January 19, 2017
Professor Josep M. Campistol
Editor-in-Chief
World Journal of Nephrology

Dear Professor Campistol,

We would like to thank the reviewers for their time and valuable comments on our manuscript entitled "Targeting Cannabinoid Signaling for Peritoneal Dialysis-induced Oxidative Stress and Fibrosis" (Manuscript Number: 31213). We list the point-by-point responses to all comments in the following pages, and we have made relevant changes in our manuscript accordingly. For the convenience of the reviewers, we have marked the revised parts of the manuscript in red and bold type. We sincerely hope the revised manuscript will fulfill the requirements for publication in the *World Journal of Nephrology*. Thank you again for considering publication of our report.

Yours sincerely,

An-Hang Yang, on behalf of the authors.
Corresponding author: An-Hang Yang, Department of Pathology, National Yang-Ming University and Taipei Veterans General Hospital, Taipei, Taiwan
Tel: +886-2-2875-7090 Fax: +886-2-2875-7056 E-mail: ahyang@vghtpe.gov.tw

Responses to the comments of the reviewers

Reviewer 1 (code: 00646291)

Well written report addressing the issue of increased risk of inflammation and oxidative stress occurring in patients receiving peritoneal dialysis therapy. Authors might consider indicating the significance of the problem mentioning numbers of patients receiving PD therapy and percentages of these patients presenting peritoneal fibrosis.

=> **Responses:** We truly appreciate the reviewer's important comments. We found that a recent survey indicated that approximately 11% of dialysis patients undergo peritoneal dialysis (PD) therapy worldwide, estimating to be more than 272,000 patients with an 8% annual growth rate globally (Li *et al.* 2016). The above content and its related citation have been added to the introduction section on page 3 lines 4-7.

As to the prevalence of peritoneal fibrosis, we have added the relevant content in the introduction section as follows. "After long-term exposure to various GDPs and AGEs, mesothelial cells undergo a dedifferentiation process, followed by peritoneal fibrosis and ultrafiltration failure (De Vriese *et al.* 2006;Jimenez-Heffernan *et al.* 2004;Radisky 2005;Yanez-Mo *et al.* 2003;Selgas *et al.* 2006). In a large peritoneal biopsy study, peritoneal tissue samples from 212 subjects including healthy controls, hemodialysis and PD patients were examined. They found that peritoneal fibrosis was absent in normal individuals but was present in 28% of samples from hemodialysis patients and up to 56% of biopsies from PD patients (Williams *et al.* 2002)." The above content has also been added in the introduction section on page 3 lines 11-18.

Reviewer 2 (code: 00051227)

This is well written and well organized mini-review on an interesting topic relating to metabolic disturbance leading to peritoneal fibrosis associated with peritoneal dialysis. The part of this mini-review regarding the potential role of cannabinoid signaling pathway on inflammation and fibrosis is important from both theoretical and practical point of view. Overall, the manuscript provides useful information for nephrologist and other readers. Specific comments: The title of the manuscript should be slightly modified. It seems to me that the effect of cannabinoid signaling pathway on inflammation and fibrosis should be mentioned in title. Some abbreviations are missing.

=> **Responses:** We would like to thank the reviewer for the valuable comments. We have amended our title from "Peritoneal Dialysis-induced Oxidative Stress and Fibrosis" to "Targeting Cannabinoid Signaling for Peritoneal Dialysis-induced Oxidative Stress and Fibrosis" to include cannabinoid signaling pathway in the title as

suggested by the reviewer. As to the missing abbreviations, we have corrected our manuscript on page 4 line 4, page 6 lines 1, 3, 15, 19, 20, and page 8 line 19.

Reviewer 3 (code: 00502781)

In their manuscript, Yang et al. review the therapeutic impact of cannabinoid signaling pathway in peritoneal dialysis-induced oxidative stress and fibrosis. Major comments: The cellular sources of reactive oxygen and nitrogen species in peritoneal dialysis-induced oxidative stress could be described. The authors could mention that cannabidiol exerts anti-inflammatory and antioxidant effects independent from classical CB1 and CB2 receptors.

=> **Responses:** We truly appreciate the reviewer for pointing out this important issue. After thorough literature review, we found that current evidence is lacking regarding whether cannabidiol exerts anti-inflammatory and antioxidant effects independent from classical CB1 and CB2 receptors. As to the cellular sources of reactive oxygen and nitrogen species, we have added an independent section to review the cellular sources of reactive oxygen species in PD-induced fibrosis as follows. "*In vitro* data showed that, upon GDP and AGE exposure during PD, cellular OS of human peritoneal mesothelial cells were induced through activation of protein kinase C, nicotinamide adenine dinucleotide phosphate oxidase, and mitochondrial metabolism. In turn, the generated ROS upregulate fibronectin expression by mesothelial cells (Lee *et al.* 2004). An *in vivo* study demonstrated 8-hydroxy-2'-deoxyguanosine (8-OHdG)-positive cells, indicating cells with increased OS, were observed throughout the fibrotic peritoneal tissue. Further immunofluorescent analysis revealed that 8-OHdG-positive cells also co-stained with mesothelin (mesothelial cells), CD68 (macrophages), CD31 (vascular endothelial cells), and α-smooth muscle actin (fibroblasts) (Wakabayashi *et al.* 2015), suggesting that OS was also increased in cells other than mesothelial cells. However, whether these fibroblasts with increased cellular OS were derived from an EMT-like process of mesothelial cells is unknown. As aforementioned, it has been reported that CB_2R is located on immune cells and modulates cytokine release (Demuth and Molleman 2006;Pertwee 2006). The CB_2R expression of human lymphocytes was downregulated by TGF-β stimulation (Gardner *et al.* 2002), which was not seen in human mesothelial cells (Yang *et al.* 2013). These findings suggest that TGF-β1 might have different physiological function in different cell types. Meanwhile, CB_2R activation might exert its anti-fibrotic effects not directly to cells undergoing fibrotic change but indirectly through modulating the immune cells. Such interaction among different types of cells underlines the pathophysiological role of CBR signaling pathway in uremic and/or dialysis injuries, which is partly supported by a recent study showing that systemic administration of

interleukin-10, an anti-inflammatory cytokines secreted by M2 macrophages, significantly reduced fibrous peritoneal thickening (Onishi *et al.* 2015). Therefore, current evidence indicates that beyond mesothelial cells, macrophages and vascular endothelial cells also contribute to ROS production during PD-induced peritoneal fibrosis." The above content has been added to the manuscript on page 7 lines 4-25 and page 8 lines 1-7. We would like to thank the reviewers once again for improving our manuscript.

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