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Original Research Article

Effect of antidepressants on bone mineral density and bone metabolism in ovariectomized depressed rats

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Abstract

Purpose: *To investigate the effect of antidepressants on bone mineral density and bone metabolism in ovariectomized rats with depression.*
Methods: *48 female rats were randomly and equally assigned to six groups, namely, sham (Sn), ovariectomized depression (OD), ovariectomized depression rats treated with sertraline (ODs), citalopram (ODx), reboxetine (ODr), and venlafaxine groups (ODw). Behavioral alterations and bone-related parameters were evaluated before and after treatment.*
Results: *After treatment, ODs, ODx, ODw, and ODr groups had higher levels of horizontal and vertical exercise scores, sugar water consumption, osteoclasts, and serum CTX-1 ($p < 0.05$) compared to OD group. Osteocytes, serum serotonin (5-HT), osteocalcin (BGP), and type I procollagen N-terminal propeptide (PINP) were significantly decreased ($p < 0.05$) after treatment in ODs, ODx, ODw, and ODr groups. A positive correlation was observed between 5-HT, BGP, PINP, and whole-body bone mineral density ($r = 0.931$, 0.907, and 0.843, $p < 0.05$) respectively, while a negative correlation was observed between collagen type I C-terminal propeptide (CTX-I) and bone mineral density ($r = -0.855$, $p < 0.05$).*
Conclusion: *Antidepressants reduce bone mineral density and osteocyte proliferation, while increasing osteoclast proliferation. These effects are associated with reductions in 5-HT, PINP and BGP levels, and increase in CTX-I.*

Keywords: *Antidepressants, Ovariectomize, Depression, Bone mineral density*

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INTRODUCTION

Menopause which is a crucial shift from reproductive to post-reproductive life is often associated with psychological challenges like depression, affecting up to 20 % of menopausal women [1,2]. Severe depression may substantially impair a woman's occupation, potentially leading to employment termination [2]. Therefore, it is recommended that menopausal women diagnosed with depression should consider early treatment with antidepressants [2]. Studies have indicated that while antidepressants effectively alleviate depressive symptoms, they also impact bone metabolism in depressed patients, increasing the risk of fractures [3]. Moreover, different types of antidepressants have varying effects on bone

metabolism, and the underlying mechanisms remain unclear. Generally, antidepressants include selective serotonin reuptake inhibitors (SSRIs) (fluoxetine, paroxetine), dual reuptake inhibitors of serotonin (5-HT) and norepinephrine (NE) (venlafaxine), and selective NE reuptake inhibitors (reboxetine) [4]. This study investigated the effects and possible mechanisms of antidepressants on osteoblasts and osteoclasts in ovariectomized depressed rats.

EXPERIMENTAL

Animals

Adult Sprague-Dawley (SD) rats aged 3 months old (250 ± 30 g), were provided by the Third Military Medical University of the Chinese People's Liberation Army. They were placed under standard conditions (23 ± 2 ^{o}C and 12 h light - dark cycle. The animals were allowed to acclimatize for two weeks. During this period, all the rats were fed with normal feeds and water. All animal experiments were approved by the Ethics Committee of The First Hospital of Jiaxing for the use of animals (approval no. 2022043), and conducted in accordance with the National Institutes of Health Laboratory Animal Care and Use Guidelines [5].

Drugs, equipment, and reagents

A handmade open box (Material: cardboard, size: 80 × 80 × 40 cm) was divided into 25 squares. Discovery-Hologic dual-energy X-ray bone densitometer (Hologic, USA) and 680 Type BIO-RAD microplate reader (Awareness, USA) were used for analysis bone density. Enzyme-linked immunosorbent assay (ELISA) kits for rats, 5-hydroxytryptamine (5-HT), osteocalcin (BGP), type I collagen C-telopeptide (CTX-I), and type I procollagen N-terminal propeptide (PINP) were purchased from Wuhan Boster Bioengineering.

