Author's response to reviews

Title: In vitro metabolism studies of erythraline, the major spiroalkaloid from Erythrina verna

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Author's response to reviews: see over
Dear Editor of BMC Complementary & Alternative Medicine

You will find below the answers for the reviewers regarding the manuscript entitled “In vitro metabolism studies of erythraline, the major spiroalkaloid from Erythrina verna”, which was submitted to the BMC Complementary & Alternative Medicine as a research article. We made some additions on the manuscript as suggested by the reviewers in order to clarify possible questions. We thank for the contribution of Dr. Kazuhiro Takemoto and Dr. Horacio Heinzen that has improved the quality of the manuscript and look forward that it would be accepted for publication on BMC Complementary & Alternative Medicine.

Answers for Dr. Kazuhiro Takemoto:

Q1: This analysis assumes the same composition of microbiota between pig cecum samples. Is this assumption really satisfied? The reviewer is afraid that this result is a very special case because of few samples (n=2). If the authors cannot show the validity of this assumption, they should emphasize this limitation.

Answer: These experiments used the ceca of 2 animals and each one was carried out in duplicate. In all experiments (2 cultures and each one in duplicate) we have the positive control (incubation of quercetin), which shows the complete consumption after 480 min for the active ceca and no considerable degradation when incubated with deactivated ceca (result not showed). This procedure is standard at Prof. Humpf laboratory to confirm the culture activity. In our case, in which any considerable degradation was observed for erythraline on the active ceca (not autoclaved) and able to degrade quercetin, we assumed that this number of ceca (pigs) used was enough to represent the variety of the microbiota found in different animals. The low standard deviations observed both for erythraline and quercetin in both ceca were another data that made us comfortable to assume the reproducibility and repeatability of the model, even if more ceca are employed. The results found by colleagues when using this model found similar results for all the ceca used (n=3 or n=4), reinforcing our conclusions about the reliability of the methodology employed. Please, for more details consult the following references:


Q2: Moreover, this analysis assumes the same composition of microbiota between pigs and humans. However, the authors did not mention whether this assumption is really satisfied or not. At least, the authors should discuss this point.

Answer: The assumption of the similarity of the human and pig microbiota is supported by the studies developed by the group of Prof. Humpf on the last decade, in which they have already demonstrated the suitability of the pig cecum model. For example, Hein et al. (2008) said:

“The pig more closely resembles the human than any other nonprimate mammalian species because of the similarities in digestive anatomy, physiology, and nutrition. The cecum as the connection between the small and large intestine contains more than 400 bacterial species, most of them strict anaerobes. Besides the proximal colon the cecum is described as the segment with the highest fermentation rate. Owing to sampling difficulties microbial incubation studies often are performed rather with human stool samples. This experimental design has many disadvantages: human stool samples do not represent the microbiota in the middle intestine due to a more anaerobic atmosphere and a lower luminal pH in the proximal than in the distal colon, which affect the growth of bacteria in the intestine markedly. Furthermore, sampling of the stool specimen under strict anaerobic conditions represents another critical step.”

On the same paper they have described the characterization of the pig cecal microbiota by fluorescence in situ hybridization (FISH), and the results achieved showed a corresponding composition between human intestinal microbiota and pig cecum for the main bacterial groups. It is important to remark that we used animals from the same biodynamic farm (Kurzen family, Senden, Germany) which has been used by Hein et al. on their experiments. For more details, the following references are available:

HEIN, E.-M. et al. Deconjugation and degradation of flavonol glycosides by pig cecal microbiota characterized by fluorescence in situ hybridization (FISH). Journal of
Q3: The evaluation of cytotoxic activity against tumor cells is a little too sudden. The authors should provide a deeper explanation why cytotoxic activity against tumor cells should be evaluated in this study. The reviewer guesses that alkaloids generally show cytotoxic activity against tumor cells in addition to anxiolytic and sedative effects; however, he could not easily understand such a research background.

Answer: Dr. Takemoto is correct. I did a mistake at the introduction. There is a previous work about the cytotoxicity of some analogous spiro-alkaloids. These data stimulate us to discuss if our compounds have or not the cytotoxic activity. We have included this information at the introduction and we have also included a new reference to reinforce our investigation.

Reviewer: Horacio Heinzen

The paper is consistent by itself. It is written in good english, the experiments and findings are well described. The supporting material is adequate and informative. The synthesis of the metabolite is an interesting and valuable procedure. Although the need of looking for possible toxic metabolites produced by gut microbiota, it seems particular to me to look to this point as first step when investigating erythraline pharmacokinetics. It sounds more convinient to look for the possible routes of elimination of the alkaloid, before facing such a complicated protocol. It would be worth to study it even in a smaller animal, like mouse or rat, simply investigating animal's urine and feces. What about if erythraline is completely absorbed in the small intestine, glucoronidated and fully eliminated through the kidney and never reaches the gut? Perhaps a further experiment showing the routes of the alkaloid elimination could improve the scientific quality of this paper which I find very interesting, but rather incomplete at this level of knowledge of erythraline pharmacokinetics.

Answer: We agree with Dr. Heizen about the need of continuing this investigation. The possible mechanism of action of these alkaloids has recently been discussed in the literature (Setti-Perdigão, P., Plos One, volume 8, 2013). These findings reinforce the importance to investigate the Pharmacokinetics of erythralin alkaloids. We agree that the compound can be absorbed at the intestine, but it is really useful to obtain in vitro the possible metabolites (Phase I) before proceed the Pharmacokinetics studies. If we have the metabolites, the methodology for quantification can be performed for both compounds and if we have Phase one metabolism, it is possible to get all data at the same experiments, reducing the number of animals. Then, our group, following other groups around the world, assumed the protocols to run all in vitro procedures, before in vivo experiments.