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Effects of combined treatment with clorgyline and selegiline on extracellular noradrenaline and serotonin levels

Kitaichi Y, Inoue T, Nakagawa S, Boku S, Koyama T. Effects of combined treatment with clorgyline and selegiline on extracellular noradrenaline and serotonin levels.

Objective Combined treatment with clorgyline, an irreversible monoamine oxidase (MAO)-A inhibitor, and selegiline, an irreversible MAO-B inhibitor, reportedly increases extracellular serotonin levels in the raphe nuclei more than clorgyline does alone. However, the effects of combination of these MAO inhibitors on extracellular noradrenaline have not been reported.
Methods Using *in vivo* microdialysis, we measured extracellular noradrenaline and serotonin levels after administration of clorgyline and/or selegiline in the medial prefrontal cortex of rats.
Results Administration of clorgyline (10 mg/kg) significantly increased both extracellular serotonin and noradrenaline levels. Combined treatment using clorgyline (10 mg/kg) and selegiline (3 mg/kg) increased extracellular serotonin and noradrenaline levels more than each drug alone did.
Conclusions These findings of this study suggest the augmented antidepressant action of the combination of MAO-A inhibition and MAO-B inhibition. The addition of a MAO-A inhibitor to selegiline or increasing dose of selegiline to achieve full MAO-A inhibition might be the promising strategy for the antidepressant treatment in partial responders or non-responders to selegiline.

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Keywords: clorgyline; *in vivo* microdialysis; noradrenaline; selegiline; serotonin

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Significant outcomes

- Combined treatment with clorgyline and selegiline increased extracellular noradrenaline and serotonin levels more than each drug alone did.

Limitations

- We measured extracellular noradrenaline and serotonin levels only after acute administration of clorgyine and/or selegiline, but not after chronic treatment with these drugs.

Introduction

Monoamine oxidase inhibitors (MAO inhibitors) were first developed as antidepressants. Recently, antidepressants that inhibit monoamine re-uptake have been used more as first-line antidepressants for depression treatment than MAO inhibitors have been used (1). Nevertheless, some reports of studies have described that MAO inhibitors were effective

not only for bipolar depression, but also for atypical depression (2,3). Today, MAO inhibitors remain in use as important antidepressants, although they are no longer regarded as first-line depression treatment.

Several reports have described that MAO-A inhibition is the main contributor to the antidepressant mechanism of MAO inhibitors (4,5). However, Lotufo-Neto et al. (6) described the possibility that non-selective MAO inhibitors are more effective than reversible MAO-A inhibitors. It is suggested that MAO-B plays an important role for an antidepressant effect especially when it is inhibited simultaneously with MAO-A (7). Recently, high-dose transdermal application of selegiline, which inhibits both MAO-A and MAO-B in the brain at a high dose but inhibits only MAO-B at a low dose, was approved as an antidepressant (8). Celada and Artigas (9) reported that combined treatment with clorgyline, an irreversible MAO-A inhibitor, and selegiline, an irreversible MAO-B inhibitor, increased extracellular serotonin levels in the raphe nuclei markedly compared with clorgyline alone. Their finding suggests the role of MAO-B inhibition for the antidepressant action of MAO inhibitors, especially selegiline and non-selective MAO inhibitors. However, they did not study the effect of the combined treatment on extracellular levels of noradrenaline, another important neurotransmitter for antidepressant action.

Two aims of this study were (a) to confirm whether the results of serotonin increases in the raphe nuclei obtained by Celada and Artigas (9) are observed in the medial prefrontal cortex, a projection area of serotonergic neurons and (b) to investigate effects of combined treatment with a MAO-A inhibitor and a MAO-B inhibitor on not only extracellular serotonin levels but also extracellular noradrenaline levels, which were not examined by Celada and Artigas (9). We measured extracellular noradrenaline and serotonin levels after administration of clorgyline, selegiline and both together in the medial prefrontal cortex of rats using *in vivo* microdialysis method.