Modeling for ovariectomized depressed rats

Depressed rats with bilateral ovariectomies were used. A total of 48 female SD rats were randomly divided into 6 groups (8 rats/group) based on different treatment methods: sham-operation (Sn), ovariectomized (OD), ovariectomized + sertraline (Pfizer Pharmaceutical Co. Ltd. New York, USA) (ODs), ovariectomized + citalopram (Danish Lundbeck Pharmaceutical Factory and Xi'an Janssen Pharmaceutical) (ODx), ovariectomized + reboxetine mesylate (Chongqing Yaoyou Pharmaceutical Co. Ltd., China) (ODr), and ovariectomized + venlafaxine (Wyeth Pharmaceutical Co. Ltd. China (ODw). All antidepressants were administered at a dosage of 2 mg/kg for 28 consecutive days. This study adhered to the ethical standards of the American Psychological Association (APA) and the care and health guidelines set forth by international organizations for the use of experimental animals.

Evaluation of parameters/indices

Behavioral observation

The open-box test (Material: cardboard, size: 80 × 80 × 40 cm was made and divided into 25 squares) was employed to document behavioral parameters, including horizontal and vertical movements exhibited by all rats for 5 mins before and after treatment. Lower scores suggested more erratic behavior.

Sugar water consumption

The sugar water intake (SI) was measured before and after treatment. After 24 h of fasting and water deprivation, each rat was provided with a bottle of 1 % w/v sucrose water and purified water, and allowed to freely drink for 60 min. Subsequently, the amount of liquid consumption was quantified, and their preference (P) or inclination towards the solution was assessed using Eq 1.

P (%) = (SI/TI)100 …….. (1)

where TI is total liquid intake

Serum 5-HT, BGP, PINP, CTX-1 concentrations

Blood samples were collected from the femoral artery of the rats before and after treatment, centrifuged to isolate the serum. Subsequently, serum 5-HT, BGP, PINP, and CTX-1 concentrations were measured using ELISA kit (Wuhan Boster Bioengineering, Wuhan, China).

Bone density

After treatment, the rats were anesthetized and fixed for examination of bone density in the lumbar spine, proximal, middle and distal femurs, as well as the whole body, using dual-energy X-ray absorptiometry (Discovery, Hologic Company, USA).

Numbers of osteocytes and osteoclasts

Number of osteoblasts and osteoclasts in the right proximal femur of the rats were recorded

after treatment. Attached muscles and connective tissue from the right proximal femur were generally anesthetized, fixed with neutral formalin, prepared with paraffin-embedded cells, and stained. Counts of osteoblasts and osteoclasts were recorded using a 100x light microscope across 10 fields of view, and the average was taken.

Statistical analysis

The data were analyzed and processed using Statistical Packages for Social Sciences (SPSS 17.0, IBM, Armonk, NY, USA). Measurement data was presented as mean ± standard deviation (SD) and analyzed using one-way analysis of variance (One-way ANOVA) to compare multiple groups. Pairwise comparisons between groups were carried out using LSD-t test. $P<0.05$ was considered statistically significant.

RESULTS

Behavior of rats

There was no significant difference in the horizontal and vertical movement scores before treatment in all groups ($p > 0.05$). After treatment, OD group exhibited lower horizontal and vertical movement scores compared to Sn group ($p < 0.05$), whereas ODs, ODx, ODw, and ODr groups showed significantly increased movement scores ($p < 0.05$). However, there was no significant difference among in movement scores of ODs, ODx, ODw, and ODr groups ($p > 0.05$) (Table 1).

Sugar water consumption

There was no significant difference in sugar water consumption among all groups ($p > 0.05$) before treatment. After treatment, sugar water consumption was significantly lower in OD group and significantly higher in ODs, ODx, ODw, and ODr group compared to Sn group ($p < 0.05$). Also, there was no significant difference in sugar water consumption among ODs, ODx, ODw, and ODr groups following treatment ($p > 0.05$) (Table 2).

Bone density

Bone densities of various parts of OD, ODs, ODx, ODw, and ODr groups were significantly reduced compared to Sn group ($p < 0.05$). Similarly, bone densities of ODs, ODx, ODw, and ODr groups significantly decreased compared to OD group ($p < 0.05$). In addition, bone densities decreased in this order ODs > ODx, > ODw > ODr, with the most significant decrease observed in the proximal femur (Table 3).

