Review in response to manuscript 33241

Manuscript Review

Round	Number ID	Reviewer	Review Time	Classification	Language Evaluation	Conclusion	
1	00495228	Anonymous	2017-06-23 14:30	Grade B (Very good)	Grade B: minor language polishing	Minor revision	View Detail
1	01427317	Anonymous	2017-06-26 15:50	Grade A (Excellent)	Grade A: priority publishing	Major revision	View Detail

We thank the reviewers for their comments and suggestions to strengthen the manuscript. We have modified the manuscript accordingly.

Reviewer ID#00495228 comments to Authors:

In the manuscript entitled "Pharmacological inhibition of DGAT1 and insights into postprandial gut peptide secretion" Maciejewski at al. described the effects of the pharmacological DGAT1 inhibitor on release of gut hormones. The manuscript confirms the previous published reports demonstrating that inhibition of the DGAT1 enzyme increases secretion of GLP-1 and PYY via augmented delivery of lipids to the distal portion of the gut where enteroendocrine L-cells are located. The experimental work presented in the manuscript is well designed and of good quality. The data are presented in the logical manner. However, there are some minor issues with data presentation and interpretation. 1. The Abstract is hard to read. It is too long and contained very detailed summary of the experimental data repeating the results section. Trimming down the size of the abstract and leaving out the exact values for the plasma gut peptide concentrations would help reading the abstract. 2. Fig. 1 does not show error bars in any of the presented graphs. This needs to be fixed. Did authors test the DGAT1 inhibitor in the DGAT1 knockout mice and what was the effect of the compound on GLP-1 secretion in the knockouts? 3. In Figs. 2-5 the vehicle group symbols and lines are very light and difficult to see in the printed version of the manuscript. The tone of the symbols needs to be adjusted. 4. Authors write that combination of the DGAT1 and DPPIV inhibitors leads to synergy and increased secretion of PYY (Abstract and main body of manuscript, pages 2, 11, 13 and 15). This is incorrect statement. Combo treatment versus DGAT1 inhibitor treatment produces somewhat lower PYY secretion (Fig. 3e,f). This needs to be corrected. Was the decrease in PYY secretion by the combo therapy significant when compared to the DGAT1 effect? 5. On page 12 authors write that effects of DGAT1 inhibitor is partially dependent on GPR119 activation, whereas on page 15 it is claimed that effects of DGAT1 inhibitor are GPR119 independent. Authors need to be consistent in their conclusions.

Authors response to reviewer ID#00495228 comments:

In response to comment #1 regarding the abstract, we completely agree that the current abstract is difficult to read. As suggested, the abstract result section was streamlined by removing the exact numerical data and is much easier to read. Additionally the other sections were edited with the intent for easier reading.

Error bars were reintroduced into figure 1 as requested (comment #2). We have tested the effects of our DGAT1 inhibitor on plasma triglyceride levels, PF-04620110 in DGAT1 knockout mice following a lipid challenge. As expected, plasma triglyceride levels were significantly reduced in DGAT1 knockout mice compared to wild type mice. In response to PF-04620110, there was no significant difference in the plasma triglyceride levels between knockout mice and knockout mice treated with PF-04620110. As previously published, we believe PF-04620110 to be a selective competitive inhibitor of DGAT1.

In response to comment #3, "the vehicle group symbols and lines are very light and difficult to see in the printed version", all figures have been modified by adding color and should now be clear and visible.

In response to comment #4, we did not intend to mislead the reader and indeed there is not a synergistic effect on PYY levels so our statement was incorrect. To fix this error and represent the data and our conclusions correctly, we have changed sentences regarding synergistic effects on PYY in the abstract and main body of the manuscript (pages 2, 11,13, and 15) Also of note, the decrease in PYY secretion by the combo therapy compared to PF-04620110 is not statistically significant.

Comment #5, "On page 12 authors write that effects of DGAT1 inhibitor is partially dependent on GPR119 activation, whereas on page 15 it is claimed that effects of DGAT1 inhibitor are GPR119 independent. Authors need to be consistent in their conclusions." In response, we have left the statement on page 12 in the results the same, "that the incretin effect seen with DGAT1 inhibition is partially inhibited by knocking out GPR119 in mice." To be consistent with this statement, in the discussion on page 15, we have now changed this interpretation to reflect the notion of partial dependence. The revised sentence is now "Incretin levels and PYY were drastically decreased in these GPR119 deficient mice even in the presence of PF-04620110. This suggests that lacking GPR119 cannot be rescued by PF-04620110 and the effects of DGAT1 inhibition on gut hormone secretion are *partially* dependent on GPR119."