Materials and methods

Animals

Male Sprague–Dawley rats weighing 230–280 g were obtained from the Shizuoka Laboratory Animal Center (Shizuoka, Japan). They were housed in groups of four and maintained on a 12-h light–dark cycle (light phase: 06:30–18:30 h) in a temperature-controlled environment (22 ± 1 °C) with free access to food and water. Experiments began after a 10-day period of acclimatisation. The Hokkaido University School of Medicine Animal Care and Use Committee approved all procedures, which complied with the Guide for the Care and Use of Laboratory Animals, Hokkaido University School of Medicine.

Drugs

Clorgyline hydrochloride (*N*-methyl-*N*-propargyl-3-(2,4-dichlorophenoxy)-propylamine hydrochloride) (Research Biochemical Inc., Natick, MA, USA) and selegiline hydrochloride (*R*(-)-*N*, a-dimethyl-*N*-2-propynyl-benzenethanamine hydrochloride) (formerly L-deprenyl; Research Biochemical Inc.) were used. Clorgyline hydrochloride was dissolved in saline to achieve a final concentration of 10 mg/ml. Selegiline was dissolved in saline to achieve a final concentration of 3 mg/ml. They were injected intraperitoneally (i.p.) as a volume of 1 ml/kg. The doses of the selective MAO-A inhibitor clorgyline 10 mg/kg and the selective MAO-B inhibitor selegiline 3 mg/kg were chosen to inhibit MAO-A and MAO-B selectively and fully, respectively (9–13).

Microdialysis procedures

Experiments were performed according to a procedure described in a previous report (14). Briefly, rats were implanted stereotaxically under pentobarbital anaesthesia (30 mg/kg i.p.) using an AG-4 guide cannulae (Eicom Corp., Kyoto, Japan) leading to the surface of the medial prefrontal cortex at the following coordinates relative to the bregma from the stereotaxic atlas of Paxinos and Watson (15): $A +$ 3.2, ML + 0.8, DV + 1.0 mm. Dialysis probes with 0.22 mm outer diameter (A-I-4-03; Eicom Corp.) were then inserted into the guide cannulae so that 3.0 mm of the probe was exposed to the tissue of the medial prefrontal cortex. Rats were housed individually after these operations.

Experiments were performed using freely moving rats. On the following day, 24 h after surgery, perfusion was started using artificial cerebrospinal fluid (145 mM NaCl, 3.0 mM KCl, 1.3 mM $CaCl_2$, 1.0 mM $MgCl_2$) at a flow rate of 1 µl/min. Following initial perfusion for 2 h, dialysate samples were collected in sample vials containing 50 µl of 0.05 M acetic acid every 40 min for 480 min. Extracellular noradrenaline and serotonin levels were determined as described previously using high-performance liquid chromatography system with electrochemical detection (Eicom Corp.) (14).

Rats received a single injection (i.p.) of saline, clorgyline (10 mg/kg), selegiline (3 mg/kg) or a combination of clorgyline (10 mg/kg) and selegiline (3 mg/kg) 200 min after the first dialysate samples were collected.

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Statistical analysis

All data were given as the mean values ± standard error of the mean of individual rats from each group. The noradrenaline and serotonin contents of dialysate samples were expressed as absolute values (pg/fraction).

To analyse the combined effect with clorgyline and selegiline (2 × 2 design) on extracellular noradrenaline and serotonin concentrations, repeated measures analysis of variance (ANOVA) for absolute values was used during the 0–280-min interval after MAO inhibitors administration. When the interaction was found to be significant, subsequent *post hoc* comparisons (differences in absolute values measured at each time point of collection among the four groups) were made using Duncan's test. Differences were considered significant at $p < 0.05$.

