ORIGINAL ARTICLE

Does clozapine induce myocarditis, myocardial oxidative stress and DNA damage in rats?

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Abstract Clozapine is an atypical antipsychotic drug selectively effective in the treatment of refractory schizophrenia. However, myocarditis, as a serious cardiotoxic effect, has in several case reports been associated with clozapine therapy. An increase in the free radical production and ischemia, probably induced by clozapine-induced release of catecholamines, have been hypothesized to trigger an inflammatory response that leads to the clinically observed cardiomyopathy and sudden death even in young patients. The aim of this work is to study the role of oxidative stress in clozapine-induced myocarditis and myocardial DNA damage in a rat model.

Methods: Male Wistar rats, age ~6 weeks, were administered 5, 10 or 25 mg/kg clozapine daily for 21 days; saline-treated rats served as the control. Heart sections were stained with hematoxylin and eosin for histopathological examination. Plasma CK-MB, LDH and TNF-α concentrations were determined. Myocardial oxidative stress (MDA and NO), antioxidant (GSH and GSH-Px) parameters, and the marker of oxidative DNA damage (8-OHdG) were determined.

Results: Clozapine treatment resulted in significant dose-related increases in myocardial inflammation with increased plasma TNF-α, CK-MB and LDH levels. Myocardial MDA, NO and serum and cardiac 8-OHdG levels increased while GSH level and GSH-Px activity decreased with the highest significance seen with the largest tested dose (25 mg/kg) of clozapine.

Conclusions: Clozapine, in relatively large doses induced myocarditis consistently with increased myocardial oxidative stress, DNA damage and inflammatory cytokines in a rat model.

1. Introduction

Clozapine, a tricyclic dibenzodiazepine, belongs to the class of second-generation antipsychotics that are often called atypical antipsychotics. It has a strong affinity for D4-dopami-nergic receptors[1] and potent serotonergic, noradrenergic, histamine[2,3] and cholinergic M2 receptor blocking activity.[4] It differs from traditional antipsychotic drugs in that it has a relatively weak D2-receptor activity.[1] The main advantage of clozapine is that it rarely causes extrapyramidal symptoms, which are common with conventional antipsychotics, in addition to its effectiveness in treating resistant schizophrenia.[5]

However, some toxic effects of clozapine caused the Food and Drug Administration (FDA) to restrict its use, requiring close monitoring conditions and reserving the medication for treatment-resistant schizophrenia unresponsive to conventional antipsychotics. Among these adverse effects are agranulocytosis that occurs in about 1% of patients,[5] venous thromboembolism[6] and seizures.[7] A commonly reported and serious adverse effect of clozapine is its potential to induce cardiotoxicity and myocarditis. Myocarditis has been reported in many clinical case reports in young schizophrenic patients on clozapine therapy, without previous cardiovascular diseases.[8] Additionally, health professionals are being warned of potentially fatal myocarditis, cardiomyopathy, pericarditis, heart failure and eventually death associated with clozapine.[9]

The exact pathogenesis and incidence of clozapine-induced myocarditis is not clearly understood up till now. Previous studies have shown the role of increased catecholamines. Clozapine treatment has been associated with increased plasma levels of the catecholamines, norepinephrine and epinephrine.[10] In addition, hyper-catecholaminergic states can cause or significantly exacerbate myocarditis in animals and patients.[11] While, the increase in cardiac catecholamines by clozapine cannot be considered the sole cause, involvement of inflammatory cytokines cannot be neglected. Clozapine-induced myocarditis has been associated with an increased release of inflammatory cytokines, including interleukins and tumor necrosis factor alpha (TNF-α).[12]

Previous studies have postulated an increase in the level of reactive oxygen species (ROS) in the myocardium during the development of myocarditis and heart failure in experimental animals and in human patients.[13] Myocardial ischemia, which may result from a clozapine-induced increase in catecholamine release, can lead to cell injury with the release of ROS.[14] Cell injury in the ischemic area also causes infiltration of neutrophils, which produce oxidants and cytokines. Certain cytokines such as tumor necrosis factor-α (TNF-α) trigger the mitochondrial release of ROS.[15] In addition, an increase in ROS have been detected in various animal models of heart failure. An increase in oxidative stress due to increased production of ROS, a relative deficit in the endogenous antioxidant reservoir, or both can cause myocarditis, contractile dysfunction and cardiomyopathy.[16,17] Moreover, oxygen free radical damage has been implicated as a precipitating event in ischemia-, overload-, or drug-induced heart failure.[18]

Hence, the purpose of this study was to find an answer for this question: Does clozapine induce myocarditis via increasing myocardial oxidative stress and DNA damage in rats?

