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ARTICLE

Clozapine Induces Myocardial Inflammation Response Through Lactation Modification---Based on Novel Tree Shrew Models of Schizophrenia

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Abstract

Background: Schizophrenia is a mental disorder with a high disability rate, and patients almost lose their social functions. Clozapine is an effective drug for the treatment of refractory schizophrenia. However, recent studies have found that schizophrenic patients treated with clozapine have progressive cardiac dysfunction related to lactate accumulation. Tree shrews belong to primates, which are genetically closer to humans. Using a tree shrew to establish a schizophrenic model is helpful for the study of the pathogenesis of schizophrenia and related accompanying diseases. It is significant to reveal the mechanism of clozapine-induced cardiac damage by different models of schizophrenia.

Methods: The tree shrew schizophrenic models were established by using MK801 and virus-induced, respectively. The tree shrew-specific maze (homing maze) independently developed by the research team was used to determine the model establishment and evaluate the tree shrew's cognitive impairment. The MK801 group was treated with clozapine (gavage) for 4 weeks, and the virus-induced group was treated with clozapine (gavage) for 5 weeks. The myocardial enzymes, electrocardiogram and left ventricular ejection fraction of tree shrew model were measured, the changes of lactate level were analyzed by biochemical method, the myocardial inflammatory was analyzed by immunohistochemistry, and pan-lactated protein was detected by western-blot. The effects of clozapine and lactic acid on TNF-α, IL-1β and IL-10 were determined in vitro using cultured human cardiomyocytes.

Results: Compared with the standard control group, the two groups of schizophrenic tree shrews had apparent cognitive impairment, and the maze time (homing time) was significantly prolonged, confirming that the model was successfully established. Compared with the model group,

clozapine treatment shortened the homing time, and the difference was statistically significant (P<0.01). The electrocardiogram examination showed that compared with the model group, the tree shrews in the clozapine treatment group had a slower heart rate, longer Q-T interval, and significantly lower ST segment (P<0.01). In the clozapine groups, the Myocardial enzymes were higher than those in the model groups (CK-MB (P<0.05), LDH (P<0.05) and cTnI (P<0.01)). Echocardiography showed that the left ventricular ejection fraction of tree shrew in the clozapine groups was lower than that in the model group (P<0.05). The serum lactate in the clozapine treatment groups was significantly increased, accompanied by the accumulation of a large number of monocytes and multinucleated giant cells in myocardial tissue. After MK-8012 was discontinued for 2 weeks, the serum lactate decreased, and the accumulation of monocytes and multinucleated giant cells in myocardial tissue decreased. Western blot showed that clozapine increased the expression of the pan-lactated protein in the myocardium of tree shrews. In vitro studies showed that clozapine increased the expression of TNF-α and IL-1β in the supernatant of cultured human cardiomyocytes (P<0.01) and decreased the expression of anti-inflammatory factor IL-10 (P<0.05). Sodium lactate further exacerbates the inflammatory response caused by clozapine.

Conclusion: Both MK801 and virus can effectively establish a tree shrew schizophrenic model. Clozapine-treated tree shrew models of schizophrenia showed it decreased cardiac function, which might be related to cardiac inflammatory response due to lactic acid accumulation.

Keywords:
CIM; Schizophrenia; Lactation Modification; Tree Shrew

Introduction

Schizophrenia (SCZ), one of the ten most disabling diseases globally, is a severe chronic and disabling mental disorder with an incidence rate of about 1%[1]. The clinical characteristics of the early stage are the disorder of speech and deeds, often accompanied by clinical symptoms such as hallucinations and delusions, and negative symptoms such as withdrawal and apathy may also appear, which are accompanied by cognitive impairment as the disease progresses. Because of its characteristics of chronic recurrence, it brings a heavy economic burden to society. Treatment-resistant schizophrenia refers to the failure of SCZ to be fully relieved after antipsychotic treatment, which was once called Treatment-Resistant Schizophrenia[2] (TRS), Psychiatric Treatment Response and Resistance in Psychosis, TRRIP) the working group reviewed previous clinical studies in 2016 and proposed a consensus diagnosis of TRS, namely symptom criteria: using a standardized rating scale, symptom severity is at least moderate, and symptom duration ≥ 12 weeks, the practical scale indicates the existence of reasonable or above functional impairment; treatment criteria: therapeutic dose ≥ 600 mg/d chlorpromazine equivalent (based on equivalent dose), treatment at the therapeutic dose for ≥ 6 weeks, and use of an entire course of therapy≥ 2 different antipsychotics[3].

Clozapine (CZP) is the first-choice evidence-based treatment drug for TRS recommended by the Guidelines for the Prevention and Treatment of Schizophrenia in China[4]. CZP was first synthesized in Sweden in 1958. It was first marketed in Austria and Sweden in 1972 and used to treat schizophrenia. However, in the third year after the market, some patients had granulocytopenia and died of infection. Since then, CZP has been withdrawn from the medical system of most European countries. After being silent for nearly 20 years, an increasing number of international multi-center studies have shown that CZP effectively treats SCZ and TRS. By the early 1990s, the US FDA approved CZP for TRS and severe extrapyramidal symptoms and severe delayed onset. Patients with schizophrenia cannot tolerate other drugs with sexual movement disorders[5] .

After 30 years of clinical use, various studies have gradually revealed the pharmacological characteristics of CZP. It has an affinity for multiple receptors, including 5-HT2A, 5-HT2B, alpha-adrenergic and choline receptors. The relationship with the D2 receptor is relatively low. In contrast, the affinity for the 5-HT2 receptor is high, and it also has the agonism of 5-HT2A, so it has the effect of anti-anxiety and depression. At present, it is believed that CZP has a low affinity for D receptors but the high affinity for 5-HT, H1, and M1 receptors. At the same time, it has high selectivity for the dopamine system in the mesolimbic system and can specifically block it. 5-HT3, alpha-adrenergic receptors. Due to its multi-receptor function, while it exerts a broad-spectrum potent antipsychotic effect, it also has numerous complex and adverse reactions[6]. The most serious is leukopenia and neutropenia, other excessive sedation, salivation, central and peripheral anticholinergic effects, cardiovascular system effects, weight gain, abnormal glucose and lipid metabolism, and dose-related epilepsy. Because of its clinical irreplaceability, the side, as mentioned above, results have prompted us to accumulate a

lot of management and user experience in nearly 30 years of use[7]. For example, routinely carry out blood drug concentration monitoring, blood cell testing, related biochemical examinations, clinical symptoms and signs examinations, etc., to avoid various side effects as much as possible. At the same time, they play a role in maximizing the benefits of patients in the process of receiving treatment. However, there is insufficient clinical attention to CZP-induced myocarditis (CIM)[8], and its pathogenesis is still unclear.

In the present study, We use tree shrew to create a model of treatment-resistant schizophrenia. Validation of models using magnetic resonance MAGIC sequences, PET-CT, echocardiography. Using westernblot to examine the degree of lactylation modification of cardiac proteins with pan-lactation antibodies. Carry out a preliminary study on the relationship between lactylation modification and CIM.

