Peer Review File

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Responses to the Reviewer's comments:
Major comments

1) The authors often described some changes following CS exposure and some effects of selegiline on CS-induced oxidative stress and inflammation as a "trend" or "tendency". If there is no statistical significance, they should not report them as a "trend" or "tendency" without showing F and P-values, because it is confusing. In addition, the authors state in the Discussion (page 17, lines 3–5) that inhibition of MAO-B by selegiline restored the impaired antioxidant defense system via eliminating excessive MAO-B-derived ROS and preventing the depletion of rGSH. Although selegiline prevented CS-induced depletion of rGSH, it has nonsignificant effects on ROS production (Figure 1B and C) and activities of antioxidant enzymes, SOD and catalase in CS-exposed rat lung (Figure 2). They should revise these relevant parts.

   Thank you for the suggestion. We have now deleted the words such as "trend" or "tendency" throughout the text.
   Abstract: *Pages 2-3, lines 43-44; lines 47-51*
   Results: *Page12, lines 253-255.*
   Page 13, lines 261-266
   Page 14, lines 286-291
   Discussion: *Page 16, lines 336-339; lines 347-349; lines 353-355*
   Page 17, lines 356-358

2) In this study, the author chose i.p. injection of selegiline at a dose of 2 mg/kg for evaluating protective effect of this drug on CS-induced oxidative stress and inflammatory response. Tested dose of selegiline is higher than therapeutic doses in patients with Parkinson's disease (PD; 5–10 mg/day) and with major depressive disorder (> 10 mg/day). Thus, they should provide more information on the tested dose based on the authors' or others' previous studies. They also should briefly discuss effective doses in COPD/depression or COPD/PD, because they mentioned the potential of selegiline as a monotherapeutic agent for treating both conditions (page 20).

   We appreciate the reviewer's comment. The dose of selegiline was selected based upon one previous report (Yohn SE et al. Behav Brain Res. 2018;342:27-34) and the calculation from a simple practice guide for dose conversion between rat and human (Nair AB & Jacob S, J Basic Clin Pharm. 2016;7(2):27-31). We agree that the selected dose is higher than therapeutic doses in patients with Parkisnon's disease (PD; 5-10 mg/day) and with major depressive disorder (> 10 mg/day) respectively. However, safe and effective drug dosing to

animals is necessary, regardless to the route of administration. Human dosing of selegiline is alwasys by oral administration. Based on human dose (10 mg/day) for PD with a man weighed 50kg, this gives to 0.2 mg/kg. To convert human dose to animal equivalent dose, the dose for rat is about 1.24 mg/kg, which is close to our selected dose. We have now added text to briefly discuss the effective doses of selegiline as a monotherapeutic agent for treating COPD/depression or COPD/PD.
Materials and methods: *Page 8, lines 154-156*
Discussion: *Page 20, lines 448-449*

3) Pretreatment with selegiline at 2 mg/kg had nonsignificant ameliorating effects on CS-induced oxidative stress (except for rGSH/GSSG ratio) as shown in Figures 1 and 2. However, it significantly attenuated up-regulation of Nrf2/HO-1 or NQO1 signaling and up-regulation of NF-κB signaling by CS exposure. Although further studies using MAO-B knockout mice need to clarify the mechanisms underlying protective effects of selegiline on CS-induced oxidative stress and inflammatory responses, Xiao et al. (2011; doi:10.1016/j.tox.2011.10.007) have been reported that the MAO-B knockdown reduced the accumulation of MPP+-induced ROS in PC12 cells, and selegiline pretreatment preserved the antioxidative potency in MAO-B-knock downed cells. For non-specialist readers, the authors should briefly describe about pharmacological actions including MAO-B-independent mechanisms of selegiline reported in previous studies.

Thank you for the suggestion. We have now added the relevant text to briefly describe about the pharmacological actions of selegiline from MAO-B-independent mechanism. In support, we have cited two additional references (Xiao H et al. Toxicology. 2011;290(2-3):286-94; Wu RM et al. Ann N Y Acad Sci. 2000;899:255-61).
Discussion: *Page 19, lines 421-428*

4) In the Introduction (page 7, line 6 from the bottom), the authors state that a preliminary study has suggested that MAO-B inhibition by selegiline may be a potential strategy for the development of an effective smoking cessation medication although large sample size is warranted (George et al, 2003). Although 2 preliminary small trials of oral selegiline showed trends toward improved abstinence (George et al, 2003; Biberman et al., 2003, doi:10.1046/j.1360-0443.2003.00524.x), results of two more recent trials indicate that oral or transdermal selegiline administration was not an effective aid for smoking cessation: an 8-week randomized placebo-controlled trial of oral selegiline 5 mg twice/day (n=101) from Weinberger et al. (2010; doi:10.1016/j.drugalcdep.2009.10.009), and an 8-week placebo-controlled trial of selegiline transdermal system with cognitive behavioral therapy (n = 243) from Killen et al. (2010; doi:10.1111/j.1360-0443.2010.03020.x). Thus, I suggest that this sentence in the Introduction should be rewritten.