2. Materials and methods

2.1. Chemicals

Clozapine (8-chloro-11-(4-methyl-1-piperazinyl)-5H-dibenzo [be] [1,4] diazepine) (Sigma Aldrich, St. Louis, MO) was dissolved in 0.1 M HCl and pH balanced in phosphate buffered saline (PBS) (Sigma Aldrich, St. Louis, MO). Solution was administered intraperitoneally (i.p.) daily in 0.1 ml doses. Thiobarbituric acid, reduced glutathione (GSH), Griss reagent, Ellman's reagent [5,5-dithiobis (2-nitrobenzoic acid), DTNB] and bovine serum albumin (BSA) were purchased from Sigma (Germany). Cayman's 8-hydroxy-2-deoxy guanosine (8-OHdG) assay kit was purchased from Cayman's Chemical Co. (USA). All the reagents used in this study were of analytical grade.

2.2. Animals

Male Wistar rats with 160–180 g body weight from the animal house of the King Saud University, Riyadh, Saudi Arabia were used in this study. Animals were housed in groups of 5 rats in standard clear polycarbonate cages with food and water available ad libitum. Animals were kept on a 12-h light–dark schedule (6:00 am–6:00 pm), and all experimental testing was conducted during the light phase from 9:00 am to 12:00 pm. All experiments were carried out in accordance with the National Institutes of Health guide for the care and use of laboratory animals (NIH Publications No. 8023, revised 1978). The experimental protocol was approved by the Institutional Animal Use and Care Committee. All efforts were made to minimize animal suffering and to reduce the number of animals used.

2.3. Experimental protocol

The animals were divided into four groups, ten rats for each. Clozapine was administered in doses of 10, 15 and 25 mg/kg/day i.p. for 21 days in three groups. The fourth group was used as the control and was treated with physiological saline. Moderate to high doses of clozapine were used based on previous reports.[11] Mortality and animal weights were recorded during the course of treatment as an index for clozapine toxicity. On the last day of clozapine or saline administration, animals were anesthetized with 45 mg/kg ketamine and 5 mg/kg xylazine i.p. (Sigma Aldrich, St. Louis, MO), blood was drawn by cardiac puncture. Blood samples were centrifuged at 1300 g at 25 °C for 15 min. and serum was obtained. Animals were sacrificed by decapitation and hearts were excised, washed with ice-cold saline, blotted with a piece of filter paper and divided longitudinally into two halves. One-half of each heart was homogenized using a Bio-Gen pro 200 homogenizer (Pro Scientific, Oxford, UK) in phosphate buffer (pH 7). The homogenates were centrifuged at 1300g at 4 °C for 15 min. The supernatant of the homogenate and serum were used for biochemical assays.

2.4. Histopathology

Ventricles of the second half of each heart were fixed in a 10% neutral formalin solution, then embedded in paraffin,

sectioned at a thickness of 5 lm and stained with hematoxylin and eosin (H/E), and examined by light microscopy. The ventricle specimens were evaluated for typical histopathological features associated with clozapine-induced cardiotoxicity (including inflammation, myocyte vacuolar degradation, necrosis of myofibers, and interstitial fibrosis). Histological evidence of myocarditis was classified in terms of the degree of cellular infiltration and graded on a 5-point scale ranging from 0 to 4+ .[19] A zero score indicated no or questionable presence of lesions in each category. A 1+ score described a limited focal distribution of myocardial lesions. A 2+ to 3+ score described intermediate severity with multiple lesions, whereas a 4+ score described the presence of coalescent and extensive lesions over the entire examined heart tissue.