Materials and methods

Tree shrew were housed in the animal facility at the Institute of Kunming medical University. Select 1.5-year-old tree shrew, male and female, with a bodyweight of 120-170 g, and are raised in the maze under normal conditions. After the tree shrew is familiar with the environment, the up and down of the valve controller baffle is used to change the maze path. When the tree shrew moves in the living cage, The loudspeaker in the device will make a sudden noise, and the tree shrew will automatically run back to the resting bin when the noise stimulates it. To focus on investigating the working memory of the tree shrew, the maze control device uses a switch baffle to ensure that the path of each homing changes. But with the same difficulty. The terminal control device records the time as the tree shrews pass through the labyrinth. After the maze experiment, compared with the time of the tree shrew in the regular control group, the time was longer than that of the tree shrew in the standard control group. It was considered that its cognitive ability decreased. Conventional ECG and Cardiac Ultrasound Detection of Tree Shrews. Tree shrew detection using MRI magic sequences

Tree Shrew Maze Experiment

Train the touch screen task until the animals can complete the correct number of times more than 30 times per day, and then divide the animals into a drug-induced model group (MK-801+vehicle) and a clozapine-treated group (MK801+clozapine). There was no difference in the number of times the task was completed, the time and the correct number of times. Afterwards, two groups of animals were given MK-801 (0.225 mg/kg·day) for 21 consecutive days to induce modelling. At the same time, the treatment group was assigned clozapine, the control group was given solvent, and the completion of the touch screen was detected every day. The task completion time and the number of tasks prompted the animal's willingness to complete the job. If the animal's activity decreased and the animal lost the desire to complete the task, it was manifested as a decrease in the number of completion times and a longer time to complete the task.

Tree Shrews Touch Screen Experiment

Tree shrew touch screen learning stage (Day 1-23): set a two-way selection learning task in the experimental task box to train tree shrew: when the tree shrew touches the worm picture, it will be recorded as the correct result, and the operation box will automatically prompt a sound and give Pine nuts as a reward; touch the apple picture as a mistake, and flashing light in the operation box as a punishment. The set number of training is 40 times for 30 minutes, once a day. The experiment ended if the tree shrew completed 40 training sessions within 30 minutes; if the tree shrew did not meet the training within 30 minutes, the exercise ended automatically. After the training, the correct rate of tree shrew operation was counted. The experimental animals whose correct rate reached 80% continued to train for three days and ended the touch screen experiment. Extraction and detection stage (Day 24): A paired selection learning task is set in the same operation box. The detection process is the same as the training process. Model processing, modelling processing and intervention are performed on animals before detection. The tree shrew's task completion time, the number of tasks completed, and the number of correct assignments completed was counted.

PCET-CT imaging comparison

18 Fluoro-fluorinated deoxyglucose (18F-FDG) injection: Each tree shrew injected 18F-FDG (18.5mbq/100g body weight) through the tail vein. Behavioral testing was performed 10 minutes after the infusion was completed. PET-CT scanning: After the behavioural test was conducted, the tree shrew was anesthetized with isoflurane (the induction concentration was 5%, and the maintenance concentration was 1.5-2%), and the tree shrew was placed prone on the scanning bed. A tree shrew brain was scanned. The PET image obtained after scanning is used for attenuation correction, normalization correction, dead time correction, photon attenuation correction, scatter correction and random coincidence modification using two-dimensional ordered subset expectation maximization, and the size of the reconstructed image matrix is 256×256 ×63, and the voxel size is 0.5×0.5×1mm3. And adopt a cone-beam reconstruction algorithm to reconstruct CT image with a voxel size of 0.25×0.25×0.5mm3 in 512×512×126 matrix. The mRNAs

chip was screened according to the abnormal brain area indicated by PET-CT.

Virus tracing and chemogenetics

The tree shrew was injected intraperitoneally with sodium pentobarbital 80 mg/kg. After anesthesia, the body was placed on a surgical paper pad, the head was fixed with a stereotaxic instrument, and an oxygen-filled nose clip was worn. Trim the hair of the tree shrew head, wipe the oil on the scalp with alcohol, cut the scalp, and clean the subcutaneous tissue and blood after cleaning the surface of the tree shrew's skull, exposing the mid skull suture. Use a positioning rod to adjust the bregma and posterior fontanel to be at the same level, and position the head based on the coordinates calculated from the tree shrew brain atlas and PET-CT data. RV-N2C-ΔG-EGFP virus 120nL was propelled into the brain region with a microinjection pump at a speed of 30nL/min. After the injection was completed, the needle was stopped for 5 minutes. After the virus spread slowly, the hand was pulled out to suture the scalp, and the wound was disinfected with hydrogen peroxide. After the experiment was completed, the tree shrew was put back into the cage, a certain amount of antibiotics were added to the food to reduce inflammation, and mealworms were fed to enhance nutrition after awakening. One week after the virus was expressed, the tree shrew heart was perfused, the brain tissue was taken for frozen section, and the RV-N2C-ΔG-EGFP virus expression was observed by confocal microscope. Select the critical brain regions obtained by PET-CT data and virus tracing analysis to inject the virus (AAV-CaMKIIa-hM3Dq-GFP/AAV-CaMKIIa-hM4Di-mCherry), and the injection method is shown in the virus tracing section. At least one week after recovering from surgery. Virus expression time is 2-3 weeks. Behavioural training or rest in the cage can be carried out during the period according to the different behavioural experiment times. CNO-activating virus was administered intraperitoneally 30 minutes before the desired behavioural assay to activate or depress key brain regions. After the experiment, the tree shrew heart was perfused, and the brain tissue was taken for the frozen section to detect the virus expression range and efficiency.

Immunohistochemistry:

The tree shrews were perfused and fixed, and then frozen sagittal slices were made. The slice thickness was 40um. The brain slices were stored in antifreeze buffer at -20°C. Select the brain slices required for the experiment, rinse six times for 5 minutes each time, and dilute the primary antibody with an antibody diluent containing 1% (w/v) BSA and 0.3% (v/v) triton-100 prepared in 1×PBS solution, followed by overnight incubation at 4°C. The next day, rinsed six times with 1×PBS solution for 5 min each time, incubated with fluorescent secondary antibody at room temperature, rinsed six times with 1×PBS solution after two h, 5 min each time, and blocked the patch with DAPI-containing blocking solution after drying. Laser confocal microscopy imaging. All brain slices were photographed with uniform parameters. The tree shrew brain atlas was used for transparency processing. The original images collected by laser confocal microscopy were superimposed to determine the range of each brain area and count the target cells in the corresponding brain area.

Detection of panlactylated proteins

Electrophoresis: After the gel is fully solidified, rinse the stacking gel with water, wash off the remaining gel on the outside of the glass, and put it into the electrophoresis tank. (The small glass plate faces inward, and the large glass plate faces outward. If only one piece of glue is used, a plastic container should be placed on the other side of the groove, and the side with the words should face outward). Prepare the samples and cook them in a metal bath at 100°C for 5-8 minutes. Add the electrophoresis solution to the designated mark, and start to prepare for loading. Gently pull out the comb, and after the protein concentration was determined by the BCA method, the protein loading amount was 20ug (10-well comb, 10μl/well). Electrophoresis time and voltage can be manipulated according to individual laboratory practice. Generally, the concentration gel 90V30mA and the separating gel 120V1.5-2h are used. (Bromophenol blue indicator stops when it reaches the bottom of the gel.) Transfer membrane: Membrane processing: Cut out a PVDF membrane of a specific size (6.5 cm × 8.5 cm), slowly immerse it in methanol for activation for 5-30s, and the NC membrane directly Immersion in pure water does not require methanol activation. Glue peeling: You must first pry off the glass plate before peeling the glue. When prying, the action should be light, and it should be gently and repeatedly prayed on both sides. After poking for a while, the glass plate begins to loosen until the glass plate is removed by prying off the small glass plate, and then the sample loading tank is partially removed, and then the gel is peeled off and placed in deionized water. Transfer membrane: Take the membrane out of methanol and put it in the transfer solution for 30 s, open the membrane transfer clip and lay it flat on the experimental bench, then lay a dry sponge pad on the positive pole of the membrane transfer clip, and place the balanced membrane Put the gel on a dry sponge pad, take the gel out of deionized water and spread it on the membrane, and use a transfer roller to remove air bubbles. Spread a dry sponge pad on the gel, close the transfer film clip, and place the gel containing the gel. Insert the gel and

