Author's response to reviews

Title: Ascorbic acid antagonizes resveratrol mediated heme oxygenase-1 but not paraoxonase-1 induction in cultured hepatocytes - role of the redox-regulated transcription factor Nrf2

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Version: 2 Date: 4 November 2010

Author's response to reviews:

Dear Editorial Office of BMC Complementary and Alternative Medicine,

please find enclosed a revised version of our article entitled

“Ascorbic acid antagonizes resveratrol mediated heme oxygenase-1 but not paraoxonase-1 induction in cultured hepatocytes – role of the redox-regulated transcription factor Nrf2.”

by Anika E. Wagner, Christine Boesch-Saadatmandi, Dorothea Breckwoldt, Charlotte Schrader, Constance Schmelzer, Frank Doering, Koji Hashida, Osamu Hori, Seiichi Matsugo, Gerald Rimbach.

We are grateful to the valuable reviewers´ suggestions which helped us to improve the quality of our manuscript. We hope that the revised version of this manuscript is now suitable for publication in BMC Complementary and Alternative Medicine.

The following changes have been made as indicated below.

Yours sincerely,

Gerald Rimbach

Reviewer 1 (André Quincozes-Santos)

• The order of resveratrol and ascorbic acid in figure 2 (cytotoxicity data) has been changed as suggested by the reviewer.
• The title of figure 4 has been changed into “Effects of resveratrol and ascorbic acid on Nrf2 transactivation” as suggested by reviewer #1 and also reviewer #2. The results regarding figure 4 have been described in more detail and the fold increase in Nrf2 transactivation is now given in the manuscript.

• We fully agree with the reviewer’s suggestion that the FOX assay is not specific for hydroperoxides. This has now been mentioned in the discussion section. We have added the following paragraph: “It needs to be taken into account that the FOX assay is not specific for hydroperoxides; also cyclic peroxides and serial-cyclic peroxides may give positive FOX response (Yin and Porter, Anal Biochem. 2003, 313(2):319-26).”

• We have included more references regarding the effect of resveratrol on HO-1 induction (Yu et al. 2010 Surgery 148: 103-109; Zheng et al. 2010 Mol Vis 16:1467-1474; Chen et al. 2005 Biochem Biophys Res Commun 331:993-1000).

• A two-way ANOVA was not feasible since there was no treatment with ascorbic acid only. Furthermore, our statistical analysis revealed that most of the collected data were not normally distributed. Therefore an ANOVA was not feasible and we applied a non-parametric test (Mann-Whitney-U) for statistical analysis. Thus control and resveratrol treatments have been tested for statistical significance. The treatment ascorbic plus resveratrol has only been tested against the resveratrol induction. Therefore the treatment with ascorbic acid is not labelled as significantly induced compared to control cells.

• We have checked the manuscript for typos and grammatical errors as suggested by the reviewer.

Reviewer 2 (Klaus Eder)

• We have used HUH7 liver cells since we found that, under the conditions investigated, HUH7 cells are slightly more sensitive than HepG2 cells regarding the induction of Nrf2 dependent gene expression by resveratrol.

• A citation regarding Figure 4 (line 233) has now been made as suggested by the reviewer.

• Furthermore the title of Figure 4 has been changed into “… Nrf2 transactivation” as suggested by the reviewer.

• The effect of alpha-tocopherol on resveratrol induced Nrf2 signalling has been removed from the discussion section as suggested by the reviewer.

Reviewer 3 (Sorimuthu Subramanian)

• The relevant literature regarding the use of resveratrol and vitamin C in complementary and alternative medicine has now been considered in the revised version of our manuscript (Gonzalez 2005 Integr Cancer Ther 4: 32-44; Mainardi 2009 J Allergy Clin Immunol 123: 283-294; Vojdani 2009 Evid Based Complement Alternat Med; Padayatty 2010 PLoS One 5:e11414).

• Beside antioxidant also prooxidant effects of ascorbic acid have been described in the literature. The corresponding studies have now been quoted in our manuscript (e.g, Chen et al. PNAS 102: 13604-9 as well as Chen et al. PNAS
• We fully agree with the reviewer’s suggestion that it would be interesting to test whether resveratrol cytotoxicity may be counteracted by ascorbic acid. In fact we did such experiments but could not find a significant protection of ascorbic acid against resveratrol induced cytotoxicity in our HUH7 cells.