2.5. Biochemical assays

2.5.1. Determination of serum creatine kinase (CK-MB) activity

Creatine kinase activity was estimated in serum by commercially available CK-MB assay kit (Bioassay Systems, USA) adopting the method of Bishop.[20] This assay is based on the conversion of creatine phosphate and ADP by creatine kinase to creatine and ATP. The ATP and glucose are then converted to ADP and glucose-6-phosphate by hexokinase (HK). Glucose-6-phosphate dehydrogenase (G6PD) then oxidizes glucose-6-phosphate and reduces the nicotinamide adenine dinucleotide (NAD) to NADH. The rate of NADH formation, measured at 340 nm, is, therefore, directly proportional to creatine kinase activity.

2.5.2. Determination of serum lactate dehydrogenase (LDH) activity

Lactate dehydrogenase activity was estimated in serum by commercially available LDH kit (Linear Chemicals, S.L., UK) according to the method of Whitaker.[21] Using this method, lactate dehydrogenase (LDH) catalyzes the reduction of pyruvate to lactate in the presence of reduced nicotinamide adenine dinucleotide (NADH) at pH 7.5. The reaction is monitored kinetically at 340 nm using an UV–Visible spectrophotometer (Shimadzu, Japan) by the rate of decrease in absorbance resulting from the oxidation of NADH to NAD^+ which is proportional to the activity of LDH present in the sample.

2.5.3. Determination of tumor necrosis factor alpha (TNF-α)

The quantitative measurement of TNF-α in the cardiac homogenate was performed using commercially available ELISA kits (Quantikine ELISA kit; R&D Systems, Minneapolis, USA) according to manufacturer's instructions.

2.5.4. Determination of lipid peroxidation

The quantitative measurement of lipid peroxidation in the cardiac homogenate was performed according to the method previously described by Ohkawa.[22] The amount of malondialdehyde (MDA)–a measure of lipid peroxidation–was measured by reaction with thiobarbituric acid at 532 nm using an Optima SP 3000 plus spectrophotometer (Indogama, Japan).

2.5.5. Determination of nitric oxide

The level of NO was measured in the cardiac homogenate by assaying nitrite, one of the stable products of NO oxidation. The method was carried out as described by Green.[23] Nitrite concentration was measured spectrophotometrically using the Griss reagent [1% sulfanilamide in 5% phosphoric acid (sulfanilamide solution) and 0.1% N-1-naphthylethylenedi-amine dihydrochloride in double distilled water]. A standard curve was plotted. Nitrite concentrations in samples were expressed as micromoles per gram protein (lmol/g protein).

2.5.6. Determination of GSH level and GSH-Px activity

For determination of intracellular GSH, an equal volume of perchloric acid (1 mol/l) was added to a part of cardiac homogenate and mixed by vortexing. The mixture was allowed to stand for 5 min at room temperature. After centrifugation for 5 min, the supernatant was collected. The GSH content of the neutralized supernatant was assayed using Ellman's reagent [5,5-dithiobis-2-nitrobenzoic acid (DTNB solution)] according to the method of Griffith.[24]

GSH-Px activity was measured by the method of Paglia.[25-] The enzymatic reaction, which contained b-nicotinamide adenine dinucleotide phosphate (NADPH), GSH, glutathione reductase and a sample or a standard, was initiated by the addition of hydrogen peroxide. The change in absorbance was measured spectrophotometrically. A standard curve was plotted for each assay.

2.5.7. Determination of 8-OHdG levels

8-OHdG is produced by the oxidative damage of DNA by reactive oxygen and nitrogen species and serves as an established marker of oxidative stress. Cayman's 8-hydroxy-2′-deoxy guanosine assay kit purchased from Cayman's Chemical Co. (USA) was used. It is a competitive assay that can be used for the quantification of 8-OHdG in serum and tissue homogenate. It recognizes both free and DNA-incorporated 8-OHdG. This assay depends on the competition between 8-OHdG and 8-OHdG-acetylcholinesterase (ache) conjugate (8-OHdG tracer) for a limited amount of 8-OHdG monoclonal antibody. All procedures were carried out in accordance with manufacturer's instructions.

