Author’s response to reviews

Title: Expression of cannabinoid 1 and, 2 receptors and effects of cannabinoid 1 and, 2 receptor agonists on detrusor overactivity associated with bladder outlet obstruction in rats

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Author’s response to reviews:

Editor Comments:
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Reviewer reports:

(Reviewer 1): The aim of the article is clear. The references are adequate. The topic is relevant. Although these considerations, this paper has significant syntactic and grammatical errors that make reading it pretty difficult.

It should probably be reviewed by a primary English language speaker to improve readability. This should be considered as a major revision.

My suggestions are stated in each of the following sections.

☞: We thank you and the reviewers for their time and valuable comments on the manuscript. Based on the reviewer suggestions, we revised the manuscript, our manuscript was proofread by American Journal Experts (AJE) for English language and grammar and resubmit our manuscript.

It is included acertification from AJE editing company.

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My responses to comments from the reviewers are below.
Introduction:

The introduction contextualizes the main objective of this manuscript. The authors define DO and list potential role of cannabinoids.

In my opinion, the Authors should shortly explain the pathophysiological and molecular mechanisms of action of cannabinoids and their receptors pathways in BOO/DO.

☞ We shortly explain the pathophysiological and molecular mechanisms of action of cannabinoids and their receptors pathways in BOO/DO in the background section (line 11-16, line 1-4, page 5,6).

To understand the role of cannabinoid related to DO, we tried to use the BOO/DO model and included references (reference number 12, 13) to support this rationale.

“Recently, experimental studies have shown that CB receptors appear to be involved in afferent signaling pathways. Specially, Gratzke et al. noted that in rats with partial BOO treated with a CB agonist, the ability to empty the bladder was preserved, while non-voiding contraction frequency was decreased compared to that in controls [12]. In addition, Walczak et al. showed that the co-localization of CB1 receptors and P2X3 receptors in the bladders of mice declined in response to mechanically evoked bladder afferent activity in the pelvic nerve after the intravesical administration of a CB agonist [13]. Our study was designed based on the research findings discussed above. “

Methods

This section is clear although English is weak.

☞ Thank you. Our manuscript was proofread by American Journal Experts (AJE) for English language and grammar by red color marking (page 7-12).

Results

This section is well structured. The figures are clear. English is weak.

☞ Thank you. Our manuscript was proofread by American Journal Experts (AJE) for English language and grammar by red color marking (page 13-14).
Discussion

Authors should discuss in more detail the study's results, reporting efficacy, complications and possible applications in humans. Finally, they should address the problem of treating this rats population in the long term.

☞ We described several limitations of our study in discussion (page 18-20).

“First, we used one dose and type of agonist for CB1 and CB2 receptors. Second, we did not measure residual urine volume or functional bladder capacity in the rat model used here. Third, although we attempted to measure non-voiding contractions, we were unable to do so; in this study. Fourthly, both immunofluorescence staining and Western blotting showed that CB2 receptor density was not significantly higher in the BOO group than in the control group.”

We also discuss about the study’s results in the discussion section (line 10-15, line 1-2, page 20, 21).

“In addition, although treatment with CB1 and CB2 agonists can have beneficial effects on DO, they can also cause complications or side effects during long-term administration. However, our study did not reveal any serious complications. Complications may have been avoided because agonists were administered intravesically, which is a local injection rather than a systemic injection. A potential problem is that it may be difficult to determine the most appropriate method of administration and dosage of CB agonists in a future clinical studies of human patients.”

(Reviewer 2): Please include all comments for the authors in this box rather than uploading your report as an attachment. Please only upload as attachments annotated versions of manuscripts, graphs, supporting materials or other aspects of your report which cannot be included in a text format.

Please overwrite this text when adding your comments to the authors.

This manuscript reports the CB1 and CB2 receptors in the BOO SD rat models with detrusor overactivity. Both CB1 and CB2 receptors were found to increase in density in the BOO bladders compared with the sham operated controls. The authors instilled CB1 and CB2 receptor agonists into the BOO rat bladder and found the detrusor contraction frequency and contraction pressure were significantly decreased in the CB1 agonist treated bladders but not in CB2 agonist treated bladders. The authors concluded that CB1 and CB2 receptors play a role in the pathophysiology of BOO associated DO in rats.

1) This is an interesting study and the methodology is appropriate.
2) Did the authors perform CMG before instillation of CB1 and CB2 agonist into the BOO bladders? What were the data?

Yes. We did perform CMG in all groups after 3 weeks postoperatively. Then we repeated CMG in one hour after administration of each drug and think that it was not important, although, we confirmed the presence of DO in the all three groups. However, if you want to enroll this data in manuscript, we can put this in manuscript.

We mention about this in the Method section (line 5-6, line 12-15, page 8).

“CMG was performed on all rats 3 weeks postoperatively, when we confirmed the presence of DO in the all three groups undergoing BOO surgery. In addition, the bladder was emptied in the CB1 and CB2 agonist groups, and a penile clamp was used to impede urination. Then 50 μM/kg of a CB1 receptor agonist (WIN55,212-2) and 10 μM/kg CB2 receptor agonist (CB65) in 0.2 mL of saline was injected through a needle into the bladder of each rat in these two groups. Then, we repeated CMG 1 hour after the administration of each drug, with the bladder filled with saline.”

3) The effect of CB1 and CB2 agonists on the inhibitory effect of BOO associated detrusor contractility is significant. However, the IHC and western blotting all showed the density of CB2 was not significantly higher than the controls. Authors might try to explain the possible cause for this discrepancy.

We included this in the discussion section (line1-9, page 20).

We obtained inconsistent data in IHC and western blotting, while in CMG we obtained consistent results. We described possible cause explaining the discrepancies and also described concurring data.

“both immunofluorescence staining and Western blotting showed that CB2 receptor density was not significantly higher in the BOO group than in the control group. The cause to explain this discrepancy is not precisely known, but it is presumed that CB2 receptors are involved in the mechanisms discussed in this study but to a lesser degree than CB1 receptors. The factors underlying these difference were not revealed by the methods used here. However, there was a statistically significant difference in the control, CB1 agonist, and CB2 agonist group for CMG parameters relative to the BOO group (p<0.05).”
4) In conclusion, please report the most important findings of this study and clinical implications. Please delete hypothetical statement without evidence obtained from this study.

☞ We included this “the most important finding of this study and clinical implications” in the conclusion section (line 2-6, page 22) and deleted hypothetical statement.

‘CMG parameters in BOO group were significantly improved by inhibitory effect of CB1 and CB2 receptor agonists on DO associated with BOO. The expression of CB1 was significantly increased in the urothelium and detrusor muscle in DO associated with BOO, but no significant change of CB2 expression was observed.’