Osteoblasts and osteoclasts in the proximal femur

Numbers of osteoblasts in ODn, ODs, ODx, ODw, and ODr significantly decreased ($p<0.05$), while osteoclasts numbers significantly increased ($p<0.05$) compared to Sn, and OD group. Furthermore, ODn, ODs, ODx, ODw, and ODr showed significant decrease and increase in osteoblasts and osteoclasts respectively compared to OD group ($p < 0.05$). The osteoclast count decreased in this order ODs > ODx > ODw > ODr (Table 4, Figure 1).

Serum 5-HT, BGP, CTX-1, and PINP after treatment

Serum levels of 5-hydroxytryptamine (5-HT), osteocalcin bone Gla protein (BGP), and serum procollagen type I N-propeptide (PINP) decreased significantly in the OD, ODs, ODx, ODw, and ODr groups ($p<0.05$), while serum cross-linked C-telopeptide of type I collagen (CTX-1) significantly increased compared to Sn group ($p<0.05$). Additionally, the serum levels decreased from highest to the lowest: ODs > ODx > ODw > ODr (Table 5).

Table 1: Behavior of rats before and after treatment (n = 8)

Group	Horizontal movement		Vertical movement	
	Before treatment	***After treatment***	***Before treatment***	***After treatment***
Sn	88.50±8.17	92.25±11.59	65.63±4.93	65.25±2.96
OD	83.44±8.25	31.33±9.64[a]	67.44±3.01	4.33±1.58[c]
ODs	89.56±5.32	82.00±6.10[bc]	66.33±1.58	35.89±1.76[bc]
ODx	88.33±7.69	77.56±10.31[bc]	65.44±2.92	34.33±1.80[bc]
ODr	87.89±7.71	79.67±7.26[bc]	64.89±2.80	33.56±2.22[bc]
ODw	83.67±6.34	82.11±9.05[bc]	67.11±2.85	36.07±2.00[bc]

[a]$P<0.05$, [b]$p<0.05$ vs. OD group, [c]$p<0.05$ vs. before treatment

Table 3: Bone densities (n = 8 in each group, mean ± SD g/cm)

Group	Lumbar spine	Proximal femur	Middle tibia	Distal femur	Whole body
Sn	0.233 ± 0.009	0.231 ± 0.009	0.199 ± 0.010	0.178 ± 0.004	0.196 ± 0.008
ODn	0.213 ± 0.013[a]	0.207 ± 0.013[a]	0.169 ± 0.034[a]	0.160 ± 0.013[a]	0.202 ± 0.012[a]
ODx	0.156 ± 0.003[b]	0.124 ± 0.006[b]	0.141 ± 0.002[b]	0.146 ± 0.010[b]	0.183 ± 0.021[b]
ODs	0.144 ± 0.005[bc]	0.111 ± 0.006[bc]	0.111 ± 0.002[bc]	0.141 ± 0.005[b]	0.160 ± 0.019[bc]
ODw	0.189 ± 0.002[bcd]	0.148 ± 0.039[bcd]	0.113 ± 0.002[bd]	0.152 ± 0.001[bd]	0.190 ± 0.013[bd]
ODr	0.202 ± 0.008[bcde]	0.195 ± 0.038[bcde]	0.119 ± 0.006[cde]	0.148 ± 0.044[b]	0.187 ± 0.007[bd]

[a]*P*<0.05 *vs* Sn group, [b]*p*<0.05 *vs* OD group, [c]*p*<0.05 *vs* ODx, [d]*p* < 0.05 *vs* ODs, [e]*p* < 0.05 *vs* ODw

Table 2: Sugar water consumption (n = 8 in each group, mean ± SD)

Group	Before treatment	After treatment
Sn	77.28±7.17	78.93±3.57
OD	79.93±3.54	46.41±6.46[ac]
ODs	77.25±8.88	53.28±8.63[abc]
ODx	74.75±7.41	56.93±6.15[abc]
ODw	75.14±6.61	57.00±4.99[abc]
ODr	75.05±6.54	53.34±4.37[abc]

[a]*P*<0.05 vs. Sn group, [b]*p*<0.05 vs. OD group, [c]*p*<0.05 vs. before treatment

Table 4: Osteoblasts and osteoclasts in the proximal femur of rats after treatment (n = 8 in each group, mean ± SD)

