

BRIEF REPORT

Role of Inflammatory Bone Markers in the Antidepressant Actions of (R)-Ketamine in a Chronic Social Defeat Stress Model

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Abstract

Background: A recent study demonstrated that inflammatory bone markers play a role in the antidepressant functions of (R,S)-ketamine in treatment-resistant patients with depression. We examined the effect of inflammatory bone markers in the antidepressant functions of (R)-ketamine and (S)-ketamine in a chronic social defeat stress model.

Methods: Behavioral tests for antidepressant actions were performed after a single administration of (R)-ketamine or (S)-ketamine. We measured inflammatory bone marker levels in the plasma, which included osteoprotegerin, receptor activator of nuclear factor κ B ligand, and osteopontin.

Results: (R)-ketamine's antidepressant effects were more potent than those of (S)-ketamine in the behavioral tests. Furthermore, (R)-ketamine but not (S)-ketamine significantly attenuated increased plasma levels of receptor activator of nuclear factor κ B ligand in chronic social defeat stress-susceptible mice. We found a positive correlation between sucrose preference and osteoprotegerin/receptor activator of nuclear factor κ B ligand ratio.

Conclusions: Our findings demonstrate that inflammatory bone markers may play a role in the antidepressant effects of (R)-ketamine.

Keywords: antidepressant, (R)-ketamine, (S)-ketamine, inflammation, bone

Introduction

(R,S)-ketamine, the N-methyl-D-aspartate receptor antagonist, exhibits rapid and long-lasting antidepressant effects in treatment-resistant patients with major depressive disorder (MDD) or bipolar disorder (Newport et al., 2015; Kishimoto et al., 2016). Although (R,S)-ketamine is a preferred antidepressant in the treatment of treatment-resistant depression, the precise mechanisms underlying its functions remain unknown (Monteggia and Zarate, 2015; Duman et al., 2016).

(R,S)-ketamine is a racemic mixture containing equal parts of (R)-ketamine and (S)-ketamine. (S)-ketamine shows

approximately 3- to 4-fold greater anesthetic potency and undesirable psychotomimetic side effects than (R)-ketamine (Hashimoto, 2016a). Meanwhile, (R)-ketamine shows greater potency and longer-lasting antidepressant effects than (S)-ketamine in animal models of depression (Zhang et al., 2014; Yang et al., 2015; Fukumoto et al., 2017; Yang et al., 2017a, 2017b, 2018). Unlike (S)-ketamine, (R)-ketamine does not induce psychotomimetic side effects or exhibit abuse potential in rodents (Yang et al., 2015; 2016). A positron emission tomography study demonstrated a marked reduction in dopamine $D_{2/3}$ receptor

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Significance Statement

The rapid and long-lasting antidepressant effects of (R,S)-ketamine in patients with treatment-resistant depression are one of the most important discoveries in the field of depression research in half a century. Patients with depression are known to be associated with low bone mineral density. A recent study demonstrated that inflammatory bone markers play a role in the antidepressant actions of (R,S)-ketamine in treatment-resistant patients with depression. Previously, we reported that (R)-ketamine shows more potent antidepressant actions than (S)-ketamine in rodent models. Our current study suggests that inflammatory bone markers may play a role in the antidepressant functions of (R)-ketamine in a chronic social defeat stress model.

binding in the striatum of conscious monkeys after a single infusion of (S)-ketamine but not under an infusion of (R)-ketamine (Hashimoto et al., 2017). These results suggest an association between (S)-ketamine-induced dopamine release and the acute psychotomimetic and dissociative side effects in humans. (R)-ketamine could, therefore, be a safer antidepressant in humans than (S)-ketamine (Hashimoto, 2016a, 2016b, 2016c).

