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Analysis of the correlation between the expression levels of caspase-1/IL-1β in the peripheral blood and clinical efficacy in patients with first-episode MDD

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Abstract

Background Major depressive disorder (MDD) pathogenesis is associated with immune-inflammatory dysregulation. This study investigated the mechanistic role of caspase-1-mediated inflammatory pathways in MDD, exploring their therapeutic targeting potential and clinical predictive utility.

Methods Peripheral blood levels of caspase-1, IL-1β, and IL-10 were assessed in MDD patients, with longitudinal analysis of dynamic changes pre-/ post-antidepressant treatment. The predictive performance of the biomarker was validated using leave-one-out cross-validation (LOOCV) and receiver operating characteristic (ROC) analysis. A chronic unpredictable stress (CUS) rat model was established to evaluate the combined therapeutic effects of caspase-1 inhibitors with fluoxetine.

Results Clinical data revealed that expression levels of caspase-1 and IL-1β exhibited an negative correlation with clinical treatment response in patients with MDD, with varying levels observed among patients with differing depression severity. In contrast, IL-10 expression demonstrated a positive correlation with therapeutic efficacy. Following treatment, significant reductions in caspase-1 and IL-1β levels were observed, concurrent with an elevation in IL-10. Caspase-1 demonstrated superior predictive accuracy for treatment response (0.8529 ± 0.3542), yielding an area under the ROC curve (AUC) of 0.72. In animal models, co-administration of a caspase-1 inhibitor with fluoxetine accelerated the onset of antidepressant effects, while simultaneously reducing peripheral blood caspase-1 and IL-1β levels.

Conclusions The caspase-1/IL-1β axis contributes to MDD pathogenesis by disrupting the pro-inflammatory/anti-inflammatory balance. Its mediated immune response pattern represents a potential therapeutic target for novel antidepressant development. Combined caspase-1 modeling provides a reliable tool for predicting clinical efficacy in MDD, while caspase-1-targeted intervention strategies may overcome the limitation of slow onset of action associated with conventional therapies.

Keywords caspase-1; IL-1β; clinical efficacy; first-episode; MDD.

Introduction

Major depressive disorder (MDD) is a multifactorial mood disorder whose pathogenesis involves a complex interaction between genetic susceptibility and environmental factors [1,2]. With a global lifetime prevalence of approximately 19%, MDD is not only the most prevalent psychiatric cause of disability but also a major contributor to suicide, accounting for an estimated one million annual deaths worldwide [3]. The disorder severely impairs patients' quality of life and occupational functioning, while imposing a substantial socioeconomic burden on healthcare systems.

Current first-line pharmacotherapy for MDD, which is primarily based on the monoamine hypothesis, continues to be the foundation of clinical management. However, treatment resistance affects 10–30% of patients, underscoring the need to elucidate novel pathophysiological mechanisms [4]. Accumulating evidence implicates neuroinflammatory pathways as pivotal players in depression [5]. Pro-inflammatory signaling from the peripheral immune system can activate the hypothalamic-pituitary-adrenal (HPA) axis via sympathetic nervous system communication across the blood-brain barrier, establishing a bidirectional neuroimmune circuit that modulates depression-related behaviors [6,7]. Clinical observations consistently demonstrate elevated peripheral inflammatory markers in MDD patients, including increased granulocyte/monocyte counts and heightened levels of pro-inflammatory cytokines such as tumor necrosis factor-alpha (TNF-α), interleukin-6 (IL-6), and interleukin-1β (IL-1β) [8]. Furthermore, post-mortem brain analyses from depressed suicide victims reveal aberrant glial cell activation, and adjunctive anti-inflammatory therapies show efficacy in certain patient subgroups. These collective findings support the neuroinflammatory hypothesis of depression [9,10]. In stress-induced depression models, psychosocial stressors elicit not only depression-like behaviors but also increase peripheral and central inflammatory mediators such as IL-1β, TNF-α, and IL-6, while concurrently impairing synaptic plasticity and disturbing neurotransmitter homeostasis [11,12]. Emerging evidence also points to a gut-immune axis, where stress-induced gut dysbiosis promotes the differentiation of pro-inflammatory γδ17T cells and subsequent IL-1β-driven neuroinflammation [13]. These findings establish inflammatory responses as central elements in the multifactorial neurobiology of

depression and underscore inflammation-related biomarkers as promising candidates for early diagnosis and precision therapeutics[14].

Among key inflammatory mediators in MDD, IL-1β is consistently elevated in the serum and brain tissues of depressed patients [15]. Its upstream activator, caspase-1, serves as the IL-1β-converting enzyme and is critically implicated in MDD pathophysiology. Evidence shows increased caspase-1 mRNA in the peripheral blood of MDD patients, which normalizes following antidepressant therapy [16, 17]. Preclinical studies further support this link: chronic stress upregulates caspase-1 in rodent brain and plasma [18]. Mechanistically, as a core inflammasome component, caspase-1 catalyzes the proteolytic maturation of pro-IL-1β into its active form [19]. This process promotes the release of mature IL-1β and IL-18 while concurrently reducing levels of the anti-inflammatory cytokine IL-10 [20]. IL-10 counteracts this response through a negative feedback mechanism that inhibits NOD-like receptor thermal protein domain associated protein 3 (NLRP3) inflammasome activation, caspase-1 maturation, and IL-1β production [21–23]. Together, these interactions highlight a dysregulated inflammatory axis that contributes to the pathophysiology of MDD.

Despite this compelling evidence, key translational questions regarding the caspase-1/IL-1β pathway remain unresolved. The temporal dynamics of this pathway in relation to depression severity and treatment response are poorly defined, limiting its utility as a clinical biomarker. Furthermore, although IL-1β is an established inflammatory marker in MDD, the role of its upstream regulator, caspase-1, and the integrated activity of the caspase-1/IL-1β axis are inadequately characterized in first-episode, drug-naïve patients. Finally, the potential of this pathway to serve as a predictive biomarker for treatment outcomes remains largely unexplored.

To address these critical gaps, this study aims first to correlate peripheral caspase-1 and IL-1β expression levels with baseline depression severity in first-episode, drug-naïve MDD patients. We will further determine how changes in these inflammatory markers

following 4 weeks of antidepressant treatment are associated with the degree of clinical improvement. Finally, we seek to preclinically validate whether combined administration of a caspase-1 inhibitor and fluoxetine yields synergistic antidepressant effects in a chronic unpredictable stress (CUS) rodent model. Based on these objectives, We hypothesize that peripheral caspase-1/IL-1β levels not only positively correlate with baseline depression severity but also that their treatment-induced reduction predicts clinical improvement, and consequently, combined caspase-1 inhibition and fluoxetine will produce synergistic antidepressant effects in the CUS model. By integrating clinical longitudinal assessment with preclinical mechanistic validation, this research seeks to establish the caspase-1/IL-1β pathway as a robust biomarker for predicting treatment response and a novel target for therapeutic intervention, ultimately contributing to the advancement of precision medicine in depression management.

