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ESPS PEER REVIEW REPORT

Name of journal: World Journal of Gastroenterology
ESPS manuscript NO: 12330
Title: Increase of apoptosis by combination of metformin with silibinin in the inhibition of human colorectal cancer cells (colo 205)
Reviewer code: 02446029
Science editor: Su-Xin Gou
Date sent for review: 2014-07-03 14:15
Date reviewed: 2014-07-03 23:55

CLASSIFICATION	LANGUAGE EVALUATION	RECOMMENDATION	CONCLUSION
[ ] Grade A: Excellent	[ ] Grade A: Priority publishing	Google Search:	[ ] Accept
[ ] Grade B: Very good	[ ] Grade B: Minor language polishing	[ ] Existing	[ ] High priority for publication
[Y] Grade C: Good	[Y] Grade C: A great deal of language polishing	[ ] No records	
[ ] Grade D: Fair	[ ] Grade D: Rejected	BPG Search:	[ ] Rejection
[ ] Grade E: Poor		[ ] Existing	[Y] Minor revision
		[ ] No records	[ ] Major revision

COMMENTS TO AUTHORS

The investigators compare human umbilical vein endothelial cells with a colon cancer cell line (colo 205) for a response to the anti-diabetic drug, Metformin, and Silibinin showing that the drugs specifically alter survival of cancer cells, but not the normal cells. The results are interesting. Corrections and modifications should be made in the text to make this more intelligible to readers. Specific Comments: Abstract: In the abstract, the authors should explain what Silibinin and Metformin are. They also need to explain the terms PTEN and AMPK. Introduction: This introduction focuses on Metformin and Silibinin and does not explain the logic of pushing apoptosis in cancer cells compared to normal cells. Materials and Methods: These are adequate except I would recommend expansion on the assays done and also an importance of why AMPK phosphorylation and AKT phosphorylation is being studied. Discussion: The discussion is long and should be focused on the two drugs and colon cancer. Additional Comments: It would be helpful to have multiple other colon cancer cell lines to test than just simply one. If this is possible, additional data should be added. The bibliography is extremely long, and should be shortened from 74 entries to approximately half that number. Figure 1 has good statistical evaluation except for panel 1C. Figure 2A and 2B are very well displayed as well as Figure 3C and Figure 3B. Figure 4A, 4B, 4C are quite effective. I would still recommend adding additional colon cancer cell lines to show the diversity of action of this combination of drugs.

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ESPS PEER REVIEW REPORT

Name of journal: World Journal of Gastroenterology
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Title: Increase of apoptosis by combination of metformin with silibinin in the inhibition of human colorectal cancer cells (colo 205)
Reviewer code: 02446204
Science editor: Su-Xin Gou
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CLASSIFICATION	LANGUAGE EVALUATION	RECOMMENDATION	CONCLUSION
[ ] Grade A: Excellent	[Y] Grade A: Priority publishing	Google Search:	[ ] Accept
[Y] Grade B: Very good	[ ] Grade B: Minor language polishing	[ ] Existing	[Y] High priority for publication
[ ] Grade C: Good	[ ] Grade C: A great deal of language polishing	[ ] No records	
[ ] Grade D: Fair		BPG Search:	[ ] Rejection
[ ] Grade E: Poor	[ ] Grade D: Rejected	[ ] Existing	[ ] Minor revision
		[ ] No records	[ ] Major revision

COMMENTS TO AUTHORS

This paper assessed the efficacy of a combined usage of silibinin, which is a phytochemical, and metformin, which is an oral antidiabetic drug in the biguanide class. Authors successfully showed the synergistic effects of the two compounds regarding the apoptosis induction in COLO 205, a human colorectal cancer cell line. They also investigated the molecular basis for the synergism by studying activation states of Akt/PTEN and AMPK. This paper is well written, showing a possibility towards a novel therapeutic regimen for the treatment of colorectal cancers. However, there remain some points to be confirmed. If authors show additional data regarding those points, the quality of this paper will be further up-graded. Major concerns 1) The control cells used in this paper are vascular endothelial cells of fetal origin, which are not a genuine counterpart of COLO 205. At least some of the major findings, such as figure 4A, should be re-confirmed by the experiments using normal human colorectal epithelial cells. Primary human colon epithelial cells are commercially available; for example, Human Colonic Epithelial Cells (HCoEpiC, Catalog #2950) can be purchased from ScienCell Research Labortories (6076 Corte Del Cedro, Carlsbad, CA 92011 USA). http://www.sciencellonline.com/site/productInformation.php?keyword=2950 2) Descriptions regarding the mechanistic insight for the synergy of silibinin and metformin are rather speculative than verificative. To make the finding firmer, performing inhibitor analyses are encouraged; for example, experiments using caspase 3 inhibitors (Z-VAD-FMK, Z-DEVD-FMK etc) and AMPK

inhibitors (A-769662 etc) and so on. Minor concerns 1) “caspase3” in page 5 line 15, page 9 line 1, page 9 line 7 and Figure 4C should be rewritten as “caspase 3” adding a space between “caspase” and “3”.

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ESPS PEER REVIEW REPORT

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CLASSIFICATION	LANGUAGE EVALUATION	RECOMMENDATION	CONCLUSION
[ ] Grade A: Excellent	[ ] Grade A: Priority publishing	Google Search:	[ ] Accept
[ ] Grade B: Very good	[Y] Grade B: Minor language polishing	[ ] Existing	[ ] High priority for publication
[Y] Grade C: Good	[ ] Grade C: A great deal of language polishing	[ ] No records	
[ ] Grade D: Fair	[ ] Grade D: Rejected	BPG Search:	[ ] Rejection
[ ] Grade E: Poor		[ ] Existing	[ ] Minor revision
		[ ] No records	[Y] Major revision

COMMENTS TO AUTHORS

The manuscript provided by Tsai et al. demonstrated that combined treatment with metformin and silibinin caused apoptosis in colorectal cancer cells. These authors also indicated that co-treatment of metformin and silibinin enhanced PTEN expression, decreased AKT and mTOR phosphorylation and elevated AMPK activities. However, these still have some points need to be clarify before this manuscript could be accepted. Major points: 1. These authors declared that co-treatment affected several pathways such as PTEN, AKT, mTOR, and AMPK. In contrast, synergistically effect was found only in phosphorylation of AKT. Elevated PTEN expression was the same in co-treatment and silibinin alone. Increased AMPK and decreased mTOR phosphorylation was the same in co-treatment and Met alone. These authors should explain and discuss the phenomenon. 2. These authors demonstrated that co-treatment induced caspase 3 activation and AIF releasing. As known the AIF induced caspase-independent pathway whereas caspase 3 cleavage was involved in caspase-cascade. These authors still ned to perform more apoptotic-related proteins such as bax, bcl-2, PARP to verify co-treatment induced both caspase-dependent and –independent pathway. Minor point: 1. The manuscript needs editing with regards to English.