Reviewer ID#01427317 Comments to Authors:

The authors studied the effects of a DGAT1 inhibitor (PF-04620110; PF) and DGAT1 deficiency on corn oil-induced release of GLP-1 and PYY from L cells, and GIP from K cells. They clearly demonstrated that DGAT1 inhibition or deficiency enhanced the release of GLP-1 and PYY, but decreased GIP release. Combination of DGAT1 inhibition with a DPPIV inhibitor enhanced active GLP-1 levels, but had no effect on total GLP-1, PYY and GIP release. A lipase inhibitor Orlistat inhibited DGAT1 inhibition-induced changes. GPR119 knockout likely inhibited DGAT1 inhibition-induced active and total GLP-1 release. Overall data are clearly demonstrated and clinically worthy. However, some interpretations should be clarified and exact relation between DGAT1 inhibition and hormone release is still unclear; target cells (enterocytes and/or enteroendocrine cells); site of action (luminal, intracellular or basolateral); segmental responses (proximal or distal small intestine, or colon). 1. Since DGAT1 is expressed in enterocytes and DGAT1 inhibition decreases enterocyte TG synthesis, long-chain fatty acids LCFA transport (absorption into enterocytes from the lumen and/or extrusion from the cells to subepithelial spaces) might be affected. Localization of nutrient receptors (GPCRs) on the enteroendocrine cells is still controversial; apical versus basolateral expression. Therefore, luminal nutrients likely activate apical GPCRs, but also may activate basolateral GPCRs after nutrient transport (absorption) through enterocytes. One possibility of the effects of DGAT1 inhibition on hormone release is that decreased LCFA absorption by inhibition of TG synthesis may increase luminal LCFA/monoacylglycerol (MG) content that activates apical LCFA/MG receptors (GPR40/120 and GPR119, etc) on enteroendocrine cells, then increase hormone release. Other is that increased LCFA/MG transport to subepithelial spaces by simple diffusion or intercellular pathway rather than TG transport as chylomicron formed in the enterocytes, may activate basolateral LCFA/MG receptors of endocrine cells, then increase hormone release. Orlistat experiment was excellent, but only showed that Orlistat decreased luminal LCFA/MG content, resulting in decreased LCFA/MG absorption (apical entry) into the enterocytes, rather than showing intracellular mechanisms as the authors stated. Since Orlistat abolished the effects of DGAT1 inhibition, TG degradation into LCFA and MG is likely upstream of the effects of DGAT1 inhibition. Please re-write the corresponding Results and Discussion. 2. Segmental effects; GIP containing K cells are predominantly located in the duodenum, whereas GLP-1 containing L cells are mainly present in the jejunum and ileum, and PYY is in the ileum. Major segment for lipid absorption is jejunum. Therefore, the rapid GIP release (peak at 1hr after corn oil challenge) is consistent with the duodenal response to luminal lipid. Inhibition of GIP release by Orlistat and GPR119 KO suggests

that luminal LCFA/MG released by lipase in the duodenum stimulates GPR119 on the apical membrane of K cells. DGAT1 inhibition decreased GIP release, suggesting that PF may act as a GPR119 inhibitor, may accelerate duodenal transit time, then inhibit lipid exposure to duodenal K cells, or DGAT1 in K cells may act as signals to release GIP. Although PF delays gastric emptying, the accelerated intestinal transit may account for the delayed, but enhanced release of active GLP-1 (peak at 2 hr by PF, compared to peak at 1 hr by vehicle), since GLP-1 is present in the jejunum and ileum. PYY release reached to peak at 6 hr, further suggesting that more LCFA/MG reached to the ileal lumen, where PYY is more abundant. Please re-write the discussion in p14 the first and second paragraphs, accordingly. 3. Interpretation of GPR119 KO study is weird. Fig. 5 represents that active GLP-1 release enhanced by PF was abolished in GPR119 KO, total GLP-1 release enhanced by PF was reduced in KO (although total GLP-1 release stimulated by corn oil was reduced in KO), PYY release enhanced by PF was likely reduced in KO, and GIP release stimulated by corn oil was abolished in KO. Therefore, it seems that PF-induced enhanced GLP-1 and PYY release is GPR119 'dependent'. Please re-write the corresponding paragraphs (p13 the second paragraph, p15 the third paragraph), or discuss why the authors concluded GPR119 'independent'. Minor 1. Introduction, p6, line 9-10, 'the pattern of enterocyte secretion of gut hormones' should be 'enteroendocrine cell secretion'. 2. Results, p10-11, Figure 2E, 2F for PF and Sitagliptin study should be Figure 3E and 3F.

Authors response to reviewer ID#01427317 comments:

In response to comment #1 to address the exact relationship between DGAT1 inhibition and incretin release regarding target cells, site of action and spatial responses. As the data provided in these studies is suggestive but not definitive, we have revised the corresponding results and discussion section to capture all possible scenarios that could explain our observations.