Subject and Methods

Study Subjects

This study employed a consecutive enrollment method, a type of non-probability sampling, to recruit a cohort of patients with first-episode MDD. A total of 40 inpatients were initially enrolled from Chaohu Hospital of Anhui Medical University between January 2024 and January 2025. Among them, six patients were excluded from the final analysis: three due to hospitalization lasting less than four weeks and three who voluntarily withdrew from the study. Consequently, 34 patients completed the follow-up protocol. It is noteworthy that among the six excluded cases, three were discharged early due to significant clinical improvement, two had their medication changed mid-study due to insufficient therapeutic efficacy, and one discontinued treatment because of pronounced adverse effects, notably nausea. The control group included 32 cases of healthy volunteers from the medical examination center in Chaohu Hospital of Anhui Medical University during the same period. All participants signed an informed consent form approved by the Ethics Committee of Chaohu Hospital of Anhui Medical University (No.KYXM-202310-045).

Inclusion criteria: 1) Diagnosed with MDD according to the diagnostic criteria of the Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition (DSM-5) by two physicians with the title of attending physician or above; 2) Age 18-60 years; 3) Educational level of primary school and above; 4) First episode of MDD and no use of antidepressant medication before admission; 5) Clinical symptoms were assessed using the Hamilton Depression Rating Scale-24 items (HAMD-24) and the Hamilton Anxiety Scale (HAMA), and a HAMD-24 score ≥ 20 was required. 6) No history of infection in the last 2 weeks and no use of hormones, antibiotics, or other anti-inflammatory drugs.

Exclusion criteria: 1) Pregnant or breastfeeding women were excluded; 2) Patients with serious somatic diseases such as unstable cardiovascular disease, abnormal liver, and kidney function, endocrine disorders, or active/chronic inflammation were also excluded; 3) Being studied in other clinical programs; 4) Combination of other mental disorders such as bipolar disorder, schizophrenia, psychoactive substances, epilepsy, or abnormal brain development.

Experimental animals

SPF-grade male C57BL/6J mice (6-8 weeks old, Henan Skibbes Bio-technology Co., Ltd, Licence No.: SYXK (Anhui) 2020-001) were used. They were housed in a constant-temperature and constant-humidity environment (temperature 21-23°C, humidity 40-60%, 12 h circadian rhythm), and four mice per cage were free to ingest and drink. The experimental protocol was approved by the Laboratory Animal Ethics Committee of Anhui Medical University (No.LLSC20241835).

Methods

Clinical data collection

Demographic characteristics were documented for all participants. Scale assessments, including the 24-item Hamilton Depression Rating Scale (HAMD-24) and the Hamilton Anxiety Rating Scale (HAMA), were independently administered by two psychiatrists who had undergone standardized training to ensure consistency.

Depression severity was classified using the HAMD-24 as follows: severe depression (> 35 points), mild-to-moderate depression (20–35 points), probable depression (8–20 points), and no depression (< 8 points). This scale demonstrated good internal consistency in the study sample, with a Cronbach's α of 0.86. Anxiety severity was assessed using the 14-item HAMA and categorized as: severe anxiety (≥ 29 points),

marked anxiety (≥ 21 points), moderate anxiety (≥ 14 points), possible anxiety (≥ 7 points), and no anxiety (< 7 points). The HAMA also showed high internal consistency, with a Cronbach's α of 0.89. Clinical efficacy was evaluated based on the reduction rate of HAMD scores before and after treatment , calculated as follows:

Reduction rate = [(pre-treatment score – post-treatment score) / pre-treatment score] × 100%. The outcomes were defined as: Cured: reduction rate ≥ 75%; Adequate improvement: reduction rate ≥ 50%; Effective: reduction rate ≥ 25%; Ineffective: reduction rate < 25%[24].

Drug treatment program

The clinical group was treated with individualized SSRIs (fluoxetine, sertraline, etc.), with the dose adjusted concerning the range of instructions (Refer to the Clinical Data section of the Supplementary Materials). The animal groups were given fluoxetine (HY-B0102, MedChemexpress, USA) and Ac-YVAD-cmk (HY-16990, MedChemexpress, USA) according to the literature method, which were injected intraperitoneally daily [25,26].

Animal experimental design After 1 week of acclimatization feeding, the animals were randomly divided into five groups (n=10/group): (1) Control group: conventional feeding; (2) CUS+Fluoxetine group (CUS+FXT): intraperitoneal injection of fluoxetine 10 mg/kg (3 weeks) after 4 weeks of Chronic Unpredictable Stress (CUS) modeling; (3) CUS+Ac-YVAD-cmk group (CUS+YVAD): injection of YVAD 8 mg/kg (3 weeks) after CUS modeling; (4) CUS+FXT+YVAD group: combined injection of the above two drugs after CUS; (5) CUS group: modeling alone without intervention. Behavioral assessments were conducted after CUS intervention, after 1 week of drug treatment, and after 3 weeks of drug treatment, respectively. A schematic diagram of the experimental design is provided in Figure 1.

Biological sample processing

In the clinical group, 5 ml of fasting venous blood was collected at baseline and 4 weeks of treatment and centrifuged at 1500g for 10 min at 4°C after EDTA anticoagulation, and the serum was divided and stored at -80°C. In the mouse group, 0.5-1.0 mL of blood was collected after the behavioral test and treated similarly.

Inflammatory factor assay

Serum concentrations of caspase-1, IL-1β, and IL-10 were measured using commercially available enzyme-linked immunosorbent assay (ELISA) kits (Jianglai Biotechnology Co., Ltd.), according to the manufacturer's protocols. The following kits were used: Human caspase-1 ELISA Kit (JL14728-96T): detection limit < 0.1 pg/mL, intra-assay CV < 9%, inter-assay CV < 11%; Human IL-1β ELISA Kit (JL13662-96T): detection limit < 0.1 pg/mL, intra-assay CV < 10%, inter-assay CV < 10%; Human IL-10 ELISA Kit (JL19246-96T): detection limit < 0.32 pg/mL, intra-assay CV < 10%, inter-assay CV < 10%; Mouse IL-1β ELISA Kit (JL18442-96T): detection limit 1.98 pg/mL, intra-assay CV < 10%, inter-assay CV < 10%; Mouse caspase-1 ELISA Kit (JL20273-96T): detection limit 3.1 pg/mL, intra-assay CV < 10%, inter-assay CV < 10%; Mouse IL-10 ELISA Kit (JL20242-96T): detection limit 1.45 pg/mL, intra-assay CV < 10%, inter-assay CV < 10%. Standard curves were generated using serially diluted standards included in each kit, which were run in parallel with study samples. Absorbance was read at 450 nm, and analyte concentrations were interpolated from the respective standard curves. To minimize variability, all assays were performed in a single batch by the same operator under standardized conditions. Each sample was measured in duplicate, and a common internal reference sample was included across plates to normalize inter-plate measurements and ensure reproducibility.

