CLOZAPINE AFFECTS THE INFLAMMATORY IMMUNE RESPONSE IN THE BRAINS OF SCHIZOPHRENIC RATS BY REGULATING B - CATENIN/APC/GSK-3B GENE

Xiu Li, Huirong Chen, Qiaoan Zhang*
Department of Laboratory, Hanchuan People's Hospital, Hanchuan 431600, Hubei Province, China

ABSTRACT

Objective: *To investigate the effect of clozapine on the inflammatory immune response in the brains of schizophrenic rats by regulating β-Catenin, GSK-3 β and the APC gene.*

Methods: *Twenty-one SD rats were randomly divided into a control group, a model group (using MK-801 to establish a model for schizophrenia and intraperitoneal injections of equal volume of normal saline) and a clozapine group (establishing a model for schizophrenia and treatment with 1mg/kg clozapine). The peripheral blood T cell subsets CD3 +, CD4 +, CD8a +, IgG, IgA, IgM, IL-2, IL-6, IL-1 β, TNF-α, Bcl-2, β-Catenin mRNA, and APC were compared in each group. The expression of mRNA and GSK-3 β mRNA and the number of IL-1 β, TNF-α and Bcl-2 positive cells in the rats' brains were also assessed.*

Results: *The levels of CD3 +, CD4 + and CD8a + in the model group were significantly higher than in the control group (P<0.05). The levels of CD3 +, CD4 + and CD8a + in the clozapine group were significantly lower than in the model group (P<0.05). The levels of IgG, IgA and IgM in the model group were significantly higher than in the control group (P<0.05). The levels of IgG, IgA and IgM in the clozapine group were significantly lower than in the model group (P<0.05). The water level of IL-2 and IL-6 in the model group was significantly higher than in the control group, and the level of TNF-α was significantly lower than in the control group (P<0.05). The water level of IL-2 and IL-6 in the clozapine group was significantly higher than in the model group, and the level of TNF-α in the clozapine group was significantly higher than in the model group (P<0.05). In the brain tissues of rats of the model group, the number of TNF-α and IL-1 β positive cells was significantly higher than in those of the control group, and the number of Bcl-2 positive cells was significantly lower than in those of the control group (P<0.05). In the brain tissues of rats of the clozapine group, the number of TNF-α positive cells was significantly lower than in those of the model group, and the number of Bcl-2 positive cells was significantly higher than in those of the model group (P<0.05). The expression of β-Catenin mRNA in the model group was significantly higher than in the control group, and GSK-3β and APC mRNA were significantly lower than that in the control group (P<0.05). The expression of β-Catenin mRNA in the clozapine group was significantly lower than in the model group, while GSK-3β and APC mRNA were significantly higher than in the model group (P<0.05).*

Conclusion: *Clozapine can inhibit the inflammatory immune response in the brains of schizophrenic rats by regulating the gene expression of β-Catenin, APC and GSK-3β.*

Keywords: *Clozapine, β-Catenin, APC, GSK-3β, schizophrenia, inflammatory immune response.*

DOI: 10.19193/0393-6384_2022_1_58

Received *March 15, 2020;* ***Accepted*** *October 20, 2020*

Introduction

Schizophrenia, whose cause is not clear, is a common severe mental illness. It usually manifests as a clinical syndrome with a variety of symptoms, and most patients experience cognitive dysfunction and mental disharmony, which are characterized by prolonged and difficult healing, recurrent attacks, high disability rates, and great social impediments [1, 2]. In China, schizophrenia accounts for about 70% of all psychiatric hospitalizations; surveys show that the total number of schizophrenia cases in the country exceeds 20 million, representing the brunt of the burden of mental illnesses[3]. Drug therapy is the primary clinical treatment route for schizophrenia. Chlorpromazine led the first generation of antipsychotic drugs mainly used to treat the illness in the past, with the adverse reactions

of these drugs having mostly been eliminated[4]. At present, clozapine leads the second generation of antipsychotics being used as a mainstream clinical treatment option for schizophrenia, and has served as the basis for the development of quetiapine and olanzapine[5]. However, not all patients receive the curative effects expected from antipsychotics: About 40% of patients' conditions are still not effectively being bought under control, although clozapine has been found to be the most effective drug currently available for treating schizophrenia, and most patients show significant improvements after treatment[6]. Part of the issue is that clozapine cannot be the first choice for treatment, since its mechanism of action is not completely clear and it can have many adverse side effects, including intestinal obstruction and cardiomyopathy[7].

