Kontoangeli K, Loizos S, Kanakis K, et al. Myocarditis after administration of clozapine. *Eur Rev Med Pharmacol Sci* 2014; 18: 2383–2386.

Korkmaz H, Korkmaz S. Hearts and minds: real-life cardiotoxicity with clozapine in psychosis. *J Clin Psychopharmacol* 2019; 39: 694.

Lang UE, Willbring M, von Golitschek R, et al. Clozapine-induced myocarditis after long-term treatment: case presentation and clinical perspectives. *J Psychopharmacol* 2008; 22: 576–580.

Marti V. Sudden cardiac death due to risperidone therapy in a patient with possible hypertrophic cardiomyopathy. *Ann Pharmacother* 2005; 39: 973.

Peters S, Klein HU. Life-threatening ventricular arrhythmias due to atypcal mid-ventricular tako tsubo cardiomyopathy in a patient with chronic QT interval prolongation under anti-psychotic medication. *Int J Cardiol* 2014; 172: e534–e536.

Polciwiatrycz M, Sneider B, Graff C, et al. The cardiac safety of atipiprazole treatment in patients at high risk for torsade: a systematic review with a meta-analytic approach. *Psychopharmacology (Berlin)* 2015; 232: 3297–3308.

Polciwiatrycz M, Krugholm K, Schjerme O, et al. Cardiovascular safety of atypical antipsychotics: a clinical overview. *Expert Opin Drug Saf* 2016; 15: 679–688.

Serrano A, Rangel N, Carrizo E. Safety of long-term clozapine administration. Frequency of cardiomyopathy and hypotension: two cross-sectional, naturalistic studies. *Aust N Z J Psychiatry* 2014; 48: 183–192.

Sicouri S, Antzelevitch C. Sudden cardiac death secondary to antidepressant and atypical antipsychotic drugs. *Expert Opin Drug Saf* 2008; 7: 181–194.

Smolders DME, Smolders WAP. Case report and review of the literature: cardiomyopathy in a young woman on high-dose quetiapine. *Cardiovasc Toxicol* 2017; 17: 478–481.

Vieweg WV. New generation antipsychotic drugs and QTc interval prolongation. *Prem Care Companion J Clin Psychiatry* 2003; 5: 205–215.

Zhu J, Hou W, Xu Y. Antipsychotic drugs and sudden cardiac death: a literature review of the challenges in the prediction, management, and future steps. *Psychiatry Res* 2019; 281: 112598.

Arunagam S, Thandavaryan RA, Veeraveedu PT. Beneficial effects of edaravone, a novel antioxidant, in rats with dilated cardiomyopathy. *J Cell Mol Med* 2012; 16: 2176–2185.

Auger F, Martin F, Pérault O. Risperidone-induced metabolic dysfunction is attenuated by Curcuma longa extract administration in mice. *Metab Brain Dis* 2018; 33: 63–77.

Dogan HO, Ersan EE, Aydin H. Thiol disulfide homeostasis in schizophrenic patients using atypical antipsychotic drugs. *Clin Psychopharmacol Neurosci* 2018; 16: 39–45.

Fehsel K, Leeffler S, Krieger K. Clozapine induces oxidative stress and proapoptotic gene expression in neutrophils of schizophrenic patients. *J Clin Psychopharmacol* 2005; 25: 419–426.

Hendouei N, Farnia S, Mohseni F, et al. Alterations in oxidative stress markers and its correlation with clinical findings in schizophrenic patients consuming perphenazine, clozapine and risperidone. *Biomed Pharmacother* 2018; 103: 965–972.

Nikolic-Kokić A, Tatalović N, Nestorov J, et al. Clozapine, ziprasidone, and sertindole-induced morphological changes in the rat heart and their relationship to antioxidant enzymes function. *J Toxicol Environ Health A* 2018; 81: 844–853.

Padurariu M, Ciobica A, Dobrin I, et al. Evaluation of antioxidant enzymes activities and lipid peroxidation in schizophrenic patients treated with typical and atypical antipsychotics. *Neurosci Lett* 2010; 479: 317–320.

Vidović B, Đorđević B, Milovanović S, et al. Selenium, zinc, and copper plasma levels in patients with schizophrenia: relationship with metabolic risk factors. *Biol Trace Elem Res* 2013; 156: 22–28.

Abu-Elsaad N, El-Karef A. The falconoid luteolin mitigates the myocardial inflammatory response induced by high-carbohydrate/high-fat diet in wistar rats. *Inflammation* 2018; 41: 221–231.

Akao M, O’Rourke B, Teshima Y, et al. Mechanistically distinct steps in the mitochondrial death pathway triggered by oxidative stress in cardiac myocytes. *Circ Res* 2003; 92: 186–194.

Brown DA, O’Rourke B. Cardiac mitochondria and arrhythmias. *Cardiovasc Res* 2010; 88: 241–249.