Meta-analyses have demonstrated that depression is associated with low bone mineral density, especially in women (Wu et al., 2009; Yirmiya and Bab, 2009; Schweiger et al., 2016). The osteoprotegerin (OPG)/receptor activator of nuclear factor κ B (RANK)/RANK Ligand (RANKL)/pathway contributes to bone formation and resorption. Kadriu et al. (2017) demonstrated that (R,S)-ketamine significantly increased both the OPG/RANKL ratio and plasma osteopontin (OPN) levels and significantly decreased RANKL levels. Given the role of the OPG/RANKL ratio as an index of the balance between bone resorption and bone formation (Boyce and Xing, 2008), it is likely that the OPG/RANKL/RANKL system may play a role in the serious bone abnormalities associated with MDD (Kadriu et al., 2017). However, the role of the OPG/RANK/RANKL system in the antidepressant functions of these 2 ketamine enantiomers in animal models has not been reported.

The current study was undertaken to examine whether inflammatory bone markers (e.g., OPG, RANKL, and OPN) could influence the antidepressant effects of (R)-ketamine and (S)-ketamine in a chronic social defeat stress (CSDS) model.

Methods and Materials

Animals

Male adult C57BL/6 mice ($n=70$), aged 8 weeks (body weight 20–25 g, Japan SLC, Inc., Hamamatsu, Japan) and male adult CD1 (ICR) mice ($n=20$), aged 13–15 weeks (body weight >40 g, Japan SLC, Inc) were used. Animals were housed under controlled temperatures and 12-hour-light/-dark cycles (lights on between 7:00 AM and 7:00 PM) and given ad libitum food (CE-2; CLEA Japan, Inc., Tokyo, Japan) and water. The protocol was approved by the Chiba University Institutional Animal Care and Use Committee. This study was carried out in strict accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health, USA. Animals were deeply anaesthetized with isoflurane before being killed by cervical dislocation. All efforts were made to minimize suffering.

Materials

(R)-ketamine hydrochloride and (S)-ketamine hydrochloride were prepared by recrystallization of (R,S)-ketamine (Ketalar, ketamine hydrochloride, Daiichi Sankyo Pharmaceutical Ltd., Tokyo, Japan) and D-(-)-tartaric acid and L-(+)-tartaric acid, respectively (Zhang et al., 2014). The dose (10 mg/kg as

hydrochloride) dissolved in the physiological saline was used as previously reported (Yang et al., 2015, 2016, 2017a, 2017b, 2018; Zhang et al., 2018). Other reagents were purchased commercially.

Chronic Social Defeat Stress (CSDS) Model

The procedure of CSDS was performed as previously reported (Yang et al., 2015, 2016, 2017a, 2017b, 2018; Zhang et al., 2018). The C57BL/6 mice were exposed to a different CD1 aggressor mouse for 10 min/d for consecutive 10 days. When the social defeat session ended, the resident CD1 mouse and the intruder mouse were housed in one-half of the cage separated by a perforated Plexiglas divider to allow visual, olfactory, and auditory contact for the remainder of the 24-hour period. At 24 hours after the last session, all mice were housed individually. On day 11, a social interaction test was performed to identify subgroups of mice that were susceptible and unsusceptible to social defeat stress. This was accomplished by placing mice in an interaction test box (42 × 42 cm) with an empty wire-mesh cage (10 × 4.5 cm) located at one end. The movement of the mice was tracked for 2.5 minutes followed by 2.5 minutes in the presence of an unfamiliar aggressor confined in the wire-mesh cage. The duration of the subject's presence in the interaction zone (defined as the 8-cm-wide area surrounding the wiremesh cage) was recorded by a stopwatch. The interaction ratio was calculated as time spent in an interaction zone with an aggressor/time spent in an interaction zone without an aggressor. An interaction ratio of 1 was set as the cutoff: mice with scores <1 were defined as susceptible to social defeat stress and those with scores ≥ 1 were defined as resilient. Approximately 70% to 80% of mice were susceptible after CSDS. Susceptible mice were randomly divided in the subsequent experiments. Control C57BL/6 mice without CSDS were housed in the cage before the behavioral tests.