membrane transfer clips into one channel of the membrane transfer instrument, generally choose 300mA, 90-120min for membrane transfer. Blocking of membranes: Each membrane was blocked with about 20 ml of 5% nonfat dry milk (prepared with 1×TBST), gently shaken on a shaker (the speed of the shaker was 45-65rpm), and blocked for 1hr. The amount of 5% skimmed milk powder depends on the size of the specific box and is subject to the cover film. After the end of blocking, rinse three times with TBST on a shaker for 10 min each time (the speed of the shaker is 80-100rpm). Add the primary antibody according to the recommended dilution ratio of the antibody for incubation or according to the antibody dilution ratio you have found. Incubate overnight at four °C or two h at room temperature. Secondary antibody incubation: After the primary antibody incubation, rinse three times with TBST for 10 min each time (the speed of the shaker is 80-100rpm). After rinsing:

- Incubate the secondary antibody at a 1:5K dilution.
- Add about 20 ml to each membrane.
- Ensure that the membrane is completely submerged.
- Shake gently on a shaker.
- Incubate for 45 min (shaker speed 45-65rpm).

The amount of the secondary antibody depends on the size of the specific box, and the incubation with the secondary antibody is determined by covering the membrane. Rinse three times with TBST for 10 min each time (the speed of the shaker is 80-100 rpm). Allow the substrate to equilibrate at room temperature for 5 min while rinsing the secondary antibody. Exposure: After rinsing, lay the membrane flat on an exposure pad, drain, and dry the TBST around the edges. Add 1.5 mL of substrate (1:1 formulation) per complete membrane, placing the membrane face down when finished, making sure there are no air bubbles. Incubate for 1-2 min at room temperature, absorb excess liquid with absorbent paper, and then perform chemiluminescence imager detection.

Detection of Cardiac Enzymes in Tree Shrews by ELISA

The basis of ELISA is the immobilization of antigen or antibody and the enzymatic labelling of antigen or antibody. The antigen or antibody bound to the surface of the solid phase carrier still retains its immunological activity, and the enzyme-labelled antigen or antibody includes both its immunological activity and the enzymatic activity. During the measurement, the test sample (the antibody or antigen in it) reacts with the antigen or antibody on the solid support surface. The antigen-antibody complex formed on the reliable phase carrier is separated from other substances in the liquid by washing. The enzyme-labelled antigen or antibody is then added, and it is also bound to the solid-phase page through the reaction. At this time, the amount of enzyme on the solid phase is proportional to the amount of the tested substance in the sample. After adding the substrate of the enzyme reaction, the substrate is catalyzed by the enzyme into a coloured product, and the amount of the product is directly related to the amount of the tested substance in the sample, so qualitative or quantitative analysis can be carried out according to the colour depth.

Statistical analysis

All experimental results were expressed as the mean ± S.E.M, and the data were analyzed using one-way analysis of variance (ANOVA), where appropriate. If any statistically significant difference was detected, posthoc comparisons were performed using Tukey's test. A value of $P < 0.05$ was considered statistically significant.

Results

Imaging suggests that dysfunction of different brain regions can lead to cognitive decline and increase the expression of serum lactate concentration

The magic sequence of MRI indicated that the conduction ability of the cortex, thalamus and dorsal extrathalamus of the SCZ tree shrew was lower than that of the regular group. Serum lactate expression is elevated after cognitive impairment. It is detected by PET-CT, suggesting that the glucose metabolism capacity of the dorsolateral prefrontal cortex, lateral geniculate nucleus and thalamus is decreased.(Fig1)

Evaluation results of cognitive function in tree MK-801 shrew SCZ model

It was found that the number of tasks completed by animals after MK-801 was significantly reduced ($^{\#}P<0.05$ vs Day 0), and still not all animals were able to complete 40 training tasks by day 21; Animals can complete 40 missions. In terms of the time to complete the task, after modelling, the time to complete the task almost reached the most extended time limit (1800 seconds). Still, at 21 days, the task completion time of the clozapine group was significantly different from that of the MK-801 group ($^{**}P<0.01$); it indicated that the activity and willingness to complete the task of the animals in the treatment group were significantly improved. Most importantly, we found that clozapine was very effective in correcting the number of functions and was substantially different from the solvent control group on the first day of modelling ($^{*}P<0.05$). Although the cognitive impairment

caused by MK-801 could be partially overcome by daily training, the correct number of times in the modelling group could not return to the pre-modelling level until day 21. Still, the right number of times in the clozapine treatment group exceeded that in the modelling group. Pre-level and the statistics are statistically different ($^{*}P<0.05$). (Fig2)

Characteristics of a chemogenetic tree shrew model of schizophrenia

Posterior cingulate cortical virus tracer results (highly consistent with PET-CT results).Positive areas include TI temporal inferior area,CLA claustrum, IRd infraradiata dorsalis, TC temporal cortex, PPc posterior parietal caudal area, DLG dorsal lateral geniculate nucleus, Pc central nucleus of the pulvinar, V1 primary visual cortex, V2 secondary visual cortex.(Fig 3)

Pan-lactate modification of myocardial tissue leads to severe CIM

Extensive lactation modification in myocardium detected by western blot pan-lactation resistance assay. Comparison of dynamic electrocardiogram parameters in two groups, QT interval dispersion and Sustained ventricular tachycardia, showed marked abnormalities in the tree shrew CIM model. (Fig4; Table1) Using cardiac ultrasound to examine the tree shrew model, a comparison of cardiac function in two groups: Left ventricular end-diastolic volume and left ventricular ejection fraction, showed marked abnormalities. (Fig5; Table2) The study found that the myocardial tissue with more severe lactation had increased myocardial enzyme expression.The expression of IL-1B was significantly increased in CIM (Fig6 $^{*}P<0.05$;$^{\#}P<0.01$).

Discussion

Schizophrenia (SCZ), one of the ten most disabling diseases globally, is a severe chronic and disabling mental disorder with an incidence rate of about 1%. Its clinical features are the disorder of speech and deeds, often accompanied by clinical symptoms such as hallucinations and delusions. Negative symptoms such as withdrawal and apathy may also appear, accompanied by cognitive impairment as the disease progresses. Because of its characteristics of chronic recurrence, it brings a heavy economic burden to society. Treatment-resistant schizophrenia refers to the failure of SCZ to be fully relieved after antipsychotic treatment, which was once called Treatment-Resistant Schizophrenia (TRS), Psychiatric Treatment Response and Resistance in Psychosis, TRRIP) the working group reviewed previous clinical studies in 2016 and proposed a consensus diagnosis of TRS, namely symptom criteria: using a standardized rating scale, symptom severity is at least moderate, and symptom duration ≥ 12 weeks, the practical scale indicates reasonable or above functional impairment; treatment criteria: therapeutic dose ≥ 600 mg/d chlorpromazine equivalent (based on equivalent dose), treatment at the therapeutic dose for ≥ 6 weeks, and use of a sufficient and complete course of treatment for ≥ 2 different antipsychotics.

