Reviewer's report

Title: Compound K, a metabolite of ginseng saponin, induces apoptosis via caspase-8-dependent pathway in HL-60 human leukemia cells

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Reviewer: Christian Ploner

Reviewer's report:

This study aimed to elucidate the molecular mechanism of compound K, a metabolized form of protopanaxadiol ginsenoside, induced cell death in leukemic cells. The authors chose the human promyelocytic leukemia HL-60 cell model to address this question and found that compound K mainly act via the caspase 8 controlled extrinsic pathway. Although several findings are already published this study shows in well performed experiments that a) compound K acts mainly via caspase 8 activation, b) capase 9 is activated by tBid/mitochondrial pathway and c) DISC formation is involved in caspase 8 activation.

Minor Essential Revisions:

1) Compound K induced apoptosis happens within hours and more remarkable at the highest concentration used in this study all cells become AnnexinV positive within 30 minutes and AnnexinV/PI positive within 4h. Using cycloheximide experiments the author hypothesized that de novo protein synthesis is required for compound K induced cell death. However, rapid apoptosis induction argues somehow against de novo protein synthesis (and immediate transcriptional response?). If cycloheximide inhibits activation of caspase 8, these cells should be protected from compound K induced cell death, what supports the hypothesis. The authors shall include data of apoptosis of cycloheximide-treated vs. non-treated cells exposed to compound K and shortly discuss the obtained data.

2) page 20, line 3: “….or by cleaving BH3,….”. The author must clarify what is meant by ‘BH3’ (BH3-only protein Bid?)

Discretionary Revisions

1) The authors showed ‘specific’ apoptosis in figure 1B rather than ‘total’ apoptosis, which include basal apoptotic levels of cell culture cells. In this figure the legend of the y-Axis shall be changed to ‘specific apoptosis (%)’.

2) Apoptosis induced by compound K is mainly mediated by caspase 8 activation, as specific inhibition of caspase 8 protected the cells from apoptosis. The finding that the intrinsic pathway is involved via tBid and caspase 9 activation might also be explained by the hypothesis that tBid/caspase 9 activation is a consequence of active caspase 3, which is processed within 1h after treatment (figure 2A).
**Level of interest:** An article whose findings are important to those with closely related research interests

**Quality of written English:** Acceptable

**Statistical review:** No, the manuscript does not need to be seen by a statistician.

**Declaration of competing interests:**
I declare that I have no competing interests.