Treatment and Behavioral Tests

The CSDS-susceptible mice were divided into 3 groups. Saline (10 mL/kg), (R)-ketamine (10 mg/kg), or (S)-ketamine (10 mg/kg) was administered i.p. into mice (day 12) (Figure 1A). Furthermore, saline (10 mL/kg) was administered i.p. into control mice (day 12) (Figure 1A). Behavioral tests, including locomotion test (LMT), tail suspension test (TST), forced swimming test (FST), and 1% sucrose preference test (SPT) were performed. LMT and TST were performed 2 and 4 hours after a single injection of saline, (R)-ketamine, or (S)-ketamine, respectively. FST and SPT were performed 1 and 2 days after a single injection, respectively.

On day 15 (3 days after injection of ketamine isomers), the mice were anesthetized deeply with 5% isoflurane, and blood was placed into a tube containing ethylenediamine- N,N,N',N' -tetraacetic acid dipotassium salt dihydrate as an anticoagulant. Blood samples were immediately centrifuged (3000 × g, 3 min) to prepare plasma samples. The plasma samples were stored at -80°C until bioanalysis.

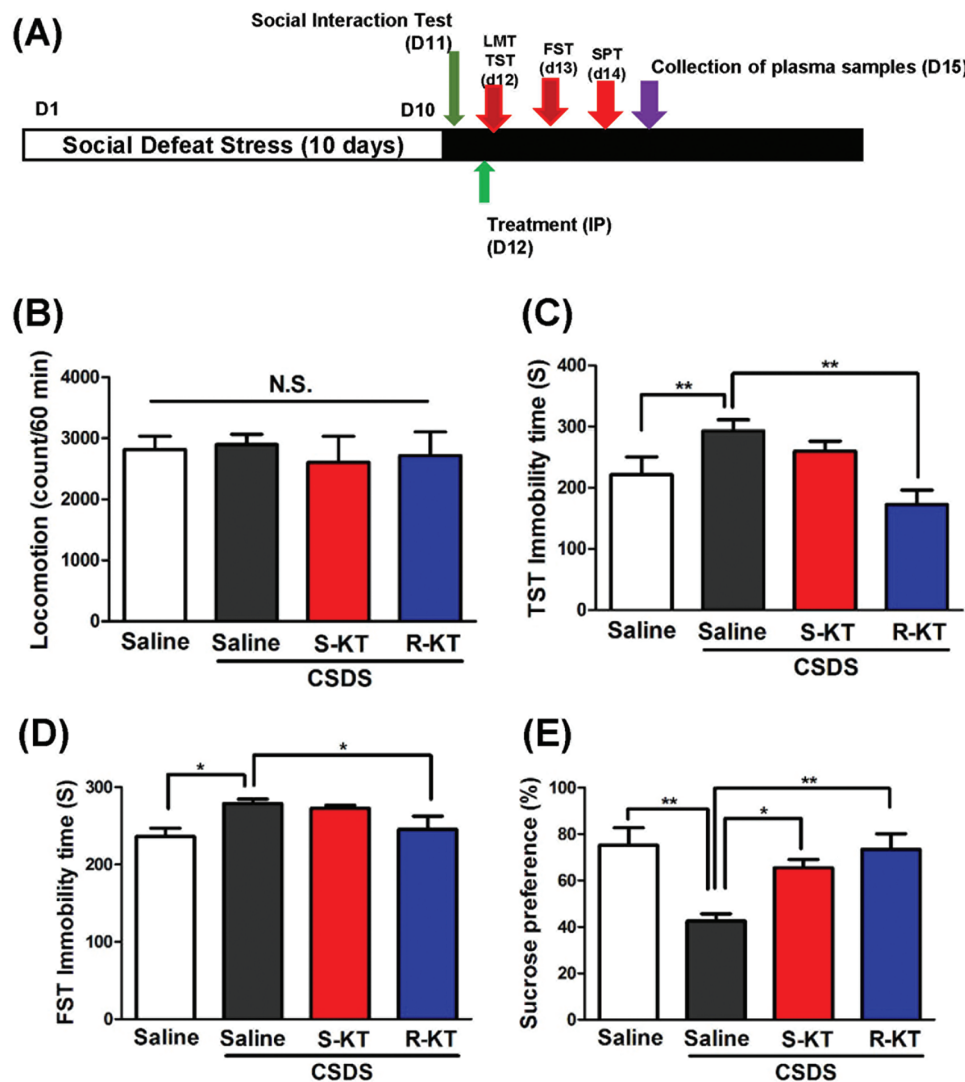


Figure 1. Schedule of a chronic social defeat stress (CSDS) model, treatment, and behavioral tests. (A) CSDS was performed from day 1 to day 10, and the social interaction test (SIT) was performed on day 11. Saline (10 mL/kg), (R)-ketamine (10 mg/kg), or (S)-ketamine (10 mg/kg) was administered i.p. in the susceptible mice on day 12. Locomotion and tail suspension test (TST) were performed 2 and 4 hours after injection, respectively. Forced swimming test (FST) and sucrose preference test (SPT) were performed 1 (day 13) and 2 days (day 14) after injection, respectively. Plasma samples of all mice were collected on day 15. (B) Locomotion. (C) TST. (D) FST. (E) SPT. The values represent the mean \pm SEM ($n=6$). * $P < .05$, ** $P < .01$, *** $P < .001$. LMT: locomotion test; N.S., not significant; R-KT: (R)-ketamine; S-KT: (S)-ketamine; SPT: 1% sucrose preference test; TST: tail suspension test; FST: forced swimming test.

We examined the effects of (R,S)-ketamine (10 mg/kg), (R)-ketamine (10 mg/kg), and (S)-ketamine (10 mg/kg) in control (no CSDS) mice. Behavioral tests and blood sampling were performed as described above.

Locomotion

The locomotor activity was measured by an animal movement analysis system SCANETMV-40 (MELQUEST Co., Ltd., Toyama, Japan). The mice were placed in experimental cages (length \times width \times height: 560 \times 560 \times 330 mm). The cumulative locomotor activity counts were recorded for 60 minutes. Cages were cleaned between testing session.