CUS procedure

In the modified classical CUS model, animals were subjected to two randomized stressor exposures per day over 4 weeks [27]. The stressors included (1) fasting for 12h; (2) water fasting for 12 h; strobe flash illumination for 12 h (19:00-07:00); (3) damp bedding for 12 h (wetting mouse bedding with 150-200ml of water); (4) behavioral restraint (restraining mice in 50ml porous centrifugal tubes); (5) iced-water swimming (5-min stimulation with ice-water at 6°C); and (6) tail pinching (Use a clip to hold the root of the mouse tail at 1cm for 5min); (7) tilting the mouse cage (45° tilting of the cage for 12h); (8) diurnal reversal (placed in a dark environment from 07:00-19:00; (9) placed in a light environment from 19:00-07:00).

Behavioural assessment

All experimental procedures were conducted within a climate-controlled behavioral testing suite. Subjects were habituated to the testing environment through a 2-hour acclimatization period prior to experimental commencement. Between trials, apparatus surfaces (including chamber interiors and flooring) were thoroughly decontaminated using 75% ethanol solution, with subsequent testing initiated only after complete solvent evaporation. Behavioral parameters were quantified and processed using SMART 3.0 behavioral analysis software (PanLab, Spain).

Sucrose Preference Test (SPT)

SPT was employed to evaluate anhedonia, which is a core symptom of depression reflecting the loss of pleasure in rewarding stimuli. Following a 24-hour fluid deprivation period, subjects received simultaneous access to two drinking solutions: 1% sucrose (w/v) and standard tap water, presented in identical graduated bottles. To control for positional bias, bottle orientation was systematically alternated at the 12-hour interval. Fluid consumption was measured gravimetrically over a 24-hour test window. Sucrose preference indices were calculated as: Sucrose preference (%) = Sucrose water consumption / (Sucrose water consumption + Tap water consumption) × 100%.

Open Field Test (OFT)

The OFT was conducted to assess general locomotor activity and exploratory behavior in a novel environment. This test is crucial for ruling out the confounding effects of altered locomotor activity on the performance in other behavioral paradigms. Exploratory behavior and spontaneous locomotor activity were assessed in a novel arena (50×50×50 cm polyvinylchloride chamber) under controlled illumination. Subjects were positioned in the central quadrant (25×25 cm demarcation) for trial initiation. Movement patterns were video-tracked for 5 minutes, with primary outcome measures comprising central zone occupancy duration (sec).

Forced Swimming Test (FST)

The FST was used to quantify "behavioral despair," a state in which mice, after initial escape-oriented movements, develop immobility when exposed to an inescapable

stressful situation, which is thought to reflect a depressive-like state. Behavioral despair was quantified using a modified Porsolt paradigm. Subjects were individually placed in a transparent cylindrical vessel (30 cm height × 12 cm diameter) containing a 23-25°C water column (15 cm depth). Trials consisted of a 2-min acclimatization phase followed by a 4 min observational period. Immobility duration (defined as cessation of escape-directed movements while maintaining necessary buoyancy) served as the principal metric of depressive-like behavior.

Tail Suspension Test (TST)

Similar to the FST, the TST is another widely used model to measure behavioral despair. It evaluates the duration of immobility when mice are subjected to an unavoidable, mildly stressful situation, which is sensitive to antidepressant treatment. TST was implemented to quantify depressive-like immobility through a modified Steru-Laborie protocol. Subjects were suspended via adhesive fixation to an elevated horizontal bracket (10 cm clearance from the platform surface), ensuring ventral orientation and unrestricted cephalic movement. Behavioral responses were video-recorded for 6 min under acoustic isolation conditions. Initial 2 min constituted habituation to the aversive stimulus, with subsequent 4 min observational window analyzed for immobility duration.

Statistical Analysis

Data analysis was performed using SPSS Statistics 26.0 (IBM Corp.) and RStudio. Continuous variables, including demographic characteristics, psychometric scale scores, and inflammatory biomarkers such as caspase-1, IL-1β, and IL-10, were normally distributed according to the Shapiro–Wilk test and are expressed as mean ± standard deviation. Group comparisons were conducted using independent-samples *t*-tests, while within-subject changes before and after treatment were evaluated with paired *t*-tests. Categorical variables, such as sex, are presented as frequencies and percentages, with baseline differences examined via chi-square tests. Prior to correlation analyses, normality was verified; Pearson correlation was applied for

normally distributed data, and Spearman rank correlation was used otherwise. Treatment response was dichotomized into significant improvement versus poor response based on HAMD score reduction rates. The predictive value of biomarker panels for antidepressant efficacy was assessed using leave-one-out cross-validation and receiver operating characteristic curve analysis. For mouse experiments, behavioral outcomes and peripheral inflammatory markers were analyzed by one-way ANOVA with Bonferroni post hoc correction. A two-tailed significance level of $\alpha = 0.05$ was set, with $p < 0.05$ considered statistically significant.

Results

Analysis of general information

There were no significant differences between the two groups in demographic characteristics such as gender, age, and years of education ($p > 0.05$). HAMD and HAMA scores were significantly higher in the depression group than in the control group ($p < 0.01$). The expression levels of caspase-1 and IL-1β in the peripheral blood of patients in the depression group were significantly higher than those in the control group ($p < 0.01$). In contrast, the expression level of IL-10 was slightly lower than that of the control group, but the difference was not statistically significant ($p > 0.05$).

Regarding the stratification within the depression group into severe depression (SD) and mild to moderate depression (MD) subgroups, no significant differences were observed in duration ($p > 0.05$) and family history ($p > 0.05$) between the two subgroups. However, the HAMD score was significantly higher in the SD subgroup than in the MD subgroup ($p < 0.01$). Additionally, peripheral blood levels of caspase-1 ($p < 0.01$) and IL-1β ($p < 0.01$) were significantly higher in the SD subgroup compared to the MD subgroup. In contrast, there was no significant difference in IL-10 level between the SD and MD subgroups ($p > 0.05$). See Table 1.

Pre- vs post-treatment changes in clinical scores and biomarkers in the depression group

After treatment, the HAMD and HAMA scores of patients in the depression group were significantly reduced ($p < 0.01$). Concurrently, the expression levels of caspase-1 and IL-1β in peripheral blood decreased significantly compared with the baseline period ($p < 0.01$), while the expression level of IL-10 increased significantly ($p < 0.01$).

When stratified into MD and SD subgroups, after 4 weeks of treatment, both subgroups showed significant reductions in HAMD scores ($p < 0.01$), and the post-treatment HAMD score was significantly lower in the MD subgroup than in the SD subgroup ($p < 0.01$). For HAMA scores, both MD ($p < 0.01$) and SD ($p < 0.01$) subgroups exhibited significant decreases after treatment, with no significant difference between the two subgroups ($p > 0.05$).