Group	Osteoblasts	Osteoclasts
Sn	30.22±3.55	1.44±1.13
ODn	27.11±2.03[a]	4.56±1.13[a]
ODx	18.22±2.05[b]	10.78±2.05[b]
ODs	15.33±1.73[bc]	12.33±2.29[bc]
ODw	20.67±1.80[bcd]	9.33±1.32[bd]
ODr	23.00±3.32[bcd]	7.44±1.51[bcde]

[a]*P*<0.05 vs. Sn group, [b]*p*<0.05 vs. OD group, [c]*p*<0.05 vs. ODx group, [d]*p* < 0.05 vs. ODs, [e]*p* < 0.05 vs. ODw group

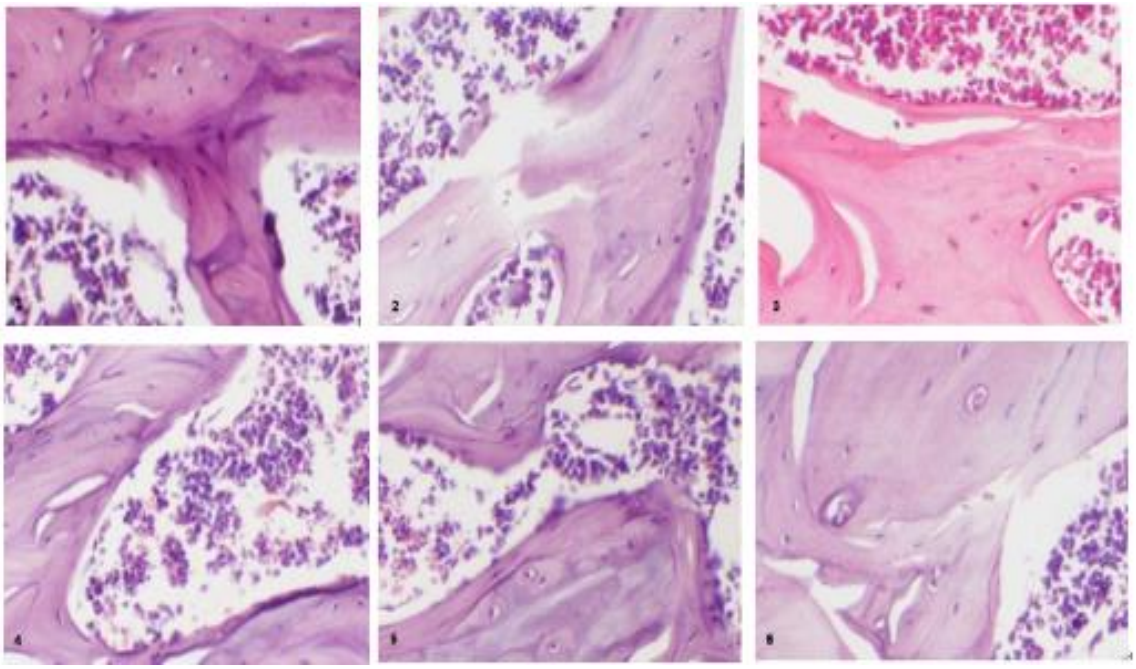

Figure 1: Osteoblasts and osteoclasts in the proximal femur of rats after treatment. Sn group: osteoblast proliferation is robust; ODn group: reduced osteoblast proliferation and rare osteoclasts; ODx group: reduced osteoblast proliferation, scattered osteoclasts; ODs group: decreased osteoclasts proliferation and scattered osteoclasts; ODw group: sparse osteoblasts and scattered osteoclasts; ODr group: sparse osteoblasts and scattered osteoclasts

Table 5: Comparison of serum 5-HT, BGP, CTX-1, and PINP of rats after treatment (n = 8, ng/mL)