TST

A small piece of adhesive tape was placed approximately 2 cm from the tip of the tail for mouse. A single hole was punched in the tape and mice were hung individually on a hook. The immobility time was recorded for 10 minutes. Mice were considered

immobile only when they hung passively and completely motionless.

FST

The FST was tested by an automated forced-swim apparatus SCANET MV-40 (MELQUEST Co., Ltd., Toyama, Japan). The mice were placed individually in a cylinder (diameter: 23 cm; height: 31 cm) containing 15 cm of water, maintained at $23 \pm 1^\circ\text{C}$. Immobility time from activity time as (total) – (active) time was calculated by the apparatus analysis software. The immobility time for mouse was recorded for 6 minutes.

SPT

Mice were exposed to water and 1% sucrose solution for 48 hours, followed by 4 hours of water and food deprivation and a 1-hour exposure to 2 identical bottles (water and 1% sucrose solution). The bottles containing water and sucrose were weighed before and at the end of this period. The sucrose preference was

calculated as a percentage of sucrose solution consumption to the total liquid consumption.

Enzyme-Linked ImmunoSorbent Assay Measurement

Plasma levels of OPN (cat number: MOST00, R&D Systems, Minneapolis, MN), OPG (cat number: MOP00, R&D Systems), RANKL (cat number: MTR00, R&D Systems) were measured using the Enzyme-Linked ImmunoSorbent Assay kits according to the manufacturer's instructions.

Statistical Analysis

The data show as the mean \pm SEM. Analysis was performed using PASW Statistics 20 (formerly SPSS Statistics; SPSS, Tokyo, Japan). The data were analyzed using the 1-way ANOVA, followed by posthoc Fisher's Least Significant Difference test. The *P* values $<.05$ were considered statistically significant.

Results

Antidepressant Effect of (R)-ketamine and (S)-ketamine in a CSDS Model

First, we examined the antidepressant effects of (R)-ketamine (10 mg/kg) and (S)-ketamine (10 mg/kg) in a CSDS model (Figure 1A). Locomotion showed no difference ($F_{3,24}=0.15$, $P=.93$) among the 4 groups (Figure 1B). One-way ANOVA of TST data showed a statistical significance ($F_{3,24}=11.08$, $P<.001$) among the 4 groups (Figure 1C). Posthoc tests showed that (R)-ketamine (10 mg/kg), but not (S)-ketamine (10 mg/kg), significantly attenuated the increased immobility times in susceptible mice after CSDS (Figure 1C).