2.5.8. Estimation of total protein

Total protein in hippocampal homogenate was estimated using the method of Lowry.[26] The absorption was read spectrophotometrically at 750 nm. Bovine serum albumin was used as standard.

2.6. Statistical analysis

The Instat version 2.0 (graph pad Prism 5, ISI Software, Philadelphia, PA, USA, 1993) computer program was used to compute statistical data. All experimental results are expressed as the mean ± SEM. Comparisons between experimental and control groups were performed by one-way analysis of variance (ANOVA) followed by Bonferroni's test for post hoc comparison when appropriate. A value of $p < 0.05$ was considered significant.

3. Results

3.1. Clozapine-induced myocarditis

Table 1 shows that rats treated with clozapine (25 mg/kg) had a higher increase in mortality (20%) and an increase in body weight (9 g ± 1.26) at the end of the experiments. Rats treated with clozapine of 10 and 15 mg/kg showed an increase in body weight 2.6 g ± 0.32 and 4 g ± 0.43, respectively. No mortality was observed in these two groups. Heart tissues revealed histological findings within normal limits in the control group (Figure 1A). In clozapine-treated groups, histological sections revealed interstitial edema, perinuclear vacuolation, evident focal sub-endocardial fibrosis and disorganization and degradation of the myocardium. Myocardial inflammation increased with increasing clozapine dose with the highest scores at dose 25 mg/kg/d (Figures 1B–D) and was significantly increased relative to controls on day 21 of clozapine treatment for all doses (Table 1). Inflammatory lesions were found in both the left and right ventricles, primarily in the myocardium below the endocardium of the left ventricle, in the posterior papillary muscle of the left ventricle and the septum, consistent with myocarditis.

3.2. Effect on Serum CK-MB and LDH activities

Results from the measurement of serum CK-MB and LDH showed significant changes in its levels among the tested groups $[F(3, 39) = 8.538, p = 0.0002]$ and $[F(3, 39) = 6.398, p = 0.0014]$, respectively. Serum CK-MB significantly increased with the dose of 15 mg/kg/d ($p < 0.05$) and with the dose of 25 mg/kg/d ($p < 0.01$) compared with the control group (Figure 2). In addition, the serum LDH level significantly increased ($p < 0.05$) with the dose of 10 mg/kg/d and ($p < 0.01$) with the doses of 15 and 25 mg/kg/d of clozapine (Figure 3).

3.3. Effect on cardiac TNF-α level

Results revealed significant changes in cardiac levels of TNF-α after treatment with clozapine $[F(3, 39) = 6.511, p = 0.0012]$. Clozapine treatment significantly increased the TNF-α level ($p < 0.05$) with the dose of 15 mg/kg/d and ($p < 0.01$) with the dose of 25 mg/kg/d relative to the control group (Figure 4).

3.4. Effect on cardiac anti-oxidants

Results obtained from the effects of clozapine on cardiac levels of MDA, NO, GSH and GSH-Px activity are shown in Table 2. Results showed that clozapine treatment significantly affected myocardial lipid peroxidation and cardiac levels of MDA $[F(3, 39) = 7.158, p = 0.0007]$. Post hoc analysis indicated that clozapine treatment significantly increased cardiac MDA levels ($p < 0.05$) with the dose of 15 mg/kg/d and ($p < 0.01$) with the dose of 25 mg/kg/d relative to the control group level. In addition, regarding the myocardial NO level, there was a significant difference between the treated groups $[F(3, 39) = 7.374, p = 0.0006]$. Clozapine treatment significantly increased cardiac NO levels ($p < 0.05$) with the dose of 15 mg/kg/d and ($p < 0.01$) with the dose of 25 mg/kg/d relative to the control group level.

Moreover, clozapine treatment significantly decreased myocardial GSH levels $[F(3, 39) = 3.512, p = 0.0248]$. The effect was statistically significant at the dose of 25 mg/kg/d relative to the control level. Furthermore, clozapine treatment significantly attenuated the GSH-Px activity $[F(3, 39) = 4.586, p = 0.0081]$, the effect that was significant with the dose of 15 mg/kg/d and ($p < 0.01$) with the dose of 25 mg/kg/d relative to the control group level.