Specifically, we changed the following in the results section

Due to our previous observations of the role of DGAT1 in the temporal and spatial absorption of dietary lipids, we speculated if the 'signal' generated by DGAT1 inhibition to enhance gut hormone secretion was generated in the lumen of the gut or intracellularly within the enterocytes. DGAT1 is expressed on enterocytes whereas incretins are secreted from enteroendocrine L and K cells. To gain some insight, we used a pancreatic lipase inhibitor which blocks the luminal absorption of dietary lipids. Coadministration of Orlistat completely blunts the ability of PF-04620110 to elevate GLP-1 and PYY levels demonstrating a requirement of luminal TG degradation and lipid absorption. Thereby, these data suggest DGAT1 inhibition enhances the

incretin response from the lumen and breakdown of lipids in the lumen are upstream of the effects of DGAT1 inhibition We then hypothesized the mechanism by which DGAT1 inhibition effects incretin release is by increasing luminal long chain fatty acids and 2-MAG which could signal via GPR119 on enteroendocrine cells. To understand if GPR119, whose ligand, 2-monoacylglycerol is presumably being elevated via DGAT1 inhibition [45], is playing a role in the ability of PF-04620110 to elevate gut hormones, we utilized GPR119 deficient mice. GPR119 deficient mice were treated with PF-04620110 and subjected to a TG tolerance test. GLP-1, PYY and GIP were all reduced postprandially and drastically reduced the PF-04620110 profile of GLP-1, PYY and GIP. The decrease in GLP-1 AUC demonstrated in GPR119 knockout mice in either vehicle or PF-04620110 treated mice is 50% of GPR119 wildtype mice, suggestive that effects of DGAT1 inhibition on gut hormone secretion are partially dependent on GPR119. We speculate that additional LCFA/2-MAG-2 GPCRs are also activated by DGAT1 inhibition. Collectively, these data suggest DGAT1 inhibition requires upstream TG hydrolysis and is partially mediated by altering GPR119.

In response to Comment #2, on effects of DGAT1 inhibition on hormonal release from the various cell types in different segments of the GI tract, we have modified the discussion section (p.14 first and second paragraphs) accordingly to incorporate these comments. The two paragraphs now read as:

The augmented plasma GLP-1 and attenuation of GIP observed with genetic deficiency and pharmacological inhibition of DGAT1 in rodents is in agreement with previously reported studies [28, 29, 44, 46, 47]. It has been hypothesized that the delay in gastric emptying and temporal shift in intestinal lipid absorption in these rodent models can contribute to increased GLP-1 and attenuation of GIP. [48]. The reduction in GIP secretion via DGAT1 inhibition is potentially affecting K cell signaling by increasing lipid transit bypassing the proximal portion of the intestine. Apical L cell stimulation via GLP-1 and PYY release would also be enhanced with increased gut transit as more lipids ends up further down the GI tract. The PF-04620110 mediated increase in PYY is consistent with other studies as well as being increased in DGAT1 deficient mice, but the mechanism of action is not well understood [44, 46]. PYY secreting L cells are located predominantly in the lower portion of the gut (ilium and colon) [49]. Since it is clear inhibiting the resynthesis of TG through DGAT1 inhibition is causing an increase in PYY concurrent to GLP-1 release, it is most likely occurring through an ancillary pathway.

The incretin response is altered via the lipid load as well as the degree of FA saturation presented to the K and L cell population in the intestine [17]. The dose responsiveness of the enhanced gut hormone response following DGAT1 inhibition suggests the 'signal' is potentially the generation of ligands for fatty acid GPCRs. The exact molecular mechanisms of how inhibition of DGAT1 in the enterocytes results in impacts on enteroendocrine cells within the intestine remains to fully understood.

While DGAT1 plays a major role in endogenous TG synthesis, it does not rule out other enzyme contributions as residual TG synthesis is present in DGAT1 deficient mice and other enzymes like DGAT2 could compensate for the absence of DGAT1 [50]. One limitation to these experiments includes measuring these gut hormones only 6 hours post corn oil administration in mice. It is possible that the GIP signal returns at later time points when the lipid load reaches the distal sections of the intestine. This has been observed in DGAT1 inhibitor treated canines where GIP signaling returned after 4 hours [46].

In response to comment #3 and the other reviewer's comment #5 on the interpretation of figure 5 GPR119 knockout data that our conclusions were confusing, we completely agree and have clarified and corrected inconsistencies. In the results we found that GPR119 global knockout in mice elicits a reduced incretin profile compared DGAT1 inhibitor treatment (enhancing GLP-1, PYY and decreasing GIP) which slightly improves but does not fully rescue this profile in GPR119 deficiency. The incretin effect seen with DGAT1 inhibition is partially inhibited (not dependent) by knocking out GPR119 in mice. The discussion has been changed to reads as follows: "Incretin levels and PYY were drastically decreased in these GPR119 deficient mice even in the presence of PF-04620110. This suggests that lacking GPR119 cannot be rescued by PF-04620110 and the effects of DGAT1 inhibition on gut hormone secretion are partially dependent on GPR119."

We have correctly the minor changes as suggested in response to minor #1 in the revised manuscript.

Dear Fang-Fang

The manuscript has been now revised to list the software used for statistical analysis

Please let us know if you need anything else

Regards

Claire