In recent years, clozapine has become a focus of clinical research due to its special therapeutic effects as well as side effects. Clinical studies have shown that the side effects of clozapine are closely related to the body's immune system[8]. With that in mind, this study set out to establish a model based on experiments with schizophrenic rats to explore the mechanism through which clozapine regulates the immune inflammatory response in such rats.

Materials and methods

Subjects and grouping

Twenty-one clean SD rats at two weeks of pregnancy were purchased from Hunan Saike Jingda Experimental Animal Co., Ltd. Seven rats in a cage were given free water and food at room temperature (26±2) °C and humidity (55±5) %. The period of light and darkness was 12h. The 21 rats were randomly divided into a control group, a model group (in which the schizophrenic rat model was established by MK-801 and given intraperitoneal injections of equal volume of normal saline) and a clozapine group (in which the schizophrenic rat model was established and given 1mg/kg clozapine). Seven rats were placed into each group.

Main reagents and instruments

Reagents

MK-801 was purchased from Shanghai Hanxiang Biotechnology Co., Ltd. Clozapine was purchased from Beijing Lvyuan Bird Biological Technology Co., Ltd. Tartaric acid was purchased from Linyi Azeroth Biological Technology Co., Ltd. Polyclonal antibodies against TNF-α, IL-1β and Bcl-2 were purchased from Shenzhen Xinbosheng Biotechnology Co., Ltd. The SP detection kit was purchased from Suzhou Renode Biotechnology Co., Ltd. Goat serum for sealing was purchased from Nanjing Senbeijia Biotechnology Co., Ltd. The primers were purchased from Nanjing KingsRui Biotechnology Co., Ltd. The DNA enzyme (without RNA enzyme) was purchased from Promega, Inc.

Instruments

The 4°C refrigerator was purchased from Beijing Wuzhou Oriental Science and Technology Development Co., Ltd. The -80°C refrigerator was purchased from Guangzhou Haohan Instrument Co., Ltd. The heparin anticoagulant tubes were purchased from the Nanjing Jiancheng Institute of Biological Engineering. The centrifuge was purchased from Tianjin Benson Health Technology Co., Ltd. The thermostatic magnetic agitator was purchased from Suzhou Abituo Biotechnology Co., Ltd. The biological image acquisition system was purchased from Guangzhou Kezhilan Instrument Co., Ltd. The electric thermostatic water tank was purchased from Beijing Ganming Gene Technology Co., Ltd.

Methods

• The levels of CD3+, CD4+ and CD8a+ of the T cell subsets in the peripheral blood of the rats in each group were determined by flow cytometry.

• The levels of immunoglobulin G (IgG), immunoglobulin A (IgA), immunoglobulin M (IgM), interleukin 2 (IL-2), interleukin 6 (IL-6), interleukin β (IL-1β), and tumor necrosis factor alpha (TNF-α) were detected by ELISA.

• The expressions of TNF-α, IL-1β and B-cell lymphoma factor 2 (Bcl-2) in the brain tissues of rats in each group were determined via the immunohistochemical method.

• Real-time quantitative PCR was used to determine the cadherin-associated protein beta (β-catenin), glycogen synthase kinase-3 beta (GSK-3β) and adenomatous polyposis coli (APC) mRNA.

Statistical methods

The levels of T cell subsets and immunoglobulin in the peripheral blood of the rats in each group were expressed by ($\bar{x}\pm s$).

A comparison between the two groups was performed via a T test, and a comparison among multiple groups was performed via a one-way analysis of variance. $P<0.05$ is considered significant.

Results

Effects of clozapine on T cell subsets in the peripheral blood of the rats in each group

The levels of CD3+, CD4+ and CD8a+ in the model group were significantly higher than in the control group (P<0.05). The levels of CD3+, CD4+ and CD8a+ in the clozapine group were significantly lower than in the model group (P<0.05). See Table 1.

Group	CD3+(%)	CD4+(%)	CD8a+ (%)
Control group	29.87±11.30	17.61±5.35	18.70±8.27
Model group	40.85±7.36[a]	34.49±4.41[a]	43.22±7.41[a]
Clozapine group	24.97±8.71[b]	23.25±4.15[b]	29.16±5.09[b]

Table 1: Effects of clozapine on T cell subsets in the peripheral blood of rats in each group ($\bar{x}$±s).
Note: a means compared with the control group, P<0.05; b means compared with the model group, P<0.05.

