Reviewer's report

Title: Citrus auraptene suppresses cyclin D1 and significantly delays N-methyl nitrosourea (MNU) induced mammary carcinogenesis in female Sprague-Dawley rats

Version: 2 Date: 18 May 2009

Reviewer: Rosalia Simmen

Reviewer's report:

This study evaluates the effects of the natural dietary compound auraptene on mammary epithelial cell proliferation in vitro and mammary tumor incidence, latency, and multiplicity in vivo, the latter using the MNU-rodent model of mammary carcinogenesis. Results provide support to the anti-carcinogenic activity of auraptene by decreasing tumor incidence and latency in a dose-dependent manner; a potential mechanism for this tumor protective effect by decreasing cyclin D1 expression in mammary tumors, and the general applicability of dietary intake of this compound given its oral bioavailability and sequestration in mammary tissues. The collective findings provide the necessary baseline for additional studies to further examine the context and molecular mechanisms for the dietary protective effects of auraptene to improve mammary health status in women.

Major Compulsory revisions:

1) The authors indicate in a subheading under Results section, that dietary auraptene did not reduce tumor incidence. This does not agree with their data showing that auraptene decreased tumor incidence at 16 weeks post-MNU administration. Authors should reconcile their own data, and provide explanation for the incidence at 18 vs. 16 weeks as a function of auraptene intake.

2) As regards the histopathology of tumors, authors indicated that auraptene did not change the histopathology of tumors, yet they described differences with auraptene intake. The authors should decide if there is or there is no difference with intake of the compound. What is the significance of the lactating carcinomas? Could you relate these histopathologies to human mammary tumors and indicate whether some of these tumors could be classified as adenocarcinoma in situ or invasive carcinoma? Were there differences in histopathology status of the tumors when harvested at 16 weeks vs. 18 weeks?

3) As regards the cyclin D1 Western data, there were two distinct bands for immunoreactive cyclin D1 in mammary tissues but only one in mammary epithelial cells. It is not clear what cyclin D1 band is authentic from the tissue Westerns? Please clarify the identity of the two immunoreactive bands.

4) Could the authors relate the tumor protective effects of auraptene after MNU-administration to developmental changes in mammary gland structure and/or differentiation status (e.g. Terminal end buds, branching density) prior to
MNU-exposure. Many previously published studies have correlated differentiation status of mammary gland prior to carcinogenic insult as predictive of mammary tumor protection of dietary factors.

5) Authors assumed from their statement "auraptene reduced cell proliferation by suppressing cyclin D1" that this is a direct effect. Indeed, the link between decreased cyclin D1 and cell proliferation may not be as direct; the authors should consider flow cytometry to demonstrate cell cycle arrest at Go/G1 stage of cell cycle.

Minor Essential Revisions:
1) Abstract should indicate the levels of auraptene in normal mammary tissues.
2) Under Results section, second paragraph, last sentence: authors should correct their statement to indicate 'total loss of cyclin D1 expression, rather than 40% reduction.
3) Figure 10 should be Figure 9.
4) There was no data shown to support the statement under Discussion that "There were no change in the concentration of auraptene between the mammary glands of the rate that developed tumors and those that did not develop tumors". This should be provided.

Discretionary Revisions- None

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.