Clozapine (CZP) is the first-choice evidence-based treatment drug for TRS recommended by the Guidelines for the Prevention and Treatment of Schizophrenia in China. CZP was first synthesized in Sweden in 1958. It was first marketed in Austria and Sweden in 1972 and used to treat schizophrenia. However, in the third year after the market, some patients had granulocytopenia and died of infection. Since then, CZP has been withdrawn from the medical system of most European countries. After being silent for nearly 20 years, an increasing number of international multi-center studies have shown that CZP effectively treats SCZ and TRS. By the early 1990s, the US FDA approved CZP for TRS and severe extrapyramidal symptoms and severe delayed onset. Sexual movement disorder in patients with schizophrenia who cannot tolerate other drugs. After 30 years of clinical use, various studies have gradually revealed the pharmacological characteristics of CZP. It has an affinity for multiple receptors, including 5-HT2A, 5-HT2B, alpha-adrenergic and choline receptors. The relationship with the D2 receptor is relatively low[9].

In contrast, the affinity for the 5-HT2 receptor is high, and it also has the agonism of 5-HT2A, so it has the effect of anti-anxiety and depression. At present, it is believed that CZP has a low affinity for D receptors but a high affinity for 5-HT, H1, and M1 receptors. At the same time, it has high selectivity for the dopamine system in the mesolimbic system and can specifically block it. 5-HT3, alpha-adrenergic receptors. Due to its multi-receptor function, while it exerts a broad-spectrum and potent antipsychotic effect, it also has very complex and numerous adverse reactions[10].

The most serious are:

- Leukopenia and neutropenia.
- Other excessive sedation.
- Salivation.
- Central and peripheral anticholinergic effects.
- Cardiovascular system effects.
- Weight gain.
- Abnormal glucose and lipid metabolism.
- Dose-related epilepsy.

Because of its clinical irreplaceability, the side, as mentioned above effects has prompted us to accumulate a lot of management and user experience in nearly 30 years of use. For example, routinely carry out blood drug concentration monitoring, blood cell testing, related biochemical examinations, clinical symptoms and signs examinations, etc., to avoid various side effects as much as possible[11]. At the same time, they play a role in maximizing the benefits of patients in the process of receiving treatment. However, there is still insufficient clinical attention to CZP-induced myocarditis (CIM), and its pathogenesis is still unclear[12].

Lactic acid (Lac) is an essential intermediate product in glycolysis, and its biological function has received extensive attention. Lac metabolism plays a vital regulatory role in the central nervous system[13]. The concentration of Lac expression in the external environment reflects nerve tissue function under different pathophysiological conditions. Under normal circumstances, the body will promote the conversion of glucose to pyruvate during aerobic metabolism. As a critical node in the tricarboxylic acid cycle, pyruvate encourages energy production[14]. Under aerobic metabolic conditions, the amount of Lac induced by pyruvate is less.

In contrast, under hypoxic conditions, the mitochondrial tricarboxylic acid cycle and oxidative phosphorylation pathway are saturated, or metabolic disorders appear, and the body produces through the glycolysis pathway. Energy and a large amount of Lac and Lac can also be converted into pyruvic acid and then enter the tricarboxylic acid cycle to participate in energy regeneration. As a metabolite produced by cellular respiration and metabolism under anaerobic conditions, Lac is often considered to have no role in the body's biological process[15]. However, growing evidence suggests that Lac may function as a significant recyclable carbohydrate fuel, at least in mammals. As a three-carbon compound pool in mammalian cells, Lac can provide a convenient source of three-carbon compounds, and circulating Lac also uncouples glycolysis from carbohydrate-driven mitochondrial energy production. Lac, together with pyruvate, also acts as a circulating redox buffer, balancing the NADH/NAD ratio in cells and tissues[16]. The accumulation of Lac between tissues is also closely related to cellular processes, such as angiogenesis, hypoxia, macrophage polarization and T cell activation, etc. It is also closely associated with various diseases, including tumour formation, sepsis, autologous immune disorders, etc. In existing studies, Lac is mainly known as a metabolite. In recent years, some new functions have been discovered one after another. For example, a large amount of Lac accumulated outside cells can be absorbed by tumour cells and transported to mitochondria for oxidative phosphorylation and energy supply. Lac in the tumour microenvironment has an inhibitory effect on the killing function of immune cells[17].

This study found that the model showed many morphological disturbances in different brain regions under MRI detection[18]. The changes in the right prefrontal cortex, bilateral thalamus, putamen and the white matter connecting the above areas were more noticeable. There was extensive cortical thinning and volume reduction in the area, and the abnormality of various tensors was suggested. At the same time, the applicant obtained the PD value of the tree shrew using the MAGIC sequence for the first time (see the previous work section)[18, 19]. Using the tree shrew-specific maze independently developed by the applicant's team, it was found that the homing time reflecting cognitive function was significantly longer than that of the control group. This model is more sensitive to CZP intervention, and a small dose of CZP can effectively shorten the homing time. On this basis, the applicant found for the first time that continuous MK-801-induced abnormal brain function could lead to a constant increase in serum Lac of tree shrew in the experimental group and remained at a higher level than the control group. At the 4th week of combined CZP intervention, it was found that the experimental group had a significantly higher heart rate than the control group. ECG showed prolonged QT interval, elevated myocardial enzymes, echocardiography showed a significant decrease in cardiac output, immunohistochemistry showed a deterioration of the inflammatory microenvironment of myocardial tissue, and a large number of monocytes multinucleated giant cells were aggregated under the microscope. MK-801 was discontinued on this basis. It was seen that serum lactate decreased progressively and dropped to the level of the control group in about two weeks (the cognitive function did not differ from the control group until the 4th week), and all parameters of myocarditis gradually recovered.

In this study, it was found that PET-CT detection and virus axonal tracing technology based on a drug-induced model indicated that glucose reuptake occurred in the tree shrew from the right prefrontal lobe to the central occipital nucleus and the lateral geniculate nucleus during cognitive impairment. If the function is abnormal, the virus (AAV-CaMKIIa-hM3Dq-GFP/AAV-CaMKIIa-hM4Di-mCherry) was injected into the critical brain regions obtained by PET-CT data and virus tracing analysis. One week after recovering from surgery, the virus expression time is 3-5 weeks. Behavioural training or rest in the cage can be carried

out during the period according to the different behavioural experiment times. Intraperitoneal administration of CNO-activated virus can activate or inhibit key brain regions for at least 6 hours. Continuous CNO stimulation and monitoring of serum Lac expression during this period show that the word of Lac increases with cognitive function decline. Constant CZP intervention until the 5th week shows myocarditis The increase of each index means that the heart rate of the experimental group is significantly lower than that of the control group, the electrocardiogram shows that the QT interval is prolonged, the myocardial enzyme is increased, the cardiac output is decreased dramatically, and the immunohistochemistry indicates that the inflammatory microenvironment of the myocardial tissue has deteriorated. Aggregation of monocytes and multinucleated giant cells. The tree shrew model induced by chemical genetics avoids the systemic receptor interference of MK-801 and more comprehensively simulates the behavioural disorders and cognitive impairment caused by abnormal clinical brain function. The high level of Lac expression was similar to the clinically observed data. The detection of pan-lactated protein suggested that the protein was significantly modified by acetylation in myocardial tissue.

The limitation of this study is that the characteristics of CZP in promoting myocardial protein lactation under high Lac conditions are not yet known[20]. Combined with quantitative lactation modification omics screening technology, we can clarify the lactation modification of myocardial protein and identify specific protein modification sites[16]. Using inflammation-related proteomic detection methods to study the changes and characteristics of a myocardial inflammatory microenvironment.