Effects of clozapine on immunoglobulin levels in each group

The levels of IgG, IgA and IgM in the model group were significantly higher than in the control group (P<0.05). The levels of clozapine IgG, IgA and IgM were significantly lower than in the model group (P<0.05). See Table 2.

Group	IgG (ng/mL)	IgA (ng/mL)	IgM (ng/mL)
Control group	49.87±8.90	206.59±9.16	118.66±10.06
Model group	61.70±10.58[a]	242.64±13.08[a]	136.75±8.01[a]
Clozapine group	37.62±10.15[b]	197.82±12.14[b]	108.37±10.02[b]

Table 2: Effects of clozapine on the immunoglobulin levels of rats in each group ($\bar{x}$±s).
Note: a means compared with the control group, P<0.05; b means compared with the model group, P<0.05.

Effects of clozapine on the serum cytokines of rats in each group

The levels of IL-2 and IL-6 in the model group were significantly higher than in the control group, while the level of TNF-α was significantly lower than in the control group (P<0.05).

There was no significant difference in the level of IL-1β between the model group and the control group (P>0.05). The levels of IL-2 and IL-6 in the clozapine group were significantly higher than in the model group, and the level of TNF-α was significantly higher than in the model group (P<0.05). See Table 3.

Group	IL-1β (pg/mL)	IL-2 (pg/mL)	IL-6 (pg/mL)	TNF-α (pg/mL)
Control group	135.15±1.46	1.36±0.04	112.40±0.56	126.65±3.94
Model group	136.13±2.90	1.55±0.04[a]	114.74±0.39[a]	114.47±2.96[a]
Clozapine group	136.21±3.39	2.10±0.06[b]	116.31±0.70[b]	124.76±2.20[b]

Table 3: Effects of clozapine on the serum cytokines of rats in each group ($\bar{x}$±s).
Note: a means compared with the control group, P<0.05; b means compared with the model group, P<0.05.

Effects of clozapine on TNF-α, IL-1β and Bcl-2 on the brain tissues of rats in each group

The number of TNF-α and IL-1β positive cells in the brain tissues of rats of the model group was significantly higher than that of the control group, and the number of Bcl-2 positive cells was significantly lower than that of the control group (P<0.05). The number of TNF-α positive cells in the brain tissues of rats in the clozapine group was significantly lower than that in the model group, and the number of Bcl-2 positive cells was significantly higher than that in the model group (P<0.05). See Figure 1.

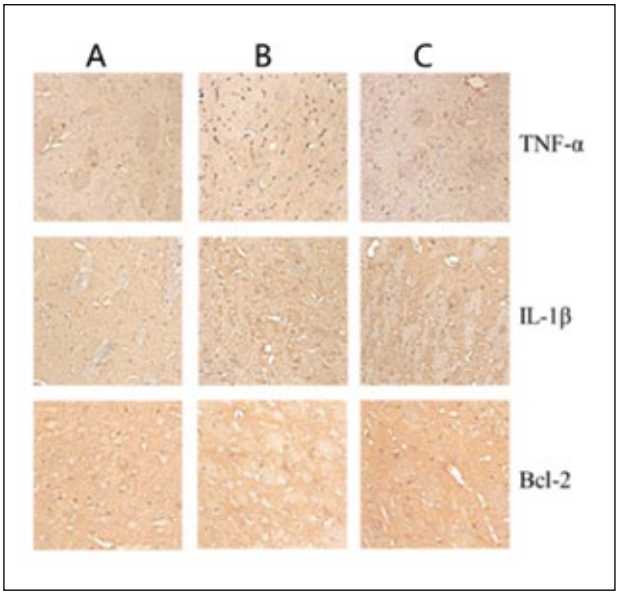

Figure 1: Effects of clozapine on TNF-α, IL-1β and Bcl-2 in the brain tissues of rats in each group.

Effects of clozapine on β-catenin, GSK-3β and APC mRNA in rats

The mRNA expression of β-catenin in the model group was significantly higher than in the control group, and the mRNA expression of GSK-3β and APC in the model group was significantly lower than in the control group (P<0.05). The mRNA expression of β-catenin in the clozapine group was significantly lower than in the model group, and

the mRNA expression of GSK-3β and APC in the clozapine group was significantly higher than in the model group (P<0.05). See Table 4.