Regarding peripheral blood biomarkers, caspase-1 levels were significantly reduced in both MD ($p < 0.01$) and SD ($p < 0.01$) subgroups post-treatment, without a significant subgroup difference ($p > 0.05$). Similarly, IL-1β levels decreased significantly in both MD ($p < 0.01$) and SD ($p < 0.01$) subgroups, with no significant between-subgroup difference ($p > 0.05$). In contrast, IL-10 levels increased significantly in both MD ($p < 0.01$) and SD ($p < 0.01$) subgroups after treatment, and there was no significant difference between the two subgroups ($p > 0.05$). See Table 2.

Blood inflammatory markers and treatment efficacy in depression

To investigate the differences in clinical indicators between groups with different therapeutic efficacy, we compared the baseline and 4-week post-treatment data of the poor response group (n=10) and the efficacy group (n=24).

At baseline, there were no significant differences in the scores of HAMD ($p > 0.05$) and HAMA ($p > 0.05$) between the two groups. However, the baseline levels of caspase-1 ($p < 0.05$) and IL-1β ($p < 0.01$) were significantly higher in the poor response group than in the efficacy group, while the baseline level of interleukin-10 (IL-10) was significantly lower in the poor response group ($p < 0.01$).

After 4 weeks of treatment, the HAMD score remained significantly higher in the poor response group compared to the efficacy group ($p < 0.01$), whereas no significant difference was observed in the HAMA score between the two groups ($p > 0.05$). For inflammatory markers, the levels of caspase-1 ($p < 0.01$) and IL-1β ($p < 0.01$) were still significantly higher in the poor efficacy group, and the level of IL-10 was significantly lower in this group ($p < 0.01$). See table 3.

HAMD score reduction rate was used to assess clinical efficacy, with a higher score reduction rate indicating better clinical efficacy. The results showed that caspase-1 (OR= -0.734, $p < 0.01$) and IL-1β (OR= -0.643, $p < 0.01$) were negatively correlated with clinical efficacy in the total depression sample, suggesting they act as risk factors for clinical efficacy. In contrast, IL-10 (OR=0.671, $p < 0.01$) was positively correlated with clinical efficacy, indicating it is a protective factor.

When stratified into MD (n=19) and SD (n=15) subgroups, consistent correlation patterns were observed. For caspase-1, negative correlations with the HAMD reduction rate were noted in the MD subgroup (OR=-0.615, $p < 0.01$) and the SD subgroup (OR= -0.860, $p < 0.01$). For IL-1β, negative correlations existed in the MD subgroup (OR= -0.478, $p < 0.05$) and the SD subgroup (OR= -0.832, $p<0.01$). Regarding IL-10, positive correlations with the HAMD reduction rate were identified in the MD subgroup (OR= 0.662, $p<0.01$) and the SD subgroup (OR=0.736, $p<0.01$). These findings consistently illustrate that caspase-1 and IL-1β are risk factors, while IL-10 is a protective factor for clinical efficacy across different depression severity subgroups. See Figure 2.

Predicting MDD treatment response using baseline inflammatory markers

Based on the HAMD score reduction rate, the patients were divided into the efficacy group (n=24) and the poor efficacy group (n=10). We evaluated the predictive performance of baseline inflammatory factors (caspase-1, IL-1β, and IL-10) individually and in combination for forecasting treatment efficacy at 4 weeks, employing machine learning models including Support Vector Machine (SVM), Logistic Regression, Random Forest, and K-Nearest Neighbors (KNN). Through leave-one-out cross-validation (LOOCV), a comprehensive comparative analysis of feature combinations and model performance revealed that the SVM model achieved optimal predictive accuracy (85.29% ± 35.42%) when utilizing baseline caspase-1 levels as a standalone feature, with precision, recall, and F1-scores all attaining high values (mean: 67.65%). Furthermore, multi-feature combinations (e.g., caspase-1 + IL-1β + IL-10) demonstrated comparable accuracy in certain models (e.g., 82.35% in SVM). However, when considering model stability and parsimony, the single-feature approach (baseline caspase-1) provided superior predictive performance with lower standard deviation . Furthermore, caspase-1 exhibited an area under the ROC curve (AUC) of 0.72, indicative of robust generalization capability. Detailed results are presented in Table 4

and Figure 3. The detailed results of the subgroup predictive modeling analysis are available in the respective MD and SD sections of the Supplementary Materials.

YVAD and fluoxetine synergistically alleviate depressive-like behavior in CUS mice

Depression-like behavior in mice after CUS modeling**.** See Figure 3A-D. After 4 weeks of CUS modeling, mice in the CUS group showed significant depression-like behaviors in the sucrose water preference experiment, the open field experiment, the forced swimming experiment, and the hanging tail experiment ($p < 0.01$).

Improvement of depressive-like behavior in CUS mice by fluoxetine combined with YVAD intervention. See Figure 3E-H. and Figure 3I-L. After 1 week of intervention, mice in the CUS+FXT+YVAD group showed a significant increase ($p < 0.05$) in sucrose-water preference ratio, timeshare in the central area of the absenteeism experiment, and a significant decrease ($p < 0.01$) in immobilization time in the forced-swimming experiment and the tail-hanging experiment. After 3 weeks of intervention, depression-like behaviors were reversed in both the CUS+FXT and CUS+YVAD groups and were not significantly different from the control group. This finding suggests that the caspase-1 specific inhibitor YVAD combined with fluoxetine can rapidly improve the effects of depressive-like behaviors and provides an important experimental rationale for its use in the treatment of MDD.See Figure 4.

Combined YVAD and fluoxetine treatment reduces peripheral inflammatory and depressive-like behavior in CUS mice

After 3 weeks of caspase-1 specific inhibitor YVAD combined with fluoxetine intervention in CUS mice, the expression levels of caspase-1 and IL-1β in the peripheral blood of mice in the FXT, YVAD, and FXT+YVAD groups were significantly lower compared with the CUS group ($p < 0.01$), and no significant difference from the control group ($p > 0.05$). Meanwhile, the expression level of IL-10 was significantly higher ($p < 0.01$). See Figure 5.

Discussion

This investigation substantiates a critical role for the caspase-1/IL-1β signaling axis in the pathophysiology of first-episode MDD, highlighting its potential clinical relevance.

Neuroinflammatory profiling demonstrated significantly elevated peripheral levels of caspase-1 and IL-1β in MDD patients, with both markers exhibiting positive correlations with disease severity. Although few studies to date have directly assessed the relationship between caspase-1/IL-1β pathway activity and depression severity, our findings align with a growing body of international research, including evidence that NLRP3/caspase-1 inflammasome activation exacerbates systemic inflammation in depression models [28, 29]. However, it is important to note that caspase-1/IL-1β pathway activity demonstrates considerable heterogeneity across depression-related comorbid conditions. For instance, persistent activation of this pathway has been implicated in the shared pathophysiology of depression and cardiovascular disease [30, 31], as well as in neuropathic pain-induced affective disorders, where its activation in key brain regions contributes to comorbid depressive-like behaviors [32].