One-way ANOVA of FST data showed a statistical significance ($F_{3,24}=3.83$, $P<.026$) among the 4 groups (Figure 1D). Posthoc tests showed that (R)-ketamine, but not (S)-ketamine, significantly attenuated the increased immobility times in susceptible mice after CSDS (Figure 1D). One-way ANOVA of SPT data showed a statistical significance (2 days after a single injection: $F_{3,24}=6.55$, $P=.003$) among 4 groups (Figure 1E). Posthoc tests showed that sucrose preference of (R)-ketamine-treated group was significantly higher from saline-treated group (Figure 1E). Furthermore, sucrose preference of (S)-ketamine-treated group was also significantly higher from saline-treated group although effect of (S)-ketamine was less potent than (R)-ketamine (Figure 1E). These results suggest that (R)-ketamine has more potent and longer-lasting antidepressant effects than (S)-ketamine in a CSDS model, consistent with previous reports (Yang et al., 2015, 2017a, 2017b, 2018).

In contrast, (R,S)-ketamine, (R)-ketamine, and (S)-ketamine did not show antidepressant-like effects in the control (no CSDS) mice (supplemental Figure 1).

Plasma Levels of Inflammatory Bone Markers

We measured plasma levels of OPG, RANKL, and OPN in the 4 groups. There was no difference ($F_{3,24}=0.72$, $P=.553$) of OPG among the 4 groups (Figure 2A). One-way ANOVA of RANKL data showed a statistical significance ($F_{3,24}=13.01$, $P<.01$) among the 4 groups. Posthoc tests showed that plasma levels of RANKL in the (R)-ketamine-treated group was significantly lower than saline-treated group (Figure 2B). One-way ANOVA of OPG/RANKL ratio data showed a statistical significance ($F_{3,24}=6.315$, $P=.003$) among

the 4 groups. Posthoc tests showed that OPG/RANKL ratio in the (R)-ketamine-treated group was significantly higher than saline-treated group (Figure 2C). One-way ANOVA of OPN data showed no statistical significances ($F_{3,24}=2.15$, $P=.125$) among the 4 groups (Figure 2D). One-way ANOVA of OPN/RANKL data showed no statistical significances ($F_{3,24}=2.69$, $P=.074$) among the 4 groups (Figure 2E). In contrast, (R,S)-ketamine, (R)-ketamine, and (S)-ketamine did not alter plasma levels of inflammatory bone markers in the control (no CSDS) mice (supplemental Figure 2).

Interestingly, there was a significantly positive correlation ($r=0.506$, $P=.012$) between sucrose preference data and OPG/RANKL ratio in the all mice (Figure 2F).

Discussion

In the current study, we demonstrated that (R)-ketamine shows greater antidepressant effects than (S)-ketamine in a CSDS model, consistent with previous reports (Yang et al., 2015, 2017a, 2017b, 2018). (R)-ketamine, but not (S)-ketamine, significantly attenuated increased plasma levels of RANKL in CSDS-susceptible mice. In addition, (R)-ketamine, but not (S)-ketamine, significantly improved the decreased ratio of OPG/RANKL in the plasma of CSDS-susceptible mice. We also found a positive correlation between sucrose preference and the OPG/RANKL ratio across all groups. These findings suggest that inflammatory bone markers may play a role in the antidepressant actions of (R)-ketamine in a CSDS model. This is the first report supporting that (R,S)-ketamine significantly increases the OPG/RANKL ratio and significantly decreases RANKL levels in treatment-resistant patients with MDD (Kadriu et al., 2017).