Group	β-catenin mRNA	GSK-3β mRNA	APC mRNA
Control group	0.007±0.001	0.035±±0.004	0.026±0.003
Model group	0.011±0.001[a]	0.024±0.003[a]	0.022±0.002[a]
Clozapine group	0.009±0.001[b]	0.042±0.003[b]	0.030±0.004[b]

Table 4: Effects of clozapine on β-catenin, GSK-3β and APC mRNA in rats of each group ($\bar{x}$±s).
Note: a means compared with the control group, P<0.05; b means compared with the model group, P<0.05.

Discussion

Clozapine is well-tolerated, on the one hand, with little change in serum prolactin levels and minimal risk of extrapyramidal adverse reactions. On the other hand, clozapine can cause reduced granulocyte numbers, myocarditis and other toxic side effects, which seriously limit its clinical applications[9]. Therefore, it is very important to further reveal its mechanism of action for clinical guidance on drug use.

Few studies have so far been published on changes in immune function in schizophrenic rat models stimulated by MK-801. Some research has shown that the levels of IgA and IgM in schizophrenic patients in that group are significantly higher than in those in the normal control group[10]. Other scholars found that IgG and IgA decreased significantly in patients with schizophrenia in remission[11]. The results of the present study showed that the levels of IgG, IgA and IgM in the model group were significantly higher than in the control group (P<0.05). Meanwhile, the levels of clozapine IgG, IgA and IgM were significantly lower than in the model group (P<0.05). These results indicated that the immune function of schizophrenic rats was disturbed, and that the effect of clozapine on the immune function played an important role in treating schizophrenia. Moreover, CD4+T cells are important immunoregulatory cells, and an increase in their levels suggests the presence of autoimmune diseases. CD8+T cells have a direct killing function and can clear target cells. CD3+T cells may participate in the signal transduction process of T cells. Regarding these aspects, the study results showed that the levels of CD3+, CD4+ and CD8a+ in the model group were significantly higher than in the control group (P<0.05). Conversely, the levels of CD3+, CD4+ and CD8a+ in the clozapine group were significantly lower than in the model group (P<0.05). These results indicated that T-lymphocyte subsets in the peripheral blood of schizophrenic rats were dysfunctional, and that clozapine could promote their functional recovery.

Many studies have been published on the role of IL-2 and IL-6 in the inflammatory response surrounding schizophrenia; it has been reported that the levels of IL-6, IL-2 and IL-8 in schizophrenic patients are significantly higher than those in the normal population[12]. Haack et al found that the level of TNF-α was significantly reduced in patients with schizophrenia, and that age could affect its level[13]. This is consistent with the results of the present study. A number of studies have shown that the expression of TNF-α and IL-1β in the affected brain tissue is significantly increased. The results of the present study showed that the levels of IL-2 and IL-6 in the model group were significantly higher than in the control group, and that the level of TNF-α was significantly lower than in the control group (P<0.05). In addition, the levels of IL-2 and IL-6 in the clozapine group were significantly higher than in the model group, and the level of TNF-α was significantly higher than in the model group (P<0.05). In this study, the levels of IL-2 and IL-6 did not recover but increased significantly after the clozapine treatment, which may suggest that they do not play immune regulatory functions through such a treatment. The number of TNF-α and IL-1β positive cells in the brain tissues of the rats in the model group was significantly higher than that of the control group, and the number of Bcl-2 positive cells was significantly lower than that of the control group (P<0.05). Furthermore, the number of TNF-α positive cells in the brain tissues of the clozapine group was significantly lower than in those of the model group, and the number of Bcl-2 positive cells was significantly higher than in those of the model group (P<0.05). These results indicated that TNF-α, IL-1β and Bcl-2 could play an important role in schizophrenia, and that clozapine could effectively promote the recovery of their levels.

APC can regulate the content of β-catenin in cells and promote the binding of β-catenin to the degradation complex. GSK-3β can promote the phosphorylation of β-catenin and eventually achieve the purpose of promoting the degradation of β-catenin[14]. Previous studies have confirmed that antipsychotics can increase GSK-3β and APC levels[15]. The present study, for its part, showed that

the mRNA expression of β-catenin in the model group was significantly higher than in the control group, and that the mRNA expression of GSK-3β and APC in the model group was significantly lower than in the control group ($P<0.05$). At the same time, the mRNA expression of β-catenin in the clozapine group was significantly lower than in the model group, and the mRNA expression of GSK-3β and APC in the clozapine group was significantly higher than in the model group ($P<0.05$).

