Author's response to reviews

Title: Effects of tamoxifen on vaginal blood flow and epithelial morphology in the rat.

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Author's response to reviews:

Dear Dr. Le,

We thank the reviewers for their evaluation of our manuscript entitled "Effects of tamoxifen on vaginal blood flow and epithelial morphology in the rat" (MS # 1145527410995937). We now submit a revised manuscript for consideration for publication in BMC Women's Health. All changes in the text of the manuscript have been highlighted. A point-by-point response to the issues raised by Dr. Labrie and the changes made are provided below. Many thanks to the editorial staff and we look forward to your final decision.

Sincerely,

Noel N. Kim
Miljan Stankovic
Abdullah Armagan
Tulay T. Cushman
Irwin Goldstein
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Response to Reviewer Labrie:

Major Compulsory Revisions

1) The approximately 20 ng/ml plasma E2 concentration found in ovariectomized animals strongly suggests a specificity problem with the assay used. Plasma E2 should be measured by mass spectrometry.

We agree with the reviewer that the levels of plasma estradiol in ovariectomized rats that we have measured in this study may be higher than those reported by other investigators. However, a cursory examination of the literature within the last 5 years indicates that plasma estradiol levels in ovariectomized female rats are reported to range from non-detectable to 25 pg/ml (mean = 10 +/- 6pg/ml; median = 9 pg/ml). Plasma estradiol levels in intact female rats range from 19 to 97 pg/ml (mean = 41 +/- 23 pg/ml; median = 34 pg/ml). In previous studies, using the same radioimmunoassay, we have observed that ovariectomized animals have plasma estradiol concentrations ranging between 8 - 28 pg/ml. Most studies suggest that ovariectomized rats have plasma estradiol values that are one-tenth to one-third of those of intact female rats. Thus, while our values may be at the upper end of the spectrum, they are not unreasonable.

More importantly, the plasma estradiol values are best interpreted as relative indications of the hormonal milieu. In conjunction with uterine wet weights, the plasma estradiol values in our study serve to verify that the ovariectomized rats were in an estrogen-deprived state, compared to intact animals. In this context, we do not feel that using highly specialized techniques such as mass spectrometry to determine estradiol levels in a more precise manner would change our interpretation of the data or alter our conclusions. In the revised manuscript, we have added additional details in the methods section regarding the radioimmunoassay that was used (pages 7-8). In doing so, we expect that readers will be able to draw their own conclusions with regard to the reliability of the assay and the relative changes that we observe.
2) The morphology should be described in greater detail: Laminar propria, muscularis?

We did not observe any overt changes in the lamina propria or muscularis layers in rats treated with tamoxifen for 2 weeks. Longer periods of treatment may result in structural changes in these layers, but this would need to be verified. We have revised the results section (page 12) to indicate that the lamina propria and muscularis were similar in control and tamoxifen-treated animals and have commented on potential future studies in the discussion (page 14).

Minor Essential Revisions

1) Fig 6 and legend - please identify the various groups in the legend and on the figure

Both the legend and the figure already include identification for the two treatment groups (CONT and TMX). We have added a sentence in the legend to indicate that representative tissue sections from three different animals are shown in any given row. Labels in the figure that appear on the left side apply to the entire row. We apologize for any confusion in interpreting the figure.

2) Page 12, second paragraph - please explain the data in more detail

This was conceived as a preliminary study to set the stage for future investigation. We did not perform any immunohistochemical or ultrastructural studies (i.e. electron microscopy) on the epithelial cells of the vagina. Thus, in the absence of further structural information or immunolocalization of markers, we do not feel that it would be appropriate to add more detail.

3) The discussion should be centered on the observations made. General discussions such as of the mechanism of action of estrogen, co-activators should be markedly reduced or removed. A total reduction of