Group	5-HT	BGP	PINP	CTX-1
Sn	10.42±0.63	9.09±0.48	15.22±0.27	5.21±0.26
ODn	8.15±0.91[a]	7.83±0.47[a]	13.28±0.27[a]	6.94±0.10[a]
ODs	1.23±0.50[b]	1.29±0.36[b]	7.78±0.31[b]	10.72±0.23[b]
ODx	2.52±0.51[bc]	3.22±0.20[bc]	9.03±0.11[bc]	10.08±0.12[bc]
ODw	4.76±0.43[bcd]	5.32±0.47[bcd]	9.94±0.24[bcd]	9.79±0.30[bcd]
ODr	6.26±0.77[bcde]	5.16±0.50[bcd]	10.11±0.18[bcd]	7.12±0.55[bcd]

[a]*P*<0.05 vs. Sn group, [b]*p*<0.05 vs. OD, [c]*p*<0.05 vs. ODx, [d]*p* < 0.05 vs. ODs, [e]*p* < 0.05 vs. ODw

Correlation analysis between 5-HT, BGP, CTX-1, PINP and bone density

The findings indicated a significant positive correlation between 5-HT, BGP, PINP, and whole-body bone density (r = 0.931, 0.907 and 0.843 respectively with $p < 0.05$), and a negative correlation between CTX-1 and whole-body bone density (r = - 0.855, $p < 0.05$).

DISCUSSION

A woman's occupation, health, behaviour and lifestyle is severely affected by severe depression [2]. As a result, antidepressants are recommended as early stage treatment in menopausal women. [2]. Although, antidepressants effectively ameliorate depressive symptoms, their impact on bone metabolism in depressed patients increases the risk of fractures [3]. Thus, this study investigated the effect of antidepressants on bone mineral density and bone metabolism in ovariectomized depressed rats. Ovariectomy-induced depressed rats exhibited significantly reduced behavioral parameters, vertical movement scores, sugar water consumption, and bone density compared to control. Additionally, there was a significant decrease and increase in osteoblasts and osteoclasts respectively. These findings validate the successful establishment of ovariectomy-induced depression model, reflecting the pathological condition observed in postmenopausal women with depression.

Depression accelerates secretion of adrenocorticotropic hormones, leading to elevated glucocorticoid levels. This increased level interacts with glucocorticoid receptors in the bone, thereby enhancing osteoclast activities and facilitating bone resorption [6] which is in tandem with this current study. Furthermore, the administration of fluoxetine, paroxetine, reboxetine, and venlafaxine to ovariectomy-induced depression rats for 28 days significantly improved their behavior and increase sugar water consumption. This suggests that these four antidepressants effectively ameliorate depressive behavior in rats experiencing ovariectomy-induced depression. Serotonin (5-HT) is a monoamine neurotransmitter that plays a crucial role in human emotional memory. Studies have demonstrated that chronic stress suppresses hippocampal 5-HT level, inducing depression [7]. Therefore, targeted suppression of norepinephrine (NE) reuptake by these antidepressants elevates central NE activity and improve mood. Both fluoxetine and paroxetine are classified as selective serotonin reuptake inhibitors (SSRIs) antidepressants. By inhibiting the function of 5-HT and preventing its reuptake, SSRIs lead to increased extracellular 5-HT concentration and prolonged action time, thereby alleviating depressive symptoms [8]. Venlafaxine acts as a dual reuptake inhibitor for both 5-HT and NE, whereas reboxetine functions as a selective NE reuptake inhibitor, alleviating depressive symptoms [9].

Additionally, these antidepressants exacerbated bone density reduction in ovariectomy-induced depressive rats, showing the most significant decline at the proximal end of the femur. Correspondingly, there was a significant decrease in osteoblasts, coupled with an increase in osteoclasts. Fluoxetine, paroxetine, venlafaxine, and reboxetine accelerated bone density reduction in that order with fluoxetine exerting the most potent effect. Fluoxetine, a typical SSRI, has a significant impact on bone metabolism, as its use in postmenopausal women has been shown to lower bone density and increase the risk of bone loss and fractures [10]. The reuptake of 5-HT from the extracellular space is blocked by SSRIs resulting in elevated extracellular 5-HT level, which stimulates bone cells by activating signaling pathways via surface 5-HT receptors, thus affecting bone cell activity, disrupting bone formation, reducing bone mass, and lowering the bone density. Additionally, SSRIs directly inhibits 5-HT activity, leading to an increase in 5-HT and inhibiting osteoblast proliferation, ultimately resulting in reduced bone density. Paroxetine, also an SSRI, has a weaker effect on bone metabolism than fluoxetine due to its effect on osteoblast progenitors and activity of osteoblasts and osteoclasts [11].