Results

Acute administration of clorgyline alone, selegiline alone and the combination of clorgyline and selegiline increased extracellular noradrenaline concentrations (Fig. 1a). Two-way ANOVA with repeated measures (0–280 min) indicated significant main effects of MAO inhibitors treatment [$F(3,23) = 29.334$, $p < 0.0001$] and time [$F(7,161) = 24.521$, $p < 0.0001$] on extracellular noradrenaline concentrations. In addition, the interaction between MAO inhibitors and time was significant [$F(21,161) = 9.863$, $p < 0.0001$]. The combined treatment group showed significantly higher concentrations of extracellular noradrenaline than the saline, the clorgyline or the selegiline groups did (Duncan's test, vs. the saline group, 40–280 min, $p < 0.01$; vs. the clorgyline group, 80–160 min, $p < 0.01$, 40, 200 min, $p < 0.05$; vs. the selegiline group, 80–280 min, $p < 0.01$). Significantly higher concentrations of extracellular noradrenaline were found for the clorgyline group than for the saline or selegiline groups (Duncan's test, vs. the saline group, 120–280 min, $p < 0.01$; vs. the selegiline group, 160–280 min, $p < 0.01$). The selegiline group showed significantly higher concentrations of extracellular noradrenaline than the saline group did (Duncan's test, 120 min, $p < 0.01$, 80, 160, 200 min, $p < 0.05$).

Acute administration of clorgyline alone and the combination of clorgyline and selegiline increased extracellular serotonin concentrations (Fig. 1b). Two-way ANOVA with repeated measures (0–280 min) indicated significant main effects of MAO inhibitors treatment [$F(3,20) = 6.903$, $p = 0.0023$] and time [$F(7,140) = 7.87$, $p < 0.0001$] on extracellular serotonin concentrations. In addition, the interaction between MAO inhibitors and time was significant

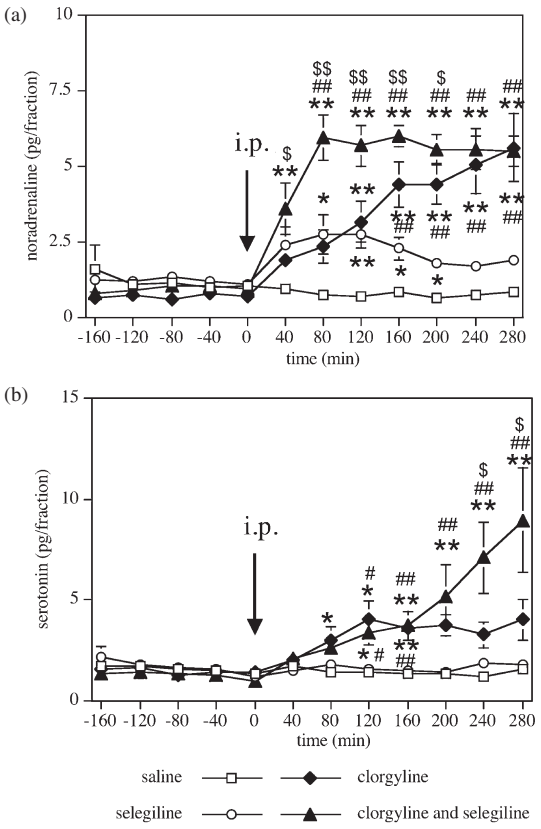

Fig. 1. Effect of combined treatment with acute clorgyline (10 mg/kg) and selegiline (3 mg/kg) on extracellular noradrenaline (a) and serotonin (b) concentrations during 0–280 min in the medial prefrontal cortex. Values represent the mean ± standard error of the mean (pg/40 min fraction). (a) $n = 8$ (saline and selegiline groups), $n = 5$ (clorgyline group) and $n = 6$ (combined treatment group). (b) $n = 6$ (saline and combined treatment groups), $n = 7$ (clorgyline group) and $n = 5$ (selegiline group). $^{**}p < 0.01$, $^{*}p < 0.05$ vs. saline group, $^{\#\#}p < 0.01$, $^{\#}p < 0.05$ vs. selegiline group, $^{\$\$}p < 0.01$, $^{\$}p < 0.05$ vs. clorgyline group.