3.5. Effect on serum and cardiac 8-OHdG levels

8-hydroxy-2′-deoxyguanosine (8-OHdG) is a product of oxidative damaged DNA formed by hydroxy radical and singlet oxygen. Results from the measurement of 8-OHdG levels revealed significant changes among clozapine-treated groups $[F(3, 39) = 8.850, p = 0.0002]$ and $[F(3, 39) = 6.512, p = 0.0012]$ in both serum and cardiac tissues, respectively (Figure 5A).

After 21 days of clozapine treatment, the serum levels of 8-OHdG significantly increased with the doses of 15 and 25 mg/kg/d at significant levels $p < 0.05$ and $p < 0.01$, respectively. The level of 8-OHdG in hearts of the tested animals significantly increased with clozapine dose of 10 mg/kg/d. The increase was at the significant level ($p < 0.01$) with the doses of 15 and 25 mg/kg/d compared with the control group levels (Figure 5B).

4. Discussion

In this study, it was investigated whether clozapine induces myocarditis and myocardial oxidative stress and DNA damage

Table 1 Histopathological scoring and percent of animals showed myocarditis in control and clozapine-treated rats in doses of 10, 15, and 25 mg/kg/d on day 21.

Treatment (mg/kg/d)	Initial body weight (g)	Final body weight (g)	Change body weight (g)	% Mortality	Myocarditis
Control	165 ± 2.34	168 ± 2.78	3.0 ± 1.44	0.00	0.00 ± 0.00
Clozapine (10)	175 ± 1.55	177.6 ± 0.32	2.60 ± 0.32	0.00	0.33 ± 0.07[a]
Clozapine (15)	170 ± 2.62	174 ± 2.19	4.00 ± 0.43[a]	0.00	0.67 ± 0.12[b]
Clozapine (25)	165 ± 1.93	174 ± 0.62	9.00 ± 1.26[b]	20.00	0.86 ± 0.12[c]

Histological scores of hearts ranged from 0 to 4 + for myocarditis.

Results represent mean ± SEM ($n = 10$).

[a] $p < 0.05$ vs. control.

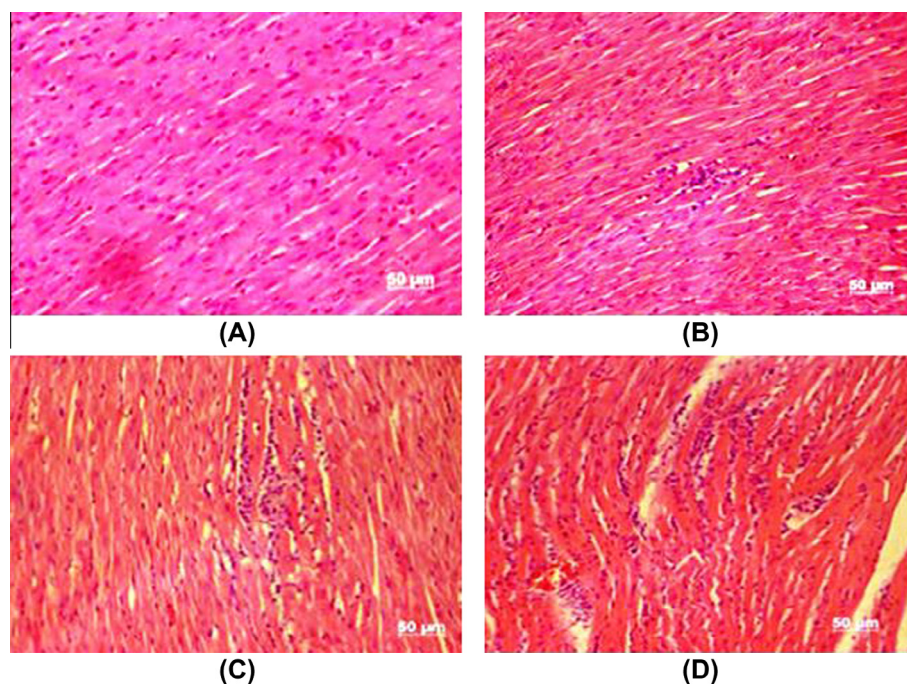[b] $p < 0.01$ vs. control.