Conclusions

Lactic modification of cardiac proteins and participate in the process of CZP-induced myocarditis by affecting the myocardial inflammatory microenvironment. Both MK801 and virus can effectively establish a tree shrew schizophrenic model. Clozapine-treated tree shrew models of schizophrenia showed it decreased cardiac function, which might be related to cardiac inflammatory response due to lactic acid accumulation

Declaration of competing interest

On behalf of the authors, no conflict of interest is present in this work.

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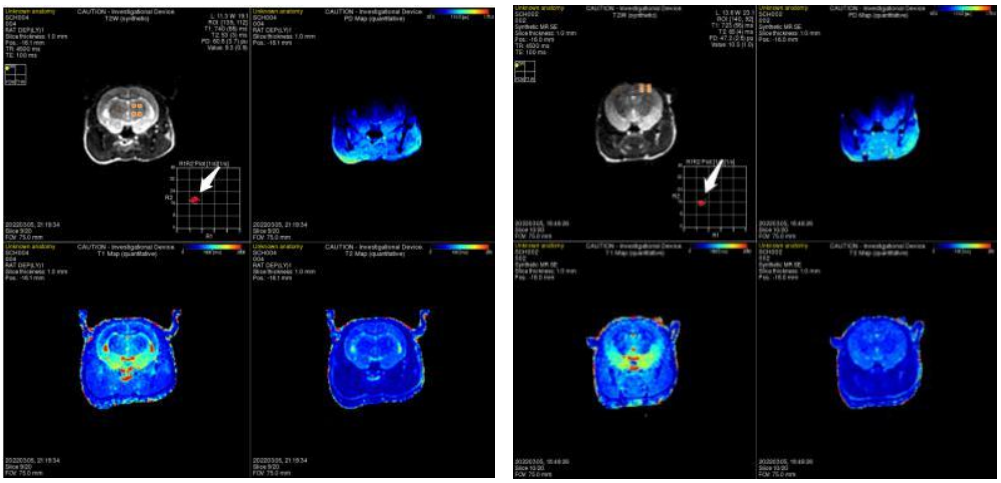
a b



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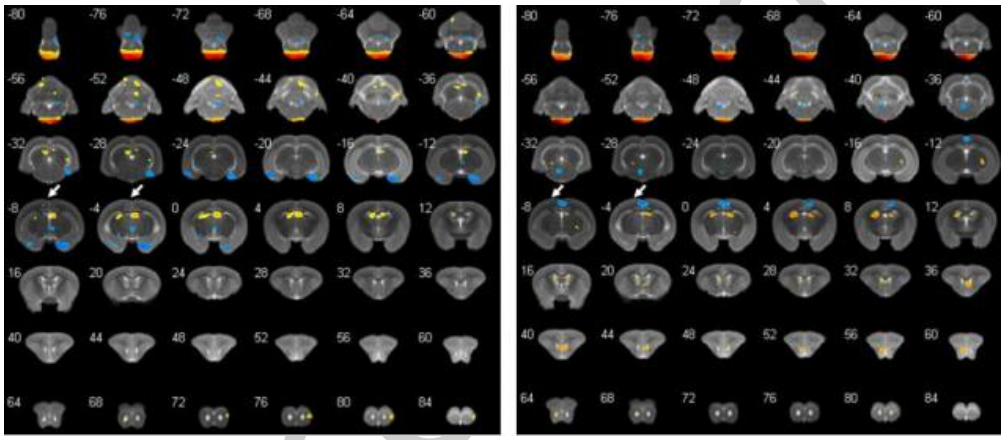

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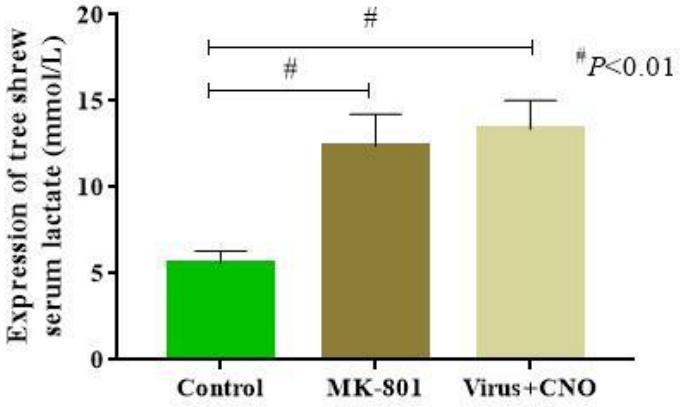
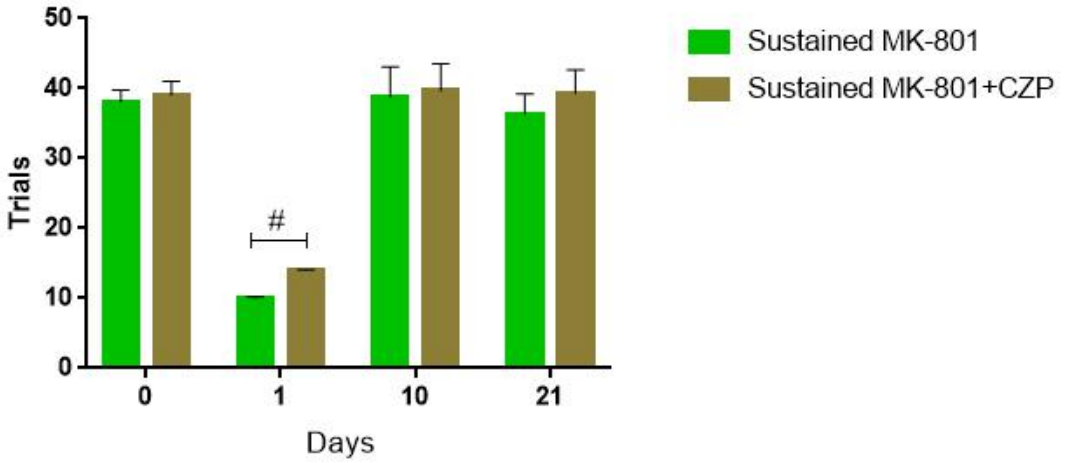


Fig1 a b The magic sequence of MRI indicated that the conduction ability of the cortex, thalamus and dorsal extrathalamus of the SCZ tree shrew was lower than that of the regular group. c PET-CT suggests that the glucose metabolism capacity of the dorsolateral prefrontal cortex, lateral geniculate nucleus and thalamus is decreased.d ompared with the mk801 group and the control group, the expression of lactate was increased in the VIRUS group.($^{\#}P<0.01$)

a



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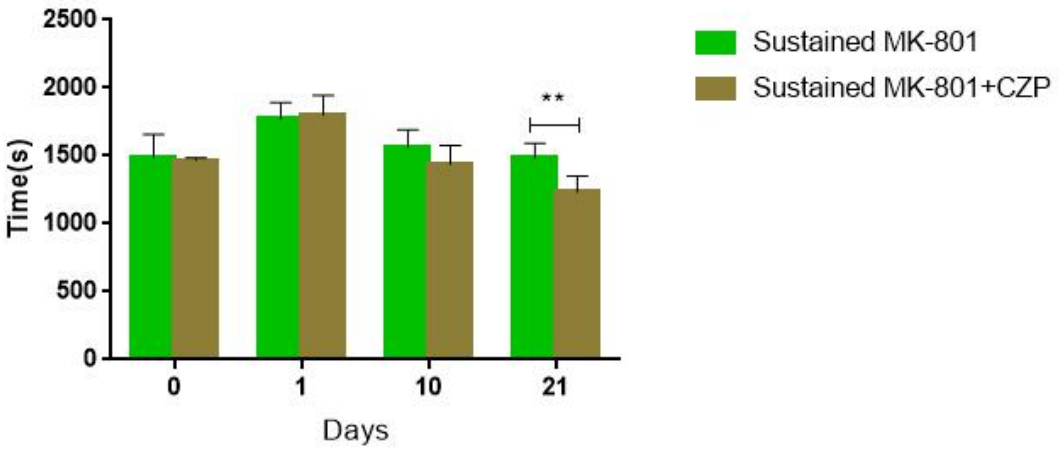