In conclusion, clozapine can inhibit the inflammatory immune response in the brains of schizophrenic rats by regulating the expression of β-catenin, APC and GSK-3β genes.

References

1) Selten JP, Cantor-Graae E. Social defeat: risk factor for schizophrenia? Br J Psychiatry 2005; 187: 101-2.
2) Myhrman A, Rantakallio P, Isohanni M, Jones P, Partanen U. Unwantedness of a pregnancy and schizophrenia in the child. Br J Psychiatry 1996; 169(5): 637-40.
3) Abraham KR, Kulhara P. The efficacy of electroconvulsive therapy in the treatment of schizophrenia. A comparative study 2018; 151(2): 152-155.
4) Duncan LE, Ratanatharathorn A, Aiello AE, Almli LM, Amstadter AB, et al. Largest GWAS of PTSD (N=20070) yields genetic overlap with schizophrenia and sex differences in heritability. Mol Psychiatry 2018; 23(3): 666-673.
5) Sun Y, Chen Y, Collinson SL, Bezerianos A, Sim K. Reduced Hemispheric Asymmetry of Brain Anatomical Networks Is Linked to Schizophrenia: A Connectome Study. Cereb Cortex 2017; 27(1): 602-615.
6) Gören JL, Rose AJ, Smith EG, Ney JP. The Business Case for Expanded Clozapine Utilization. Psychiatr Serv 2016; 67(11): 1197-1205.
7) Tanyeri MH, Buyukokuroglu ME, Tanyeri P, Mutlu O, Akar FY, et al. Effects of long-term treatment with haloperidol, clozapine and aripiprazole on mice isolated vas deferens. Int Urol Nephrol 2017; 49(9): 1561-1567.
8) Wehring HJ, Elsobky T, McEvoy JP, Vyas G, Richardson CM, et al. Adjunctive Minocycline in Clozapine-Treated Patients with Schizophrenia: Analyzing the Effects of Minocycline on Clozapine Plasma Levels. Psychiatr Q 2018; 89(1): 73-80.
9) Bener A, Dafeeah EE, Abou-Saleh MT, Bhugra D, Ventriglio A. Schizophrenia and co-morbid obsessive - compulsive disorder: Clinical characteristics. Asian J Psychiatr 2018; 37: 80-84.
10) Jordan SC, Lorant T, Choi J, Kjellman C, Winstedt L, et al. IgG Endopeptidase in Highly Sensitized Patients Undergoing Transplantation. N Engl J Med 2017; 377(5): 442-453.
11) Rajagopala S, Parameswaran S, Ajmera JS, Ganesh RN, Katrevula A. Diffuse alveolar hemorrhage in IgA nephropathy: case series and systematic review of the literature. Int J Rheum Dis 2017; 20(1): 109-121.
12) Lv LF, Jia HY, Zhang HF, Hu YX. Expression level and clinical significance of IL-2, IL-6 and TGF-β in elderly patients with goiter and hyperthyroidism. Eur Rev Med Pharmacol Sci 2017; 21(20): 4680-4686.
13) Almasy L, Gur RC, Haack K, Cole SA, Calkins ME, et al. A genome screen for quantitative trait loci influencing schizophrenia and neurocognitive phenotypes. Am J Psychiatry 2008; 165(9): 1185-92.
14) Chai S, Ng KY, Tong M, Lau EY, Lee TK, et al.Octamer 4/microRNA-1246 signaling axis drives Wnt/β-catenin activation in liver cancer stem cells. Hepatology 2016; 64(6): 2062-2076.
15) Xu Q, Xu HX, Li JP, Wang S, Fu Z, et al. Growth differentiation factor 15 induces growth and metastasis of human liver cancer stem-like cells via AKT/GSK-3β/β-catenin signaling. Oncotarget 2017; 8(10): 16972-16987.

Corresponding Author:
Qiaoan Zhang
No. 1 Renmin Avenue, Hanchuan City, Hubei Province, China
Email: p436ge@163.com
(China)