Experimental evidence further substantiates these clinical observations. Baseline caspase-1/IL-1β levels showed robust associations with MDD, consistent with chronic stress-induced caspase-1 elevation observed in both cerebral and peripheral compartments [18,33]. In chronic restraint stress (CRS) models, concomitant increases in serum and hippocampal caspase-1/IL-1β were inversely correlated with depressive-like behaviors. This aligns with domestic studies demonstrating that caspase-1 knockout attenuates stress-induced depressive-like behaviors [34], while caspase-1 knockdown reversed CRS-induced symptomatology [35]. Critically, caspase-1 inhibitors rescued anhedonic and despair behaviors in LPS- and chronic unpredictable mild stress (CUMS)-induced models [36,37], and pharmacological caspase-1 inhibition exerts antidepressant effects, partly through modulation of gut microbiota [38].

Consistent with prior evidence [11,39,40], antidepressant treatment in our study reduced pro-inflammatory mediators and concurrently promoted a compensatory upregulation of the anti-inflammatory cytokine IL-10. This dynamic reinforces the concept of disrupted inflammatory homeostasis as a fundamental pathophysiological substrate in MDD progression. Notably, exogenous IL-1β administration has been shown to induce depressive phenotypes alongside central and peripheral inflammatory

exacerbation, whereas IL-1β receptor antagonism ameliorates both behavioral and molecular deficits [41]. At the regulatory network level, IL-10 dynamics showed significant positive correlations with clinical improvement. This supports the inflammatory homeostasis hypothesis, which posits that compensatory IL-10 elevation in early-stage MDD is progressively overwhelmed by pro-inflammatory dominance as chronicity increases [42,43]. Reinforcing this paradigm, intranasal IL-10 administration alleviated depressive-like behaviors and cognitive impairments in murine models [44]. Collectively, these findings advocate for dual therapeutic strategies targeting both pro-inflammatory suppression and anti-inflammatory augmentation to restore inflammatory equilibrium.

To assess the clinical applicability of these biomarkers, we evaluated model predictive performance using leave-one-out cross-validation (LOOCV). This analysis revealed that IL-1β or IL-10 alone exhibited marginally lower predictive performance compared to caspase-1, while multi-feature combinations did not demonstrate statistically significant superiority over caspase-1 as a standalone predictor. These findings corroborate the potential clinical utility of caspase-1 for predictive applications in MDD. From a mechanistic perspective, the synergistic effect observed between caspase-1 inhibition and fluoxetine appears to operate through dual pathways: fluoxetine attenuates NLRP3 inflammasome activity via serotonin reuptake inhibition [45], while caspase-1 blockade directly impedes IL-1β maturation and enhances IL-10 production [46]. This dual-target approach mediates accelerated antidepressant effects and sustained anti-inflammatory responses through modulation of the caspase-1/IL-1β axis, offering a novel paradigm for rapid-acting antidepressant development.

Limitations

This study has several limitations. While it demonstrates an association between the caspase-1/IL-1β axis and major depressive disorder, the underlying genetic and cellular mechanisms remain unexplored, limiting insight into pathway regulation. The clinical sample size is modest, and the use of heterogeneous SSRI regimens complicates the evaluation of drug-specific anti-inflammatory effects. Additionally, the consecutive

enrollment method, a form of non-probability sampling, may affect sample representativeness and introduce selection bias. Subgroup analyses based on depression severity were also conducted with limited sample sizes, which may reduce statistical power and compromise the reliability of between-group comparisons. In preclinical models, genetic tools were not used to selectively perturb the pathway, and the blood-brain barrier penetration and long-term safety of caspase-1 inhibitors were not evaluated. Future studies should prioritize mechanistic investigations, employ probability sampling or multicenter designs to enhance generalizability, validate caspase-1 as a biomarker in larger cohorts, and develop brain-penetrant caspase-1 inhibitors to support translational progress.

Conclusion

The caspase-1/IL-1β axis serves as a central mechanism underlying neuroinflammatory processes in MDD, with its activation level demonstrating a strong association with disease severity and treatment outcomes. Caspase-1 expression exhibited significant predictive value for SSRI treatment efficacy, a finding validated through a rigorous analytical framework. Furthermore, pharmacological inhibition of caspase-1 synergistically augmented the therapeutic effects of fluoxetine by restoring inflammatory homeostasis, thereby proposing a novel mechanistic strategy for accelerating antidepressant response.

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Author Contributions

The study was designed by Huan-Zhong Liu. Data were collected by Jing-Yang Gu and Dong-Chuan Di. Results were analyzed by Meng-Yue Gu. The draft manuscript was written by Jing-Yang Gu and Dong-Chuan Di. The versions of the manuscript were revised by Huan-Zhong Liu. All authors agreed to be accountable for the content and conclusions and approved the final version of the manuscript being submitted.

Ethics Statement

All procedures involving animals were performed according to National Institute of Health (NIH) guidelines. All of the experiments were approved by the Animal Ethics Committee of Anhui Medical University (No.LLSC20241835). All participants signed an informed consent form approved by the Ethics Committee of Chaohu Hospital of Anhui Medical University (No.KYXM-202310-045). The research had been performed in accordance with the Declaration of Helsinki. Clinical trial number was not applicable.

Consent for publication

Not applicable.

Conflicts of Interest

All authors declare that they have no competing interests.

Data Availability Statement

The data from this study have been included in the supplementary materials.

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Items	Control group	Depression group	SD subgroup	MD subgroup	Control vs Depression （χ^2/t）	*p*	SD vs MD (t)	SD vs MD (p)
mean（male/female）	14/18	16/18	6/9	10/9	0.073	0.787	0.537	0.464
Age(year)	28.94±8.70	31.12± .92	29.93±8.65	32.05 ± 9.25	-1.005	0.319	-0.688	0.497
Education (year)	14.03±2.65	12.76±2.82	13.67±2.38	12.05 ± 2.99	1.880	0.065	1.752	0.089

duration （month)	-	4.09±1.64	4.53 ± 1.64	3.84 ± 1.71	-	-	1.192	0.242
family history(yes/no)	3/29	7/27	5/10	2/17	1.612	0.304	2.667	0.102
HAMD (score)	5.34 ± 3.40	34.35±9.87	43.53±7.03	27.11 ± 3.83	-15.755	<0.001	8.148	<0.001
HAMA(score)	4.91 ± 3.16	18.53±7.90	18.87±8.45	17.47 ± 7.50	-9.091	<0.001	0.861	0.396
caspase-1(pg/mL)	13.46±3.07	21.19±3.15	22.58±2.00	19.76 ± 3.46	-9.765	<0.001	2.805	0.008
IL-1β(pg/mL)	10.13±2.64	18.60±3.56	20.86±3.46	17.06 ± 2.76	-10.924	<0.001	3.559	0.001
IL-10(pg/mL)	12.42±2.49	11.21±2.69	10.70±2.36	11.89 ± 2.55	1.900	0.062	-1.385	0.176

Table 1 Comparison of Basic Information and Baseline Data

SD, severe depression; MD, mild to moderate depression. The depression group was stratified into SD and MD groups based on the severity of depressive symptom scores.