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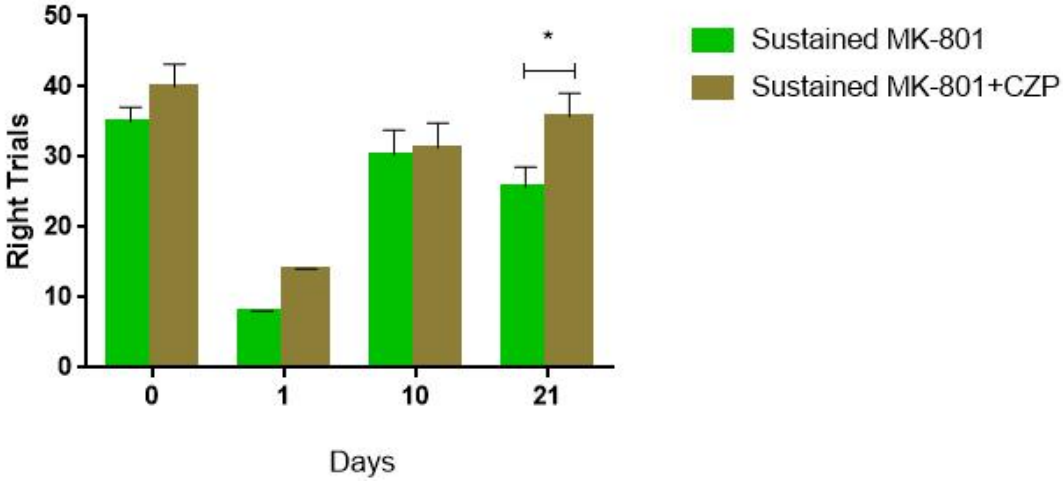


Fig2 The number of tasks completed by animals after MK-801 was significantly reduced ($^{\#}P<0.05$ vs Day 0; At 21 days, the task completion time of the clozapine group was significantly different from that of the MK-801 group ($^{**}P<0.01$); Clozapine was very effective in correcting the number of functions and was substantially different from the solvent control group on the first day of modelling ($^{*}P<0.05$).

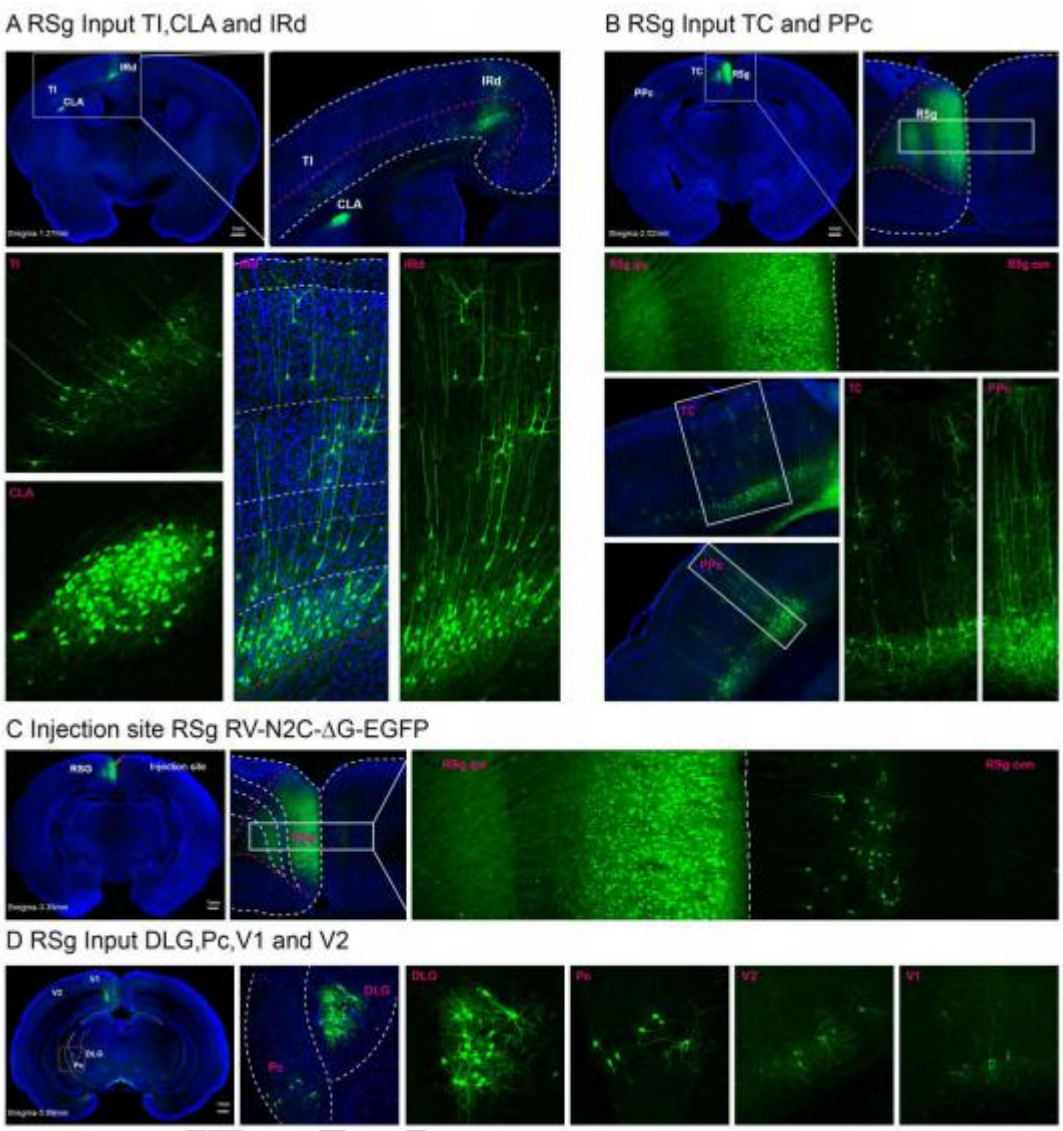
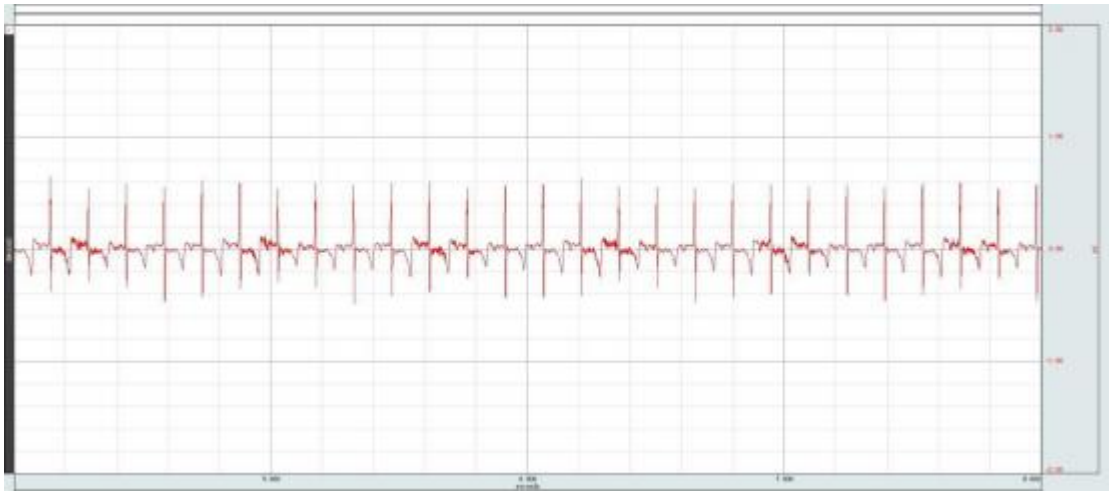