Among SSRIs, paroxetine exhibited a lesser toxic effect on the bone. Studies indicated that venlafaxine and reboxetine have milder effects on bone metabolism than fluoxetine and paroxetine. Venlafaxine effectively blocks reuptake of 5-HT and NE, thus boosting activity of 5-HT and NE in the central nervous system. Nonetheless, its effect on bone density was comparatively modest [12]. Reboxetine is a selective NE reuptake inhibitor, with no affinity for 5-HT [12]. Norepinephrine (NE) receptors are found in bone cells, and in culture of osteoblast-like cells, NE stimulates cAMP production, promoting bone resorption and regulating bone metabolism by balancing osteoclasts and osteoblasts [13]. Although venlafaxine acts as a dual receptor blocker, it has a greater effect on bone density compared to reboxetine, the NE receptor blocker. The venlafaxine group

exhibited higher serum 5-HT levels than reboxetine, suggesting that 5-HT might play a more significant role in bone strength than NE [12].

In this study, ovariectomy-induced depressive rats exhibited significant differences in peripheral blood 5-HT, BGP, PINP, and CTX-1 levels. Osteocalcin bone Gla protein (BGP) is a specific non-collagenous bone matrix protein secreted by mature osteoblasts that maintains normal bone mass and serves as a sensitive and specific marker for osteoblasts and bone formation [14]. Serum BGP is solely derived from the skeleton, and its levels increase when bone formation or bone resorption capacity is enhanced [14]. Under the action of specific enzymes, osteoblasts synthesize type I procollagen which is secreted into the extracellular space, removes peptides at both ends to form type I collagen which is thereafter aggregated into collagen fibers. Carboxyl-terminal peptide is referred to as PICP, while the amino-terminal peptide is PINP. Serum PINP level is positively correlate with bone formation, unaffected by hormones, and are more specific and sensitive indicators than BGP [15]. Also, PINP effectively predicts forearm bone loss in postmenopausal women [16].

Cross-linked C-telopeptide of type I collagen (CTX-1) is present in mature bone collagen, and when osteoclast activity is enhanced, bone collagen dissolves and releases CTX-I. After menopause, bone turnover accelerates further, osteoclast activity increases, and osteoblast function decreases [17]. As osteoclast activity increases, bone collagen dissolves and releases more CTX-I which then breaks down into NTX and CTX [17]. These are all extracellular collagen fiber degradation products detectable in serum. Studies have shown that CTX is the most sensitive marker for monitoring changes in bone resorption [18].

Results of this study indicate that changes in peripheral blood levels of 5-HT, BGP, PINP, and CTX-1 in ovariectomized depressed rats follow the same trend as changes in bone metabolism. Furthermore, correlation analysis reveals a positive correlation between 5-HT, BGP, PINP, and whole-body bone density, whereas CTX-1 showed a negative correlation with whole-body bone density. This suggests a significant correlation between peripheral blood concentrations of 5-HT, BGP, PINP, CTX-1, and bone metabolism, potentially elucidating the mechanism through which antidepressants impact bone metabolism in ovariectomized depressed rats.

CONCLUSION

Ovariectomy-induced depression rats exhibit lower bone density, osteoblast proliferation, serum 5-HT, PINP, and BGP levels, as well as higher osteoclast proliferation, and CTX-I levels. Antidepressants decrease bone density, inhibit osteoblast proliferation, and stimulate osteoclast proliferation. These changes correlate with lower levels of 5-HT, PINP, and BGP and higher levels of CTX-I in ovariectomy-induced depressive rats.

DECLARATIONS

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Ethical approval

All animal experiments were approved by the Ethics Committee of The First Hospital of Jiaxing for the use of animals (approval no. 2022043),

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Conflict of Interest

No conflict of interest associated with this work.

Contribution of Authors

We declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by the authors. Chao Liu, Chao Liang, Yiming Wang and Jie Huang designed the study and carried them out, supervised the data collection, analyzed and interpreted the data, prepared the manuscript for publication and reviewed the draft of the manuscript. All authors read and approved the manuscript.

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