Author’s response to reviews

Title: Colchicine causes prenatal cell toxicity and increases tetraploid risk

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Author’s response to reviews:

Dear editor,

We are pleased to answer the questions of the reviewers’ and the manuscript (PHAT-D-19-00198) has also been extensively revised according to the comments (resubmitted online), changes were highlighted in red.

Reviewer 1: 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.

The aim of this study was to evaluate the toxic effects of colchicine on the placenta and foetus. The study is very interesting and meaningful, however some points must be improved in order to be published.

Background, line 7, add botanical family and author scientific binomium of Colchicum autumnale

Answer:
We rewrote the sentence as following:

“Colchicine is a natural product extracted from the autumn crocus (Colchicum autumnale, the name is derives from the Greek word) plant, which is a taxonomic species within the family Colchicaceae.”

Methods: describe how colchicine solution was prepared, what were the concentrations tested and how long time the cells were incubated with colchicine. Did the authors use positive controls? How was the negative control prepared?

Answer:

We added associated part cell culture part of methods.

“Colchicine was dissolved in MEM medium, for dose-dependent, cells were treated by 0, 0.15, 0.3, 0.6, 1.2 and 2.4μg/ml colchicine for 3 hours; for time-dependent, cells were treated by 0.15μg/ml colchicine for 0, 12, 24, 48 and 72 hours. As colchicine diluted in MEM, untreated group was control.”

Data should be analyzed by ANOVA because authors present a control group and different concentrations of colchicine, or different time points.

Response:

We modified statistical analysis part of methods.

“For two groups, the data were statistically analysed by paired Student's t-tests; for different concentrations of colchicine and different time points, the data were statistically analysed by ANOVA, and a p-value of <0.05 was considered statistically significant.”

Reviewer 2: The teratogenic effects of colchicine when used at recommended dose have been extensively investigated and the majority of studies have concluded that the use of oral colchicine during pregnancy does not pose a substantial risks to the mother or fetus (see Diav-Citrin et al. 2010; Indraratna et al., 2018).

Therefore, it is unclear to this reviewer why this investigation was undertaken at this time.

Given the number of parameters measured and the failure of these investigations to demonstrate that colchicine decreases cell viability at low concentrations, the significant findings of suppression of cell proliferation and increased risk of tetraploid in a small subset of cases are difficult to extend to an expected in vivo outcome of fetal development. In addition, the reported significant results may simply false positives due to analysis of many related parameters using paired T tests with a an alpha level set at 0.05. This reviewer would therefore like to these
preliminary in vitro results extended to a relevant in vivo end point that sufficiently moves the bar our our understanding o the risks associated with use of colchicine during pregnancy.

Response:

For study purpose, we talked in the first paragraph of discussion.

“Colchicine is a clinical medicine that has been in continuous use, and epidemiological studies have suggested that clinical colchicine usage is not associated with foetal malformations or miscarriage, but its anti-mitotic properties may cause pre-term birth, short gestational age and low birth weight[7]. In this study, we used primary prenatal cells to evaluate the cell toxicity induced by colchicine and to uncover the cell biology of the colchicine-induced toxicity in the placenta and foetus.”

There were multi-factors affect the drug biological function in vivo, but it is difficult to clear the main effect of drug. Our study was the cellular biological basis of clinical phenomena, and prompt possible risk of pregnancy does. Here, we demonstrated that toxic effect of colchicine on CVCs and AFCs was inhibiting cell proliferation, which was consistent with its resulting in pre-term birth, short gestational age and low birth weight. On the other side, colchicine caused tetraploid risk increase would be the reason of its induced disorder in cell cycle.

Reviewer 3: Researchers have studied the effect of colchicine which focuses on pregnant women and fetuses. This topic fits the scope of Pharmacology and Toxicology and the results attractive.

As declared in page 6 “Primary cultures of CVCs and AFCs are widely used in prenatal genetic diagnoses”, any reference or citations justify the evaluation of the toxic effects of colchicine on the placenta and foetus?

Answer:

We rewrote the sentence as following:

“Primary cultures of CVCs and AFCs to obtain chromosomes for G-banding are widely used in prenatal genetic diagnoses.”

I suggest authors to consider toxic effect on DNA synthesis, since last century lots of literactures discussed its interaction with DNA.

Response:

Thanks for the good suggestion. One of the biochemical basis of cell proliferation arrest would be toxic effect on DNA synthesis, but there were significant increase of G2/M sphere cells but not S sphere cells by colchicine treatment, this result suggest that colchicine block cell proliferation were not induce toxic effect on DNA synthesis.
p9 line 3, why chose these 7 surface biomarkers? Any justification for it? CD29 and CD73 are broadly used for investigating cellular characteristics and the corresponding functional behaviours. What about the others?

Answer:

The surface biomarkers were chosen as the criteria of mesenchymal stromal cells (MSCs). MSCs were popular star of cytotherapy, according registration (www.clinicaltrials.gov), there were nearly thousands of MSCs project for human disease care was in progress. MSCs could be derived and propagated in most of organs and tissues, which represent a part bio-function of their sources. Here, the selected surface biomarkers were used to identify those CVCs and AFCs were kinds of MSCs.

For illustration, we add some words in the second paragraph of discussion.

“Actually, CVCs[11] and AFCs[12] were mesenchymal stromal cells (MSCs), which represent a part bio-function of their sources. Here, we check the surface biomarkers of MSCs[13],”

References


p9 line 31, In cell apoptosis assay, please list the configuration of the cell culture protocol?

Answer:

We add the process of cell drug treatment in cell apoptosis assay part.

“1×106 cells were seeded in to a 100 cm2 dish overnight for attachment. Drug treatment was accessed by change medium supplemented with 0.15 μg/ml colchicine.”

p3,9 Similar question, any known reason for the analysis of diploid and tetraploid cells? Supporting evidence from other literatures citing here will be better.
Answer:

One of the most important bio-function of colchicine was induce polyploidy in plant, to our literature, there were not any associated report in mammal cells.

We add the point and reference in the last paragraph of discussion.

“ which is common in plants[21]”

Reference


Best wishes,

Sincerely yours,

Xiaofang Sun