Fig3 Positive areas include TI temporal inferior area,CLA claustrum, IRd infraradiata dorsalis, TC temporal cortex, PPc posterior parietal caudal area, DLG dorsal lateral geniculate nucleus, Pc central nucleus of the pulvinar, V1 primary visual cortex, V2 secondary visual cortex

a



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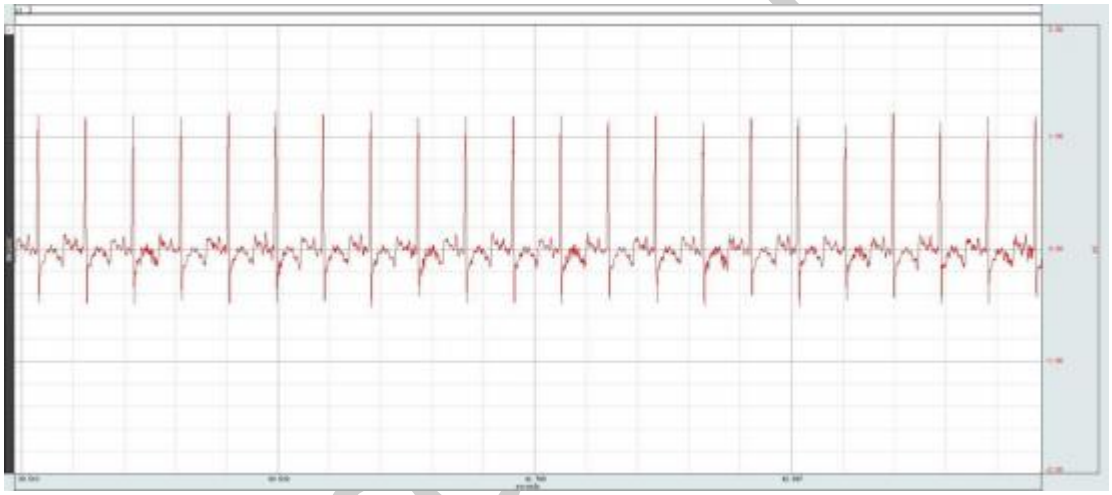


C

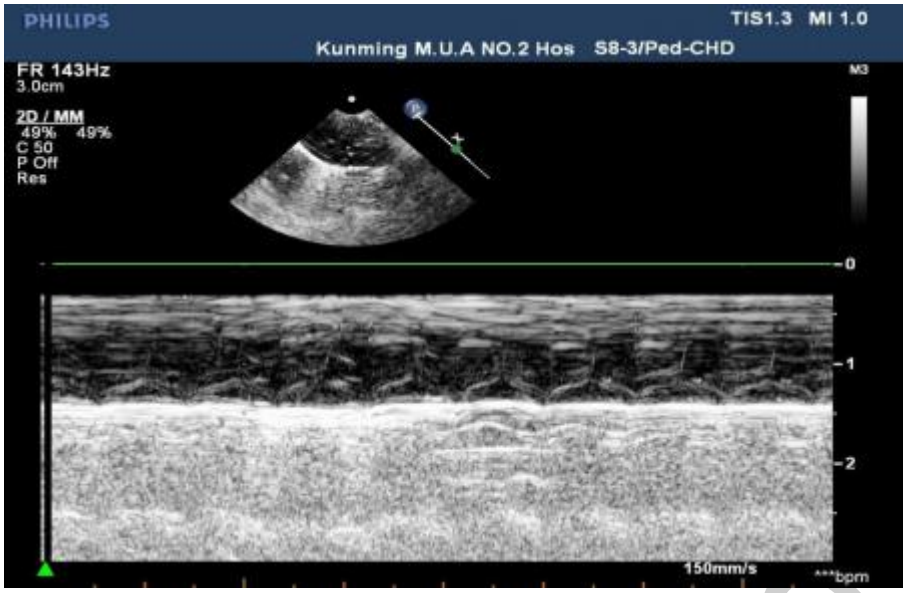
Table 1. Comparison of dynamic electrocardiogram parameter in two groups($\overline{X} \pm s$)

Item	SCZ	CIM
QTd(ms)	1170±17.31	1352±18.14*
SVT(%)	16.28±5.24	25.33±4.28*

QTd: QT interval dispersion; SVT: Sustained ventricular tachycardia;

Fig4 Extensive lactation modification in myocardium detected by western blot pan-lactation resistance assay. Comparison of dynamic electrocardiogram parameters in two groups, QT interval dispersion and Sustained ventricular tachycardia, showed marked abnormalities in the tree shrew CIM model(*P<0.05).

a



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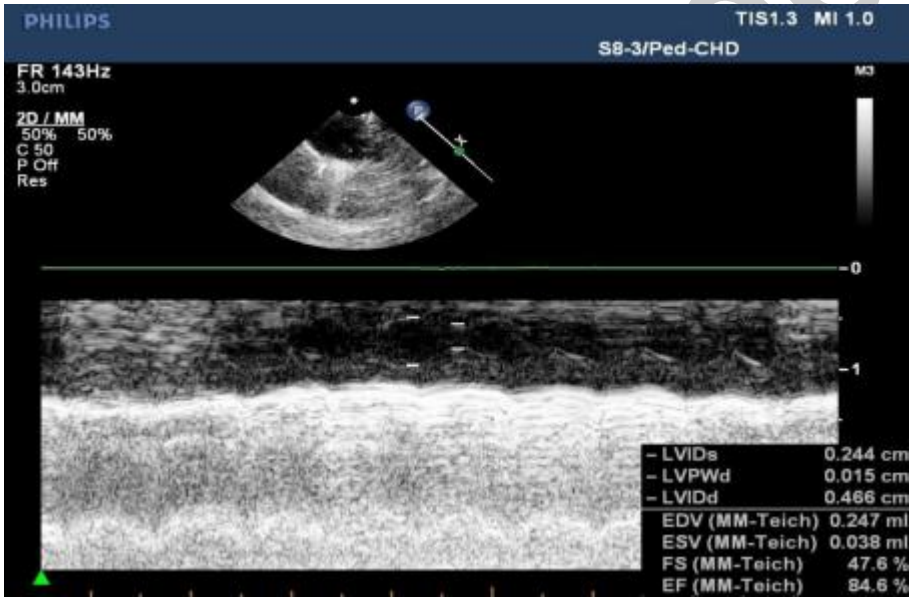