We have now rewritten the text with updated references quoted by the reviewer.
Introduction: *Page 6, lines 122-126*

5) The authors conclude that Inhibition of MAO-B may provide a promising therapeutic strategy for CS-mediated oxidative stress and inflammation in acute CS-exposed rat lung injury. However, they have not shown the histological results in lung of their rat model and selegiline-treated, CS-exposed rats. Thus, I suggest that they should change the term "acute CS-exposed rat lung injury" to "acute CS-exposed rat lung".

We agree that we haven't looked at the histological changes in lung. We have now deleted the word "injury" in the relevant text.
Abstract: Page 3, line 53.
Discussion: Page 20, lines 453-454

Minor points

1) Regarding a cigarette smoke-exposed rat model (p.8–9), there is no standardized method or protocol for CS exposure as described previously (ref. 20). Therefore, the author should give readers information about the number of cigarettes (per hour or day) and cite references for conditions you tested (TPM 2000 mg/m3 for 1 hour twice daily for 7 consecutive days). If the condition was based on results of your preliminary test, you should show it as a supplementary figure, or briefly explain it.

We understand the reviewer's concern. In this computer-controlled whole body inExpose smoking system (SCIREQ, Montreal, Canada) for mainstream CS exposure, we will have to use 20 cigarettes per hour. The level of total particulate matter (TPM) of 2000 mg/m^3 was equivalent to our homemade smoking exposure system at 4% CS in our previous publication (Chan KH et al. Respir Med. 2009;103(11):1746-54). We have now added the relevant information in the text.
Materials and methods: *Page 9, lines 160-162*

2) Discussion (page 19, lines 13–15): The author mentioned that previous findings observed that short term CS exposure (even less than 7 days) was able to produce oxidative stress and inflammation, clinical features of COPD, in Sprague-Dawley rats (ref. 45: Roh et al., 2010). In a study from Roh et al. (2010), rats were treated with chronic exposure to CS for 20 weeks (10 cigarettes for 2 h/day, 5 days/week). Thus, please cite an appropriate reference.

Thank you for pointing out our mistake. We have now cited the appropriate reference (Stevenson CS et al. Am J Physiol Lung Cell Mol Physiol. 2007;293(5):L1183-93).
Discussion: *Page 19, line 419*

3) In Figure 3A, Student's t-test is intermixed with Tukey's post hoc test (Tukey's test: Saline/air vs. Saline/CS or Saline/CS vs. Selegiline/CS; Student's t-test: Saline/air vs. Selegiline/air). Because Tukey's test was used to compare two groups in other Figures (e.g. Figure 1A: vs. the Saline/air group or vs. Saline/CS group), they should show the result of Tukey's test between the Saline/air and Selegiline/air groups in in Figure 3A.

It is actually quite different between Tukey's post hoc test after one-way ANOVA and unpaired Student's t-test (also known as an independent t-test). Student's t-test compares the averages/means of two independent or unrelated groups to determine if there is a significant difference between the two. The one-way analysis of variance (ANOVA) is used to determine whether there are any statistically significant differences between the means of three or more independent (unrelated) groups. ANOVA test tells you whether you have an overall difference between your groups, but it does not tell you which specific groups differed – Tukey's post hoc test does. Because post hoc test is run to confirm where the differences occurred between groups, they should only be run when there is an overall statistically significant difference in group means (i.e., a statistically significant one-way ANOVA result).

4) Figure Legends: The authors should change "(one-way ANOVA)" to "(Tukey's test)", because data were analyzed using one-way ANOVA followed by Tukey's test and P values for post-hoc tests were shown.

*In **Figure Legends**, we have now changed (one-way ANOVA) to "(one-way ANOVA followed by Tukey's post hoc test) as follows:*
Page 32, line 628; lines 635-636
Page 33, lines 646-648; line 656; lines 661-662
Page 34, line 675

5) The administration of selegiline (page 8, line 4 from the bottom): They mentioned that rats were intraperitoneally injected with selegiline at 2 mg/kg before CS exposure daily. How many minutes before CS exposure was this drug injected?

We have now added the relevant information to the text.
***Abstract:** Page 2, line 35*
***Materials and methods:** Page 8, line 154*

6) Introduction (page 6, line 9 from the bottom): For non-specialist readers, Please change the sentence "Two types of MAOs (MAO-A and -B) with different substrate specificity and sensitivity have been identified …" to "… with different substrate specificity and sensitivity to inhibitors have been identified …".

We have now modified the sentence accordingly.
***Introduction:** Page 5, lines 96-97*

7) Results (page 14, lines 8–10): Please revise the sentence "Selegiline alone had no significant effects…, but showed a small non-significant reduction on CS-induced inflammatory cell counts in BAL of Slg/CS group" to "Selegiline alone had …, but there was a small non-significant reduction…" .

We have now revised the sentence accordingly.
Results: *Page 14, lines 286-291*

8) Discussion (page 18, lines 14–16): Please revise the sentence "In this study, there was a significant upregulation of pro-inflammatory mediators like CINC-1, MCP-1 and IL-6, and reduction of IL-10, leading to a shift to pro-inflammatory responses" to "… reduction of IL-10 in the Saline/CS group (or following CS exposure), … ".

We have now revised the sentence accordingly.
Discussion: *Page 18, line 394*