Fineschi V, Michalodimitrakis M, D’Errico S. Insight into stress-induced cardiomyopathy and sudden cardiac death due to stress. A forensic cardio-pathologist point of view. *Forensic Sci Int* 2010; 194: 1–8.

Javadi B, Salehkar A. Natural products with anti-inflammatory and immunomodulatory activities against autoimmune myocarditis. *Pharmacol Res* 2017; 124: 34–42.

Karaguezian HS, Katzung BG. Voltage-clamp studies of transient inward current and mechanical oscillations induced by ouabain in ferret papillary muscle. *J Physiol* 1982; 327: 255–271.

Kilian JG, Kerr K, Lawrence C, et al. Myocarditis and cardiomyopathy associated with clozapine. *Lancet* 1999; 354: 1841–1845.

Kishimoto C, Shioji K, Kinoshita M, et al. Treatment of acute inflammatory cardiomyopathy with intravenous immunoglobulin ameliorates left ventricular function associated with suppression of inflammatory cytokines and decreased oxidative stress. *Int J Cardiol* 2003; 91: 173–178.

Koponen H, Alaräsänen A, Saari K, et al. Schizophrenia and sudden cardiac death: a review. *Nord J Psychiatry* 2008; 62: 342–345.

Krexi L, Georgiou R, Krexi D, et al. Sudden cardiac death with stress and restraint: the association with sudden adult death syndrome, cardiomyopathy and coronary artery disease. *Med Sci Law* 2016; 56: 85–90.

Li YC, Luo Q, Ge LS, et al. Ivabradine inhibits the production of proinflammatory cytokines and inducible
nitric oxide synthase in acute coxsackievirus B3-induced myocarditis. Biochem Biophys Res Commun 2013; 431: 450–455.

[152] Marian AJ, Senthil V, Chen SN, et al. Antifibrotic effects of antioxidant N-acetylcysteine in a mouse model of human hypertrophic cardiomyopathy mutation. J Am Coll Cardiol 2006; 47: 827–834.

[153] Mito S, Watanabe K, Harima M. Curcumin ameliorates cardiac inflammation in rats with autoimmune myocarditis. Biol Pharm Bull 2011; 34: 974–979.

[154] Miyamoto S, Kawano H, Sakamoto T, et al. Increased plasma levels of thioredoxin in patients with coronary spastic angina. Antioxid Redox Signal 2004; 6: 75–80.

[155] Octavia Y, Brunner-La Rocca HP, Moens AL. NADPH oxidase-dependent oxidative stress in the failing heart: from pathogenic roles to therapeutic approach. Free Radic Biol Med 2012; 52: 291–297.

[156] Pacher P, Szabo C. Role of the peroxynitrite-poly (ADP-ribose) polymerase pathway in human disease. Am J Pathol 2008; 173: 2–13.

[157] Raucci A, Di Maggio S, Scavello F, et al. The Janus face of HMGB1 in heart disease: a necessary update. Cell Mol Life Sci 2019; 76: 211–229.

[158] Shimada K, Uzui H, Ueda T, et al. N-acetylcysteine ameliorates experimental autoimmune myocarditis in rats via nitric oxide. J Cardiovasc Pharmacol Ther 2015; 20: 203–210.

[159] Singh OP, Chakraborty I, Dasgupta A, et al. A comparative study of oxidative stress and interrelationship of important antioxidants in haloperidol and olanzapine treated patients suffering from schizophrenia. Indian J Psychiatry 2008; 50: 171–176.

[160] Skrzypiec-Spring M, Haczkiewicz K, Sapa A, et al. Cardvedilol inhibits matrix metalloproteinase-2 activation in experimental autoimmune myocarditis: possibilities of cardioprotective application. J Cardiovasc Pharmacol Ther 2018; 23: 89–97.

[161] Sukumaran V, Veeraveedu PT, Gurusamy N, et al. Cardioprotective effects of telmisartan against heart failure in rats induced by experimental autoimmune myocarditis through the modulation of angiotensin-converting enzyme-2/angiotensin 1-7/ mas receptor axis. Int J Biol Sci 2011; 7: 1077–1092.

[162] Wu B, Li J, Ni H. TLR4 activation promotes the progression of experimental autoimmune myocarditis to dilated cardiomyopathy by inducing mitochondrial dynamic imbalance. Oxid Med Cell Longev 2018; 2018: 3181278.

[163] Yang KC, Kyle JW, Makielski JC, et al. Mechanisms of sudden cardiac death: oxidants and metabolism. Circ Res 2015; 116: 1937–1955.

[164] Yuan Z, Shioji K, Kihara Y, et al. Cardioprotective effects of carvedilol on acute autoimmune myocarditis: anti-inflammatory effects associated with antioxidant property. Am J Physiol Heart Circ Physiol 2004; 286: H83–H90.

[165] Ray WA, Chung CP, Murray KT, et al. Atypical antipsychotic drugs and the risk of sudden cardiac death. N Engl J Med 2009; 360: 225–235.