[c] $p < 0.001$ vs. control.

Figure 1 Representative H&E-stained sections (40) of the left ventricle from clozapine-treated and control rats. (A) There is no myocardial inflammation in control rats (score 0). (B) There are inflammatory lesions in the myocardium after administration of 10 mg/kg/d clozapine for 21 days (score 2). (C) There are larger inflammatory lesions in the myocardium after administration of 15 mg/kg/d clozapine for 21 days (score 3). (D) There are extensive inflammatory lesions in the myocardium after administration of 25 mg/kg/d clozapine for 21 days (score 4).
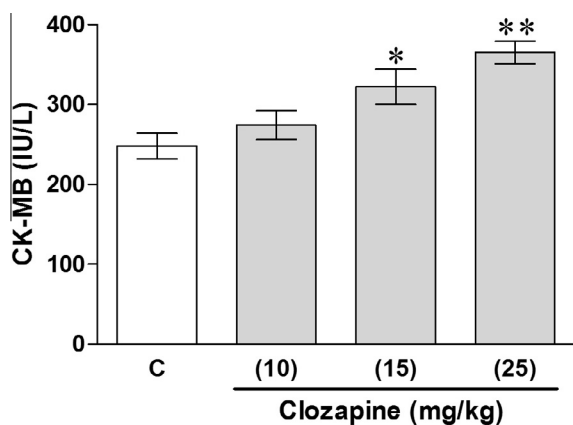

Figure 2 Serum levels of creatinine phosphokinase isoenzyme (CK-MB) of rats treated with clozapine in doses of 10, 15 ($n = 10$) and 25 ($n = 8$) mg/kg/d, i.p. for 21 days. Results in each group represent mean ± SEM. $p < 0.05$ vs. control. $p < 0.01$ vs. control.

Figure 3 Serum levels of lactate dehydrogenase (LDH) of rats treated with clozapine in doses of 10, 15 ($n = 10$) and 25 ($n = 8$) mg/kg/d, i.p. for 21 days. Results in each group represent mean ± SEM. $p < 0.05$ vs. control. $p < 0.01$ vs. control.

in rats or not. These results showed that in rats, clozapine induces myocarditis, myocardial oxidative stress and increases DNA damage.

The first finding was that clozapine-induced cardiotoxicity was manifested by histopathological changes in the heart in the form of inflammatory lesions, interstitial edema, perinuclear vacuolation and focal subendocardial fibrosis, which were dose-related and found in both the left and right ventricles, mainly in the myocardium, consistent with myocarditis. This finding is similar and related to histopathological changes
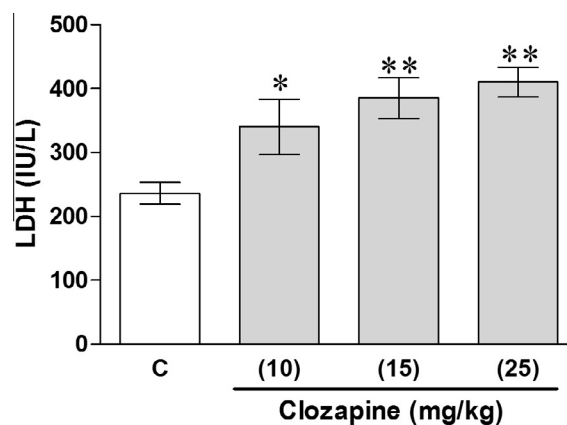
that have been shown in previous reports.[27] This cardiotoxic effect was confirmed by elevation in the activities of serum lactate dehydrogenase (LDH) and creatinine kinase (CK-MB). Elevation of serum LDH and CK-MB enzymes are considered important markers of early and late cardiac injury.