The infusion of (R,S)-ketamine had no effect on bone markers (e.g., OPN, RANKL, OPG, and eotaxin) for healthy controls, and there were no significant changes from the baseline (Kadriu et al., 2017). In contrast, OPN levels in plasma were significantly reduced in MDD patients compared with healthy controls. MDD patients showed a significant increase of OPN and OPN/RANKL ratio in response to (R,S)-ketamine infusion at days 1 and 3 after a single infusion (Kadriu et al., 2017). In this study, we found increased RANKL plasma levels in susceptible mice, although plasma levels of OPN, OPG, and RANKL at baseline in MDD patients were similar to healthy controls (Kadriu et al., 2017). However, the exact mechanism through which alterations in RANKL play a role in depression-like phenotypes after CSDS remains unknown. We reported previously that CSDS susceptible mice show inflammation since blood levels of pro-inflammatory cytokines were higher compared to control mice (Zhang et al., 2017). It seems that (R)-ketamine can attenuate an increase of RANKL in the susceptible mice through its potent antiinflammatory actions.

MDD patients who had a lower OPN/RANKL ratio compared with healthy controls showed a significant increase in the OPN/RANKL ratio in response to (R,S)-ketamine infusion at 230 minutes and days 1 and 3 after a single infusion (Kadriu et al., 2017). MDD patients also demonstrated significant decreases in RANKL levels in response to (R,S)-ketamine infusion at 230 minutes and on day 3. It therefore seems that (R,S)-ketamine may correct adverse bone metabolic state in MDD patients and return it to control levels. We found that (R)-ketamine but not (S)-ketamine significantly improved the decreased ratio of OPG/RANKL in susceptible mice. Considering the role of the OPG/RANKL ratio in the process of positive bone balance between resorption and formation, an increase in the OPG/RANKL ratio by (R,S)-ketamine in depressed patients or (R)-ketamine in mice with depression-like phenotype is of great interest.