Table 2 Comparison of Clinical Efficacy Indicators in the Depression Group After 4 Weeks of Treatment (n=34).

Indicators	group	N	baseline	4 Weeks of Treatment	4 Weeks of Treatment (All）		4 Weeks of Treatment MD VS SD	
					t	*p*	*t*	*p*
HAMD(score)	All	34	34.35±9.870	13.32±5.620	12.11	<0.001	2.859	0.007
	MD	19	27.11±3.828	11.11±4.393	14.042	<0.001		
	SD	15	43.53±7.029	16.13±5.878	10.972	<0.001		

HAMA(score)	All	34	8.53±7.900	6.15±4.400	8.945	<0.001		
	MD	19	17.47±7.501	5.74±4.241	6.593	<0.001	0.606	0.549
	SD	15	19.87±8.450	6.67±4.685	5.908	<0.001		
caspase-1(pg/mL)	All	34	21.01±3.200	14.25±3.120	10.290	<0.001		
	MD	19	19.76±3.457	14.49±2.997	5.716	<0.001	-0.500	0.621
	SD	15	22.58±1.995	13.85±3.348	12.270	<0.001		
IL-1β(pg/mL)	All	34	18.74±3.590	11.90±2.470	11.413	<0.001		
	MD	19	17.06±2.762	11.87±2.380	6.959	<0.001	0.067	0.947
	SD	15	20.86±3.457	11.93±2.666	13.876	<0.001		
IL-10(pg/mL)	All	34	1.36±2.500	16.65±2.420	-11.480	<0.001		
	MD	19	11.88±2.549	16.93±1.981	-9.774	<0.001	-0.762	0.452
	SD	15	10.70±2.360	16.29±2.925	-8.186	<0.001		

SD, severe depression; MD, mild to moderate depression. The depression group (All) was stratified into SD and MD groups based on the severity of depressive symptom scores.

Table 3 Comparison of clinical indicators between groups with different efficacy

Items	Times	poor response subgroup (n=10)	efficacy subgroup (n=24)	*t*	*p*
HAMD	baseline	33.10 ± 8.562	34.88 ± 10.494	-0.472	0.640
	4 Weeks of Treatment	19.50 ± 5.603	10.75 ± 3.082	5.875	<0.001
HAMA	baseline	20.90 ± 7.695	17.54 ± 7.934	1.134	0.265
	4 Weeks of Treatment	7.20 ± 3.882	5.71 ± 4.801	0.899	0.376

caspase-1	baseline	22.94 ± 2.954	20.20 ± 2.993	2.441	0.020
	4 Weeks of Treatment	17.87 ± 1.852	12.75 ± 2.139	6.595	<0.001
IL-1β	baseline	21.67 ± 3.647	17.52 ± 2.927	3.583	0.001
	4 Weeks of Treatment	13.79 ± 2.780	11.11 ± 1.880	3.277	0.003
IL-10	baseline	9.61 ± 2.235	12.09 ± 2.267	-2.918	0.006
	4 Weeks of Treatment	14.16 ± 2.337	17.69 ± 1.569	-5.162	<0.001

Table 4 Predictive Value of Caspase-1 and IL-1β Protein Levels in Peripheral Blood for Clinical Efficacy in MDD.

Feature Crosses （baseline）	optimization model	Accuracy (Mean ± Std)	Precision (Mean ± Std)	Recall (Mean ± Std)	F1 (Mean ± Std)
caspase-1	SVM	0.8529 ± 0.3542	0.6765 ± 0.4678	0.6765 ±0.4678	0.6765 ±0.4678
IL-1β	SVM	0.7941 ± 0.4043	0.7059 ± 0.4556	0.7059 ±0.4556	0.7059 ±0.4556
IL-10	KNN	0.7647 ± 0.4242	0.6765 ± 0.4678	0.6765 ±0.4678	0.6765 ±0.4678

caspase-1+IL-1β	Random Forest	0.8235 ± 0.3812	0.6765 ± 0.4678	0.6765 ±0.4678	0.6765 ±0.4678
caspase-1+IL-10	KNN	0.7941 ± 0.4043	0.6765 ± 0.4678	0.6765 ±0.4678	0.6765 ±0.4678
IL-1β+IL-10	Logistic Regression	0.8235 ± 0.3812	0.6471 ± 0.4779	0.6471 ±0.4779	0.6471 ±0.4779
caspase-1+IL-1β+ IL-10	SVM	0.8235 ± 0.3812	0.6765 ± 0.4678	0.6765 ±0.4678	0.6765 ±0.4678

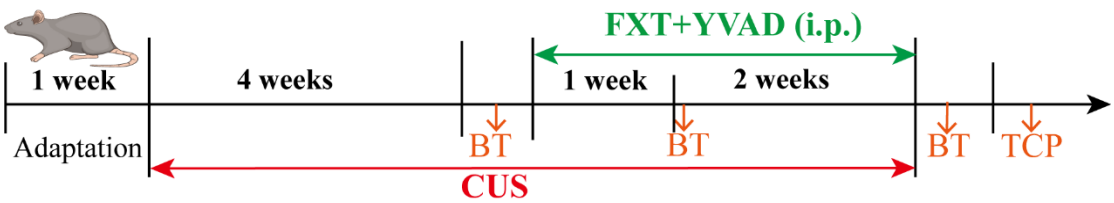

Figure1. The schematic diagram of the experimental design

FXT+YVAD (i.p.): Mice received a daily intraperitoneal (i.p.) injection of fluoxetine and Ac-YVAD-cmk for 3 weeks. TCP: Terminal cardiac puncture; CUS: chronic unpredictable stress; BT Behavioral Test included Sucrose Preference Test, Open Field Test, Forced Swimming Test and Tail Suspension Test.