Additional evidence comes from Killian,[28] who argued that clozapine-induced myocarditis possibly results from a type-I IgE-mediated acute hypersensitivity reaction. In addition, evidence of a possible linkage between myocarditis induced by clozapine and eosinophilic myocardial infiltrates was previously reported.[29]
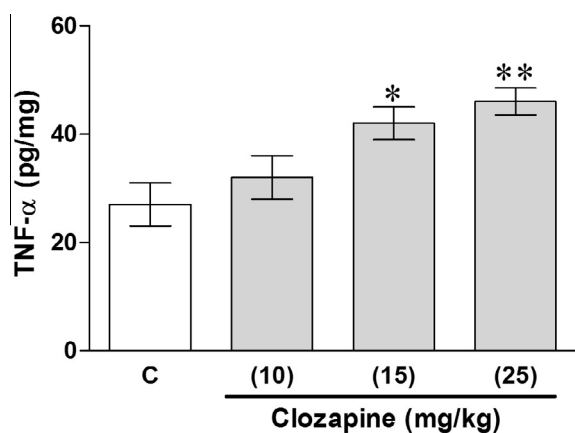
Figure 4 Cardiac levels of tumor necrosis factor alpha (TNF-α) of rats treated with clozapine in doses of 10, 15 ($n = 10$) and 25 ($n = 8$) mg/kg/d, i.p. for 21 days. Results in each group represent mean ± SEM. $p < 0.05$ vs. control. $p < 0.01$ vs. control.

Furthermore, evidence comes from recent studies showing that clozapine-induced myocarditis involves proinflammatory cytokine release, and increased level of proinflammatory cytokines such as TNF-α level.[30] Although previous studies showed that atypical antipsychotic drugs including clozapine showed the ability to decrease the levels of proinflammatory cytokines in psychotic patients in therapeutic doses,[31] these findings showed that clozapine in large doses increases TNF-α level one of the proinflammatory cytokines in a dose-related pattern. These results are inconsistent with and support the work of others.[32]

Previous studies have found that clozapine induces a rise in plasma catecholamines that correlates with the degree of myocardial inflammation.[33] Thus, the imbalance in the autonomic system with decreased parasympathetic tone and increased adrenergic drive might explain the electrophysiological effects of the drug, mainly tachycardia at rest. Persistent inappropriate tachycardia has been demonstrated to induce an impairment of left ventricular function both in animal models and in humans.[34]

These results showed that clozapine-induced cardiotoxicity was associated with marked elevation of myocardial levels of the lipid peroxidation product (MDA), with reduction of GSH content and activity of the antioxidant enzyme GSH-Px. Therefore, these results give evidence for the concept that increased oxidative stress and weakness of antioxidant defenses play an important role in clozapine-induced myocarditis.[35]
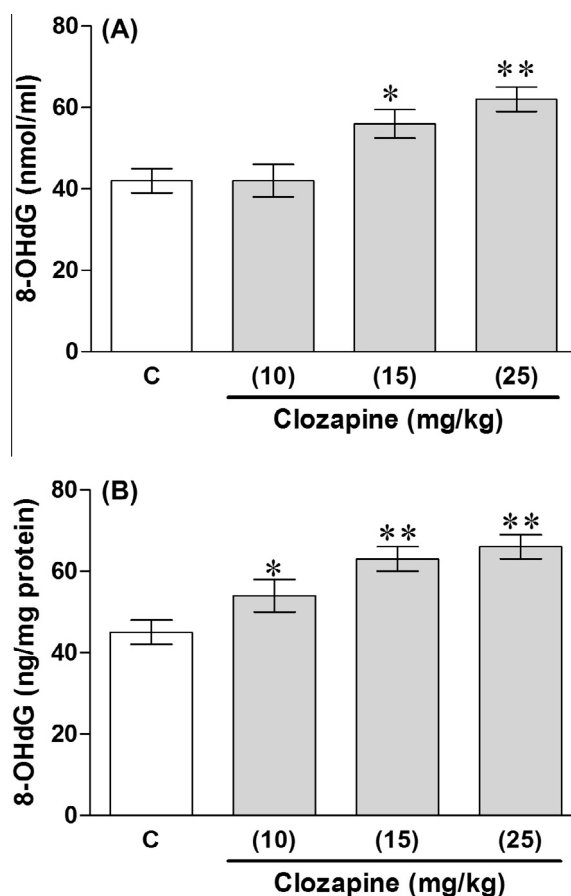

Figure 5 Effects of clozapine in doses 10, 15 ($n = 10$) and 25 ($n = 8$) mg/kg/d, i.p. for 21 days on (A) serum (B) cardiac 8-hydroxy-2-deoxyguanosine (8-OHdG) levels in rats. Results in each group represent mean ± SEM. $^*p < 0.05$ vs. control. $^{**}p < 0.01$ vs. control.