C

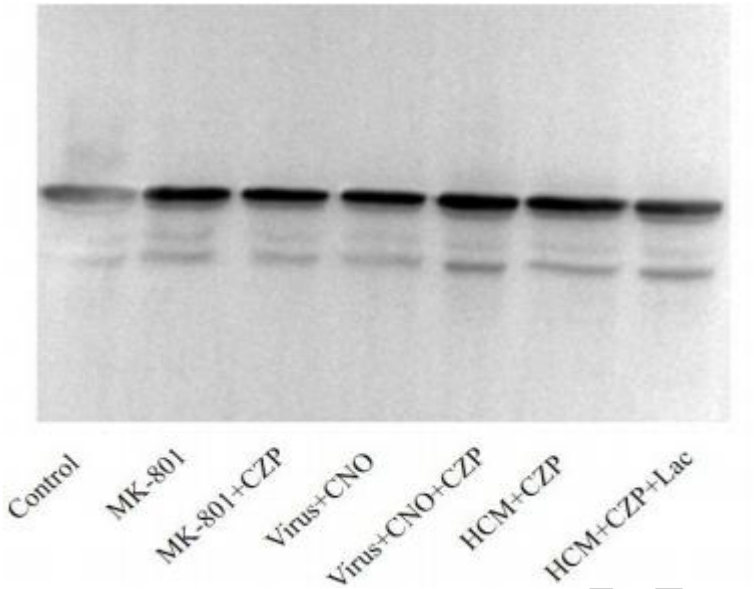
Table 2 Comparison of cardiac function in two groups($\bar{X} \pm s$)

Item	SCZ	CIM
LVEDV (ml)	0.278±0.036	0.316±0.018*
LVEF (%)	85.74±10.32	76.58±8.81*
LVESV (ml)	0.044±0.0039	0.049±0.0028

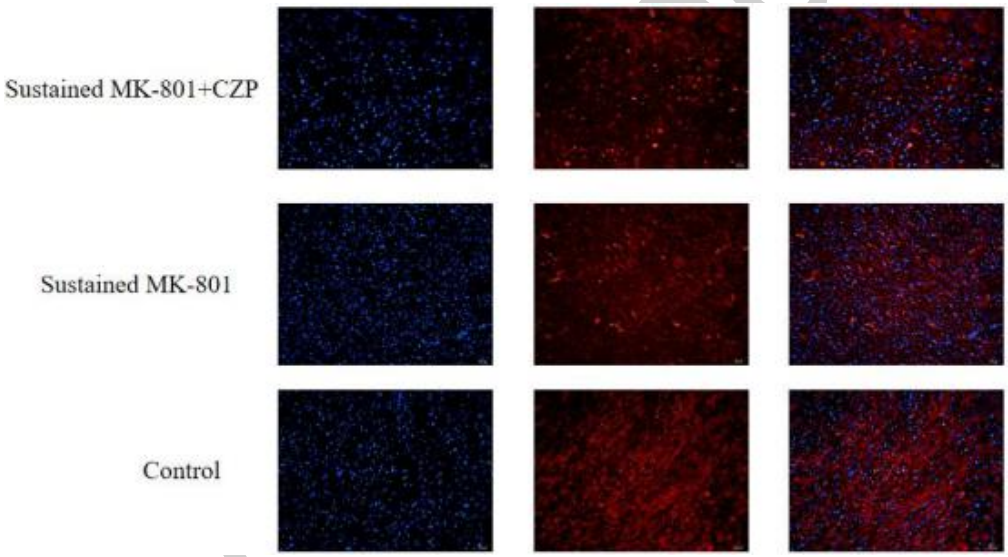
LVEDV: Left ventricular end-diastolic volume; LVEF: Left ventricular ejection fraction; LVESV: Left ventricular end-systolic volume

Fig5 Using cardiac ultrasound to examine the tree shrew model, a comparison of cardiac function in two groups: Left ventricular end-diastolic volume and left ventricular ejection fraction, showed marked abnormalities(*P<0.05)

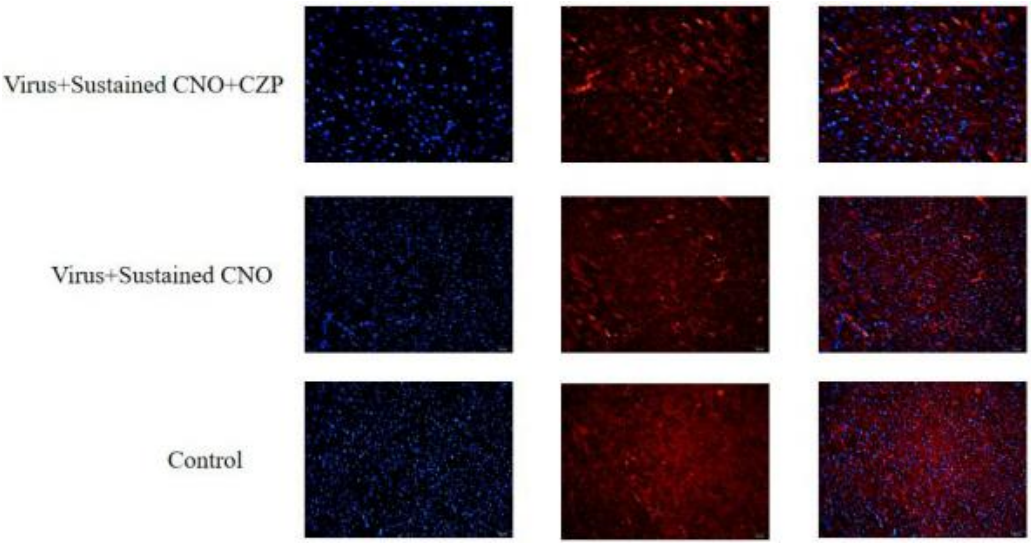
a


Control
MK-801
MK-801+CZP
Virus+CNO
Virus+CNO+CZP
HCM+CZP
HCM+CZP+Lac

b


Sustained MK-801+CZP
Sustained MK-801
Control

c


Virus+Sustained CNO+CZP
Virus+Sustained CNO
Control

d

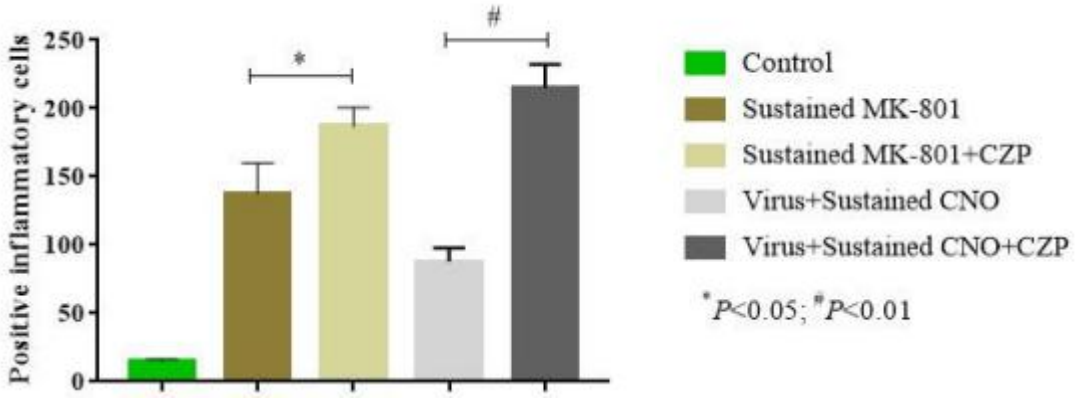


Fig6 The study found that the myocardial tissue with more severe lactation had increased myocardial enzyme expression.The expression of IL-1B was significantly increased in CIM (Fig6 $^{*}P<0.05$;$^{\#}P<0.01$).