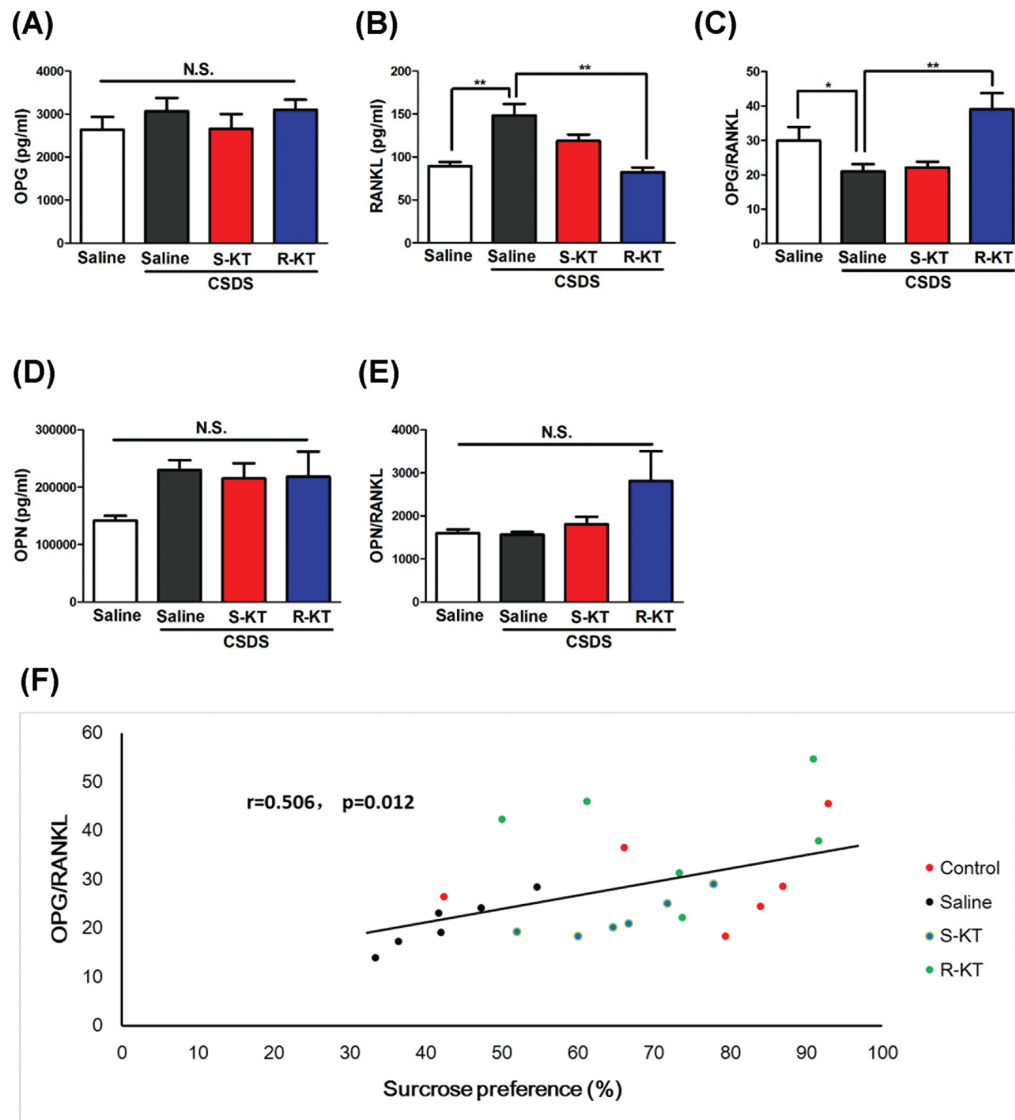


Figure 2. Plasma levels of osteoprotegerin (OPG), receptor activator of nuclear factor κ B Ligand (RANKL), osteopontin (OPN), and their ratio. (A) OPG. (B) RANKL. (C) OPG/RANKL ratio. (D) OPN. (E) OPN/RANKL ratio. The values represent the mean \pm SEM ($n=6$). * $P < .05$, ** $P < .01$ compared with saline-treated chronic social defeat stress (CSDS) mice. (F) A positive correlation between sucrose preference data and OPG/RANKL ratio in all mice. N.S., not significant. R-KT, (R)-ketamine; S-KT, (S)-ketamine.

We found a positive correlation between sucrose preference and the OPG/RANKL ratio in all groups. Given the greater antidepressant effects of (R)-ketamine compared with (S)-ketamine, it is likely that the OPG/RANKL ratio would be a peripheral biomarker of the antidepressant functions of (R)-ketamine. Nonetheless, further studies on the inflammatory bone markers of (R)-ketamine's antidepressant actions are warranted.

A longitudinal study demonstrated that proinflammatory cytokine interleukin-6 (IL-6) predicts bone loss and resorption, suggesting that antiinflammatory therapy by IL-6 antibody may have potential for the prevention of osteoporosis (Ding et al., 2008). We reported that blood levels of IL-6 in CSDS-susceptible mice were higher than in control mice and that blockage of IL-6 in the periphery showed rapid antidepressant effects in CSDS-susceptible mice (Zhang et al., 2017). We also reported that blood IL-6 level at baseline may be a predictable biomarker for (R,S)-ketamine's antidepressant action in treatment-resistant MDD patients (Yang et al., 2015). IL-6 is a stimulus promoting the production of RANKL that may work in synergy with RANKL. It is therefore likely that inflammatory cytokines such as IL-6 may

contribute to bone pathology associated with depression. Further studies underlying the role of IL-6 and the OPG/RANK/RANKL system in the antidepressant functions of (R,S)-ketamine and its enantiomers are needed. In this study, we did not use the resilient mice from the experiments. However, it is also of interest to study the role of inflammatory bone markers in the resilient mice.

In conclusion, this study found that inflammatory bone markers may play a role in the rapid antidepressant effects of (R)-ketamine in a CSDS model. The OPG/RANKL ratio could be a potentially valuable biomarker for the antidepressant actions of (R,S)-ketamine and its enantiomer, (R)-ketamine, in patients with MDD.

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Statement of Interest

Dr. Hashimoto is an inventor on a filed patent application on “The use of (R)-ketamine in the treatment of psychiatric diseases” by Chiba University. Dr. Hashimoto has received research support from Dainippon-Sumitomo, Otsuka, and Taisho. Other authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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