Increasing oxidative stress is associated with an impaired antioxidant defense status, which initiates a cascade of reactions responsible for clozapine-induced cardiotoxicity.[36] Clozapine undergoes bioactivation in the myocardial tissues converting into a chemically reactive nitronium ion metabolite. This reactive metabolite stimulates cellular injury, lipid peroxidation and free radical formation.[37] This nitronium ion binds with proteins in the myocardium leading to the formation of antigenic complex that stimulates immune response and macrophages,[38] which leads to the release of proinflammatory

Table 2 Effect of clozapine in doses 10, 15 and 25 mg/kg/day for 21 days, on myocardial malondialdehyde (MDA), nitrite, intracellular reduced glutathione (GSH) levels and glutathione peroxidase (GSH-Px) activity in rats.

Treatment (mg/kg/d)	MDA (μmol/g protein)	Nitrite (μmol/g protein)	GSH (nmol/g protein)	GSH-Px (IU/g protein)
Control ($n = 10$)	315.62 ± 15.34	3.33 ± 1.14	33.46 ± 3.52	25.45 ± 3.24
Clozapine (10) ($n = 10$)	332.26 ± 17.25	3.64 ± 2.38	30.45 ± 3.33	18.34 ± 2.48
Clozapine (15) ($n = 10$)	387.63 ± 13.26[a]	10.34 ± 1.23[a]	23.46 ± 3.25	14.67 ± 2.66[a]
Clozapine (25) ($n = 8$)	412.52 ± 21.32[b]	11.24 ± 1.12[b]	19.85 ± 3.23[b]	12.45 ± 2.13 [b]

Results in each group represent mean ± SEM.
[a] $p < 0.05$ vs. control rats.
[b] $p < 0.01$ vs. control rats.

cytokines such as TNF-α which mediate cellular inflammation and myocarditis and generates further free radicals.

Catecholamines decrease myocardial oxygen perfusion through coronary vasoconstriction.[39] In addition, these results showed that clozapine increases the cardiac level of nitrites, a stable product and indirect marker of NO, which is consistent with similar results that have demonstrated an increase in cardiac NO levels following exposure to clozapine's effect or its metabolite N-desmethylclozapine.[40] The contribution of NO to clozapine-induced cardiac damage is suggested by evidence demonstrating clozapine-mediated induction of nitric oxide synthase (iNOS) expression.[41]

These results confirmed the increase in free radical formation and the decrease in antioxidant defenses on long-term treatment with clozapine. One limitation of this study is that the experiment has thus far been conducted only on rats. It would be valuable to conduct a clinical study for comparison with these findings. The aim would be to measure the same markers of cardiotoxicity in healthy volunteers and in schizophrenic patients, without a history of preexisting cardiovascular disease.

The results of this study do not only confirm an association between clozapine treatment and the formation of myocarditis lesions, but also showed the involvement of increased myocardial oxidative stress and DNA damage in clozapine-induced myocarditis.

5. Conclusion and recommendations

In conclusion, clozapine, particularly in moderate to high doses, can produce a serious and potentially lethal cardiotoxicity in the form of myocarditis and myocardial injury in rats. The pathogenesis of this toxic reaction according to the results of this study is attributed to increased myocardial oxidative stress, inflammatory cytokines and oxidative DNA damage with attenuation in antioxidant defenses. Clinically, patients should be informed of symptoms of cardiotoxicity. It is important to note that a high degree of clinical suspicion is maintained through the duration of clozapine therapy with a low threshold for referral to a cardiologist.

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