[$F(21,140) = 4.565$, $p < 0.0001$]. The combined treatment group showed significantly higher concentrations of extracellular serotonin than in the saline, the clorgyline or the selegiline groups (Duncan's test, vs. saline group, 160–280 min, $p < 0.01$, 120 min, $p < 0.05$; vs. clorgyline group, 240 and 280 min, $p < 0.05$; vs. selegiline group, 160–280 min, $p < 0.01$, 120 min, $p < 0.05$). Significantly higher concentrations of extracellular serotonin were found for the clorgyline group than for the saline or the selegiline groups (Duncan's test, vs. the saline group, 160 min, $p < 0.01$, 80 and 120 min, $p < 0.05$; selegiline group, 160 min, $p < 0.01$ and 120 min, $p < 0.05$).

Discussion

This study showed that combined treatment with clorgyline (10 mg/kg) and selegiline (3 mg/kg)

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augmented the increases of not only extracellular serotonin levels but also extracellular noradrenaline levels in the medial prefrontal cortex compared with either drug alone. The results of this study support the idea that simultaneous inhibition of MAO-A and MAO-B can theoretically have the most robust antidepressant actions (7). These results are also consistent with the earlier results that brain tissue levels of serotonin and noradrenaline increased in MAO-A/B double knock-out mice to a much greater than in either MAO-A or MAO-B single knock-out mice (16).

Acute administration of clorgyline (10 mg/kg) alone, selegiline (3 mg/kg) alone and combined treatment all increased extracellular noradrenaline levels significantly compared with saline (Fig. 1a). The increases observed after administration of clorgyline alone appear to be the result of MAO-A inhibition primarily because various selective MAO-A inhibitors reportedly increase extracellular levels of noradrenaline, a substrate of MAO-A (7,14,17,18). Regarding effects of MAO-B inhibitor administration on extracellular noradrenaline levels, selegiline increased extracellular noradrenaline levels significantly in this study and another study (19). On the other hand, we previously reported that lazabemide, a very selective MAO-B inhibitor, did not increase extracellular noradrenaline levels (14). Hence, increased levels of extracellular noradrenaline by selegiline alone at the dose selective for MAO-B inhibition cannot be explained by its MAO-B inhibition. Selegiline has potency of noradrenaline re-uptake inhibition in addition to that of MAO-B inhibition (20). This might result in the increases of extracellular noradrenaline after selegiline administration. Acute administration of combined treatment increased extracellular noradrenaline levels more than either clorgyline alone or selegiline alone. Our previous study showed that the combination of selective MAO-A and MAO-B inhibitors, Ro 41-1049 and lazabemide, enhanced extracellular noradrenaline levels increased by Ro 41-1049 in the medial prefrontal cortex (14). Consequently, the increases of extracellular noradrenaline levels after coadministration of clorgyline and selegiline might be attributable to combined effects of MAO-A inhibition and MAO-B inhibition although noradrenaline re-uptake inhibition by selegiline might also be involved in this enhancement.

Celada and Artigas (9) reported that combined treatment with clorgyline and selegiline increased extracellular serotonin levels in the raphe nuclei markedly compared with clorgyline alone. Our results extended their results. We confirmed that extracellular serotonin increases are also potentiated in the medial prefrontal cortex, a projection area of serotonergic neurons, by coadministration of clorgyline and selegiline. However, the mechanism of action of this augmentation is still unclear. Simultaneous inhibition of MAO-A and MAO-B may not be able to explain this augmentation because the addition of the selective MAO-B inhibitor lazabemide did not enhance extracellular serotonin levels increased by the selective MAO-A inhibitor Ro 41-1049 in the medial prefrontal cortex in our previous study (14). Further studies are needed to clarify the mechanism of this serotonin augmentation.

In conclusion, combined treatment with clorgyline (10 mg/kg) and selegiline (3 mg/kg) increased not only extracellular serotonin levels but also extracellular noradrenaline levels more than each drug alone in the medial prefrontal cortex. These findings of this study suggest the more augmented antidepressant action of the combination of MAO-A inhibition and MAO-B inhibition. This study suggests a clinically important idea that the addition of a MAO-A inhibitor to selegiline or increasing dose of selegiline to achieve full MAO-A inhibition might be the promising strategy for the antidepressant treatment in partial responders or non-responders to selegiline.

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