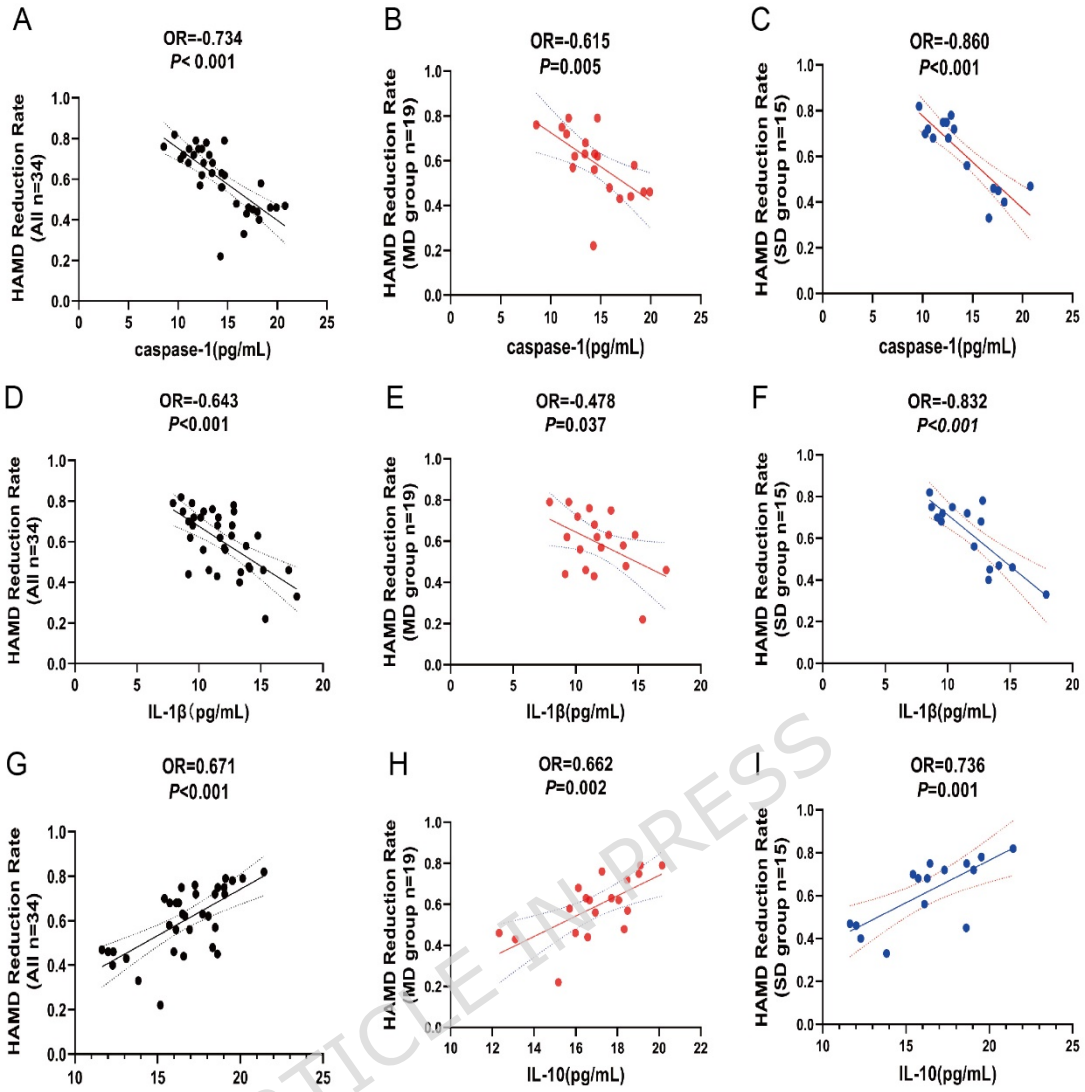

Figure 2. Correlation Analysis of caspase-1, IL-1β, and IL-10 expression Levels with HAMD Reduction Rate

After Treatment. A. Correlation analysis between caspase-1 protein levels and HAMD reduction rate after treatment in the total sample; B. Correlation analysis between caspase-1 protein levels and HAMD reduction rate after treatment in the Mild to moderate depression(MD) subgroup (n=19); C. Correlation analysis between caspase-1 protein levels and HAMD reduction rate after treatment in the severe depression (SD) subgroup (n=15); D. Correlation analysis between IL-1β protein levels and HAMD reduction rate after treatment in the total sample; E. Correlation analysis between IL-1β protein levels and HAMD reduction rate after treatment in the MD subgroup (n=19); F. Correlation analysis between IL-1β protein levels and HAMD reduction rate after treatment in the SD subgroup (n=15); G. Correlation analysis between IL-10 protein levels and HAMD reduction

rate after treatment in the total sample; H. Correlation analysis between IL-10 protein levels and HAMD reduction rate after treatment in the MD subgroup (n=19); I. Correlation analysis between IL-10 protein levels and HAMD reduction rate after treatment in the SD subgroup (n=15).

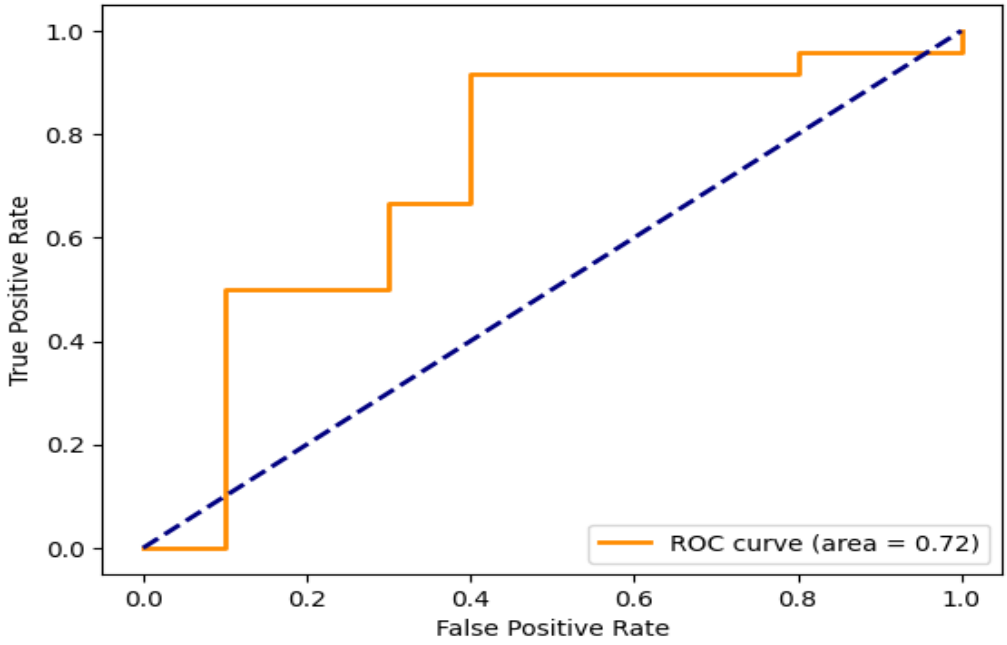

Figure 3. ROC Curves of caspase-1 in Peripheral Blood at Baseline for Predicting Clinical Efficacy of MDD

The x-axis denotes the False Positive Rate, and the y-axis denotes the True Positive Rate.The orange solid line is the ROC curve of caspase-1, with an area under the curve (AUC) of 0.72, suggesting that this indicator has a moderate predictive value for the clinical efficacy of MDD. The blue dashed line serves as a random reference line (with an AUC of 0.5), which is used to compare and assess the performance of the ROC curve.

