Author's response to reviews

Title: High glucose increased LPS induced DC apoptosis through modulation of ERK1/2, AKT and Bax/Bcl-2

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Author's response to reviews: see over
Dear Editors,

We would like to submit the enclosed manuscript entitled “BMC Gastroenterology”, which we wish to be considered for publication in “BMC Gastroenterology”. No conflict of interest exits in the submission of this manuscript, and manuscript is approved by all authors for publication. I would like to declare that the work described was original research that has not been published previously, and not under consideration for publication elsewhere, in whole or in part. All the authors listed have approved the manuscript that is enclosed.

In this work, we investigated the effect of glucose on the LPS treated dendritic cell (DC) of intestinal tract and DC2.4. Both DCs isolated from intestinal tract of Male BALB/c mouse and DC2.4 cell line was used. Pretreatment of glucose increased the LPS treated DC apoptosis both in vivo and in vitro at 24h and did not influence it at 1h and 6h. The survival proteins (AKT, ERK, Bcl-2) deceased and Bax increased at 24h. The amount of NO did not change significantly at 24h. AKT, ERK, Bcl-2 and Bax were mainly located in cytoplasm. I hope this paper is suitable for “BMC Gastroenterology”. We deeply appreciate your consideration of our manuscript, and we look forward to receiving comments from the reviewers. If you have any queries, please don’t hesitate to contact me at the address below.

Thank you and best regards.

Yours sincerely,

Yueran Zhao

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Response to reviewers

Reviewer: Daniel M Wall
Reviewer's report:
Major Compulsory Revisions:
(1) An abstract needs to be added.
Response: Abstract had been added.

(2) The figures and legends need to be improved. The figures have not reproduced well and are difficult to interpret, especially the FACS data. In addition it is unclear at times what the figures are supposed to show as the figure legend does not describe exactly is shown. In particular the concept of ‘apoptosis ratio’ is not explained or what it means or how it is calculated.
Response:
It is my fault that I have set the arrow at the wrong place in Fig1, now I have corrected it.
This chart is the record of one sample been flow-cytometry detected. As shown in the photo below, the X-axis and Y-axis represent the fluorescence density of Annexin and PI respectively. The data of four quadrants are automatically generated by machine. The scales were not clear because the photos were condensed to a smaller size. I had added the scale to the charts.

We normally regard the Annexin- PI+ cells (top left quadrant) as apoptosis cells in flow-cytometry detection. The theory of Annexin PI detecting apoptosis is as follows. The apoptotic program is characterized by certain morphologic features including loss of plasma membrane asymmetry and attachment that the membrane phospholipid phosphatidylserine (PS) is translocated from the inner to the outer leaflet of the plasma membrane, thereby exposing PS to the external cellular environment. Annexin V is a 35-36 kDa Ca2+ dependent phospholipid-binding protein that has a high affinity for PS, and binds to cells with exposed PS. Annexin V may be conjugated to fluorochromes including FITC. This format retains its high affinity for PS and thus serves as a sensitive probe for flow cytometric analysis of cells that are undergoing apoptosis. Viable cells with intact membranes exclude PI, whereas the membranes of dead cells are permeable to PI. For example, cells that are considered viable are both FITC Annexin V and PI negative while cells that are in early apoptosis are FITC Annexin V.
(3) Figure 5 is difficult to interpret as the GAPDH control appears to be stained quite randomly with regions of intense or diffuse staining. These should be addressed as I presumed the purpose was to show uniform staining in these samples as otherwise it undermines what is shown with the other antibodies.
Response: I have replaced GAPDH by actin in both ICC and western blot.

(4) There are grammatical issues throughout that need to be addressed as these at times can make understanding the data more difficult. In addition, abbreviations need to used and explained at their first use. None were included for example for DC, ECL, LPS, NC or DAB within the first 3 pages.
Response: I have added the abbreviations. Grammatical issues had been corrected with the help of experts of Edanz.

(5) The reproducibility of Western blotting should be confirmed. No mention is made of whether Westerns were repeated to ensure the observed result was not a one off.
Response: Western were repeated at least 4 times and the statistical charts had been added.

(6) The reason for measurement of nitric oxide in the supernatant of DCs is not explained and it seems a strange thing to measure. The reasons for this should be clarified.
Response: After deep consideration, I think the NO results have no relativity with this paper’s hypothysis, so I delete it.

(7) Minor Essential Revisions:
Spelling and grammatical errors throughout and the use of italics where appropriate (e.g. in vitro and in vivo)
Response: I have used the appropriate italics. Spelling and grammatical issues had been corrected with the help of experts of Edanz.

Reviewer: Jonathan Peterson
Reviewer's report:
Major Compulsory Revisions
(1) Figure 1 and 2 axis labels are unreadable. From the arrows the data indicated that the upper left corners (in fig 1A) are Live cells (An-and PI-) however you have called these apoptotic cells (An+ PI-). Why are the axes not going in the same direction for figure 1A and figure 2A? Why do the data from figures 1A and 2A not match figures 1B and 2B, respectively? How were the data in 1B and 2B obtained?
Response: It is my fault that I have set the arrow at the wrong place in Fig 1, now I have corrected it.
As shown in the photo below, the X-axis and Y-axis represent the fluorescence density of Annexin and PI respectively. The data of four quadrants are automatically generated by machine. We normally regard the Annexin-PI+ cells (top left quadrant) as apoptosis cells. Annexin+PI+ cells (top right quadrant) are dead cells while Annexin-PI- cells (left lower quadrant) are viable cells. The scales were not clear because the photos were condensed to a smaller size. I had added the scale to the charts.

(2) For your Immunoblot please include refs or detail how GAPDH has been established to be an appropriate control for these experiments. GAPDH is known to be widely variable under certain conditions (such as metabolic stress). Also figure 4 shows the representative blot; How many samples for each treatment were used? Please include a bar graph with the mean and error of the quantified data collected by the Alpha imager.
Response: Thank you for your reminding, I have replaced GAPDH by Actin in both Immuncytochemistry and western blot. Photos had been enlarged and rulers had been added in immuncytochemistry.

(3) For NO quantification please include control cells with no LPS treatment for comparisons.
Response: After deep consideration, I think the NO results have no relativity with this
paper’s hypothesis, so I delete it.

(4) Also, the variables analyzed are not always clear. Hard to judge the veracity of the presented data.
Response: Photos of paper had been optimized and statistical histograms were added.

(5) Minor Essential Revisions
Please seek help in editorial review, many grammar mistakes were noted.
Response: I had corrected grammar mistakes with the help of experts of Edanz.

(6) Please include the full name of terms the first time they are used within the article.
The Abbreviation “DC” was used in the title and second paragraph, but no explanation of what DC stood for until the material and methods section. Other abbreviations used without explanation include: BMDC, PI#K, MEK, Bcl-2, BAX, LPS, PBS.
Response: I have added the abbreviations.

(7) Please include blots of total Akt and ERK1/2.
I had finished the total Akt and ERK1/2 of DC2.4 treated by 24h LPS. There was no statistical difference between glucose negative group and glucose positive group, so I did not include these blots in chart.

(8) It is not clear in the results or in the figures when the data is from the in vivo or in vitro studies. Please clarify.
Response: I had clarified the data.
(9) For figure 5, please include magnification of the image. Also it appears CTR stands for the control staining, please verify.
Response: I had added rulers in immuncytochemistry and CTR were verified.

(10) Discretionary Revisions For figure 3, please increase the brightness of the normal staining.
Response: I had increased the brightness of Hoechst staining.