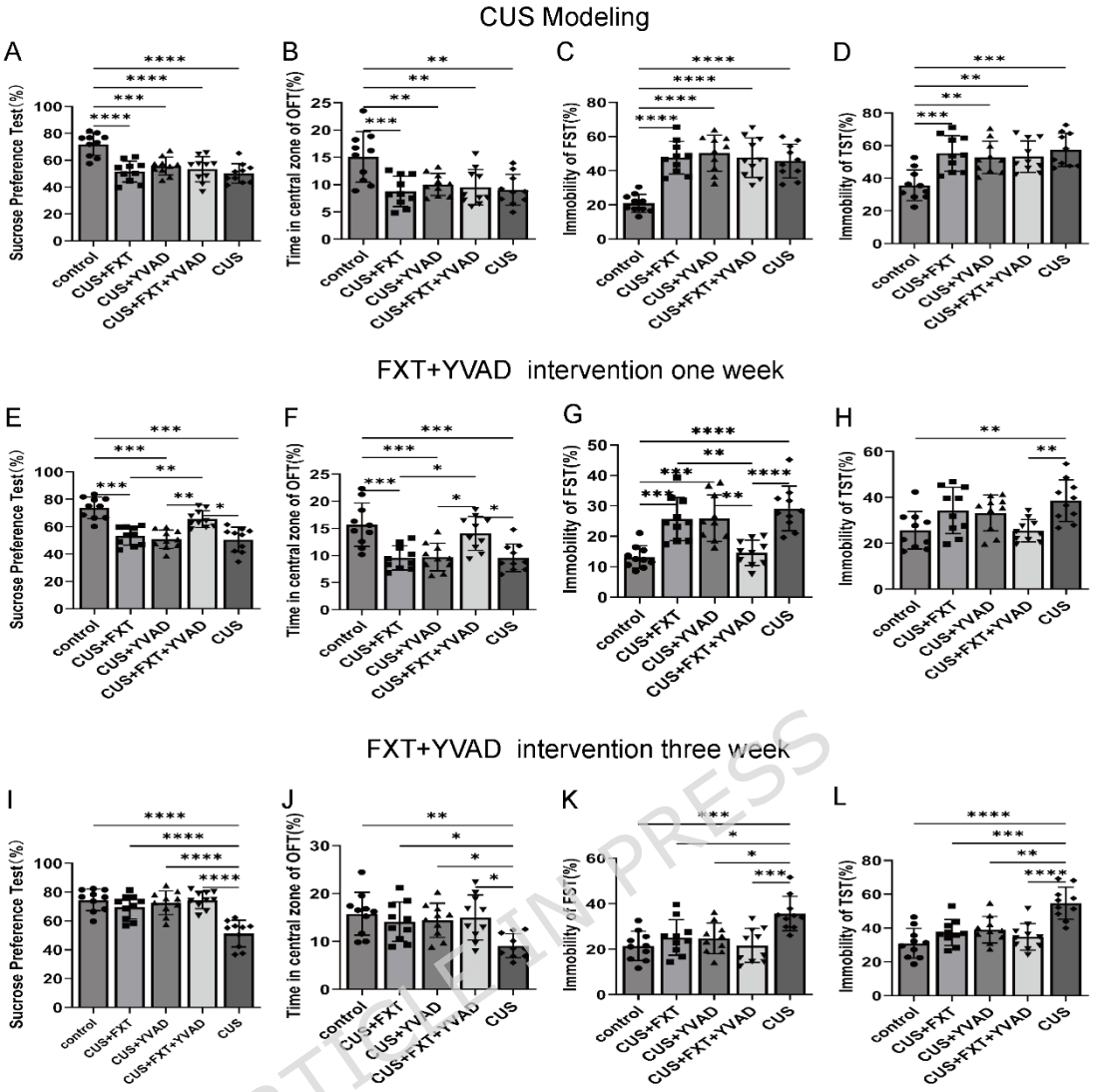

Figure 4. Fluoxetine combined with YVAD ameliorates depressive-like behavior in CUS mice. (A–D) Behavioral profiles following CUS modeling. Compared with the Control group, CUS mice exhibited: (A) significantly reduced sucrose preference in the sucrose preference test; (B) decreased time spent in the central zone in the open field test; (C) prolonged immobilization time in the forced swim test; (D) increased immobilization time in the tail suspension test.
(E–H) Behavioral effects after one week of FXT + YVAD intervention. Relative to the CUS group, CUS + FXT + YVAD mice showed: (E) increased sucrose preference; (F) more time spent in the central zone of the open field; (G) reduced immobilization time in the forced swim test; (H) decreased immobilization time in the tail suspension test.
(I–L) Behavioral outcomes after three weeks of FXT + YVAD treatment. Compared with the CUS group, the CUS + FXT + YVAD, CUS + FXT, and CUS + YVAD groups all showed: (I) a more pronounced increase in sucrose preference; (J) a significantly increased time in the central zone of the open field test; (K) a further reduction in immobilization time in the forced swim test; (L) a similarly robust decrease in immobilization time in the tail suspension test. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$; n = 10.

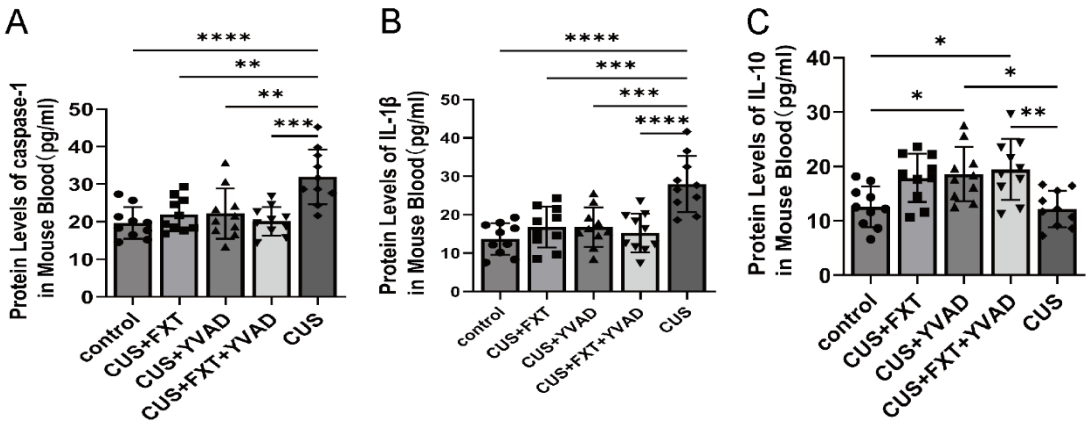

Figure 5. Changes of peripheral blood inflammatory factors in CUS mice improved by fluoxetine combined with YVAD. A. Expression level of peripheral blood caspase-1 in mice after 3 weeks of drug treatment; B. Expression level of peripheral blood IL-1β in mice after drug treatment; C. Expression level of peripheral blood IL-10 in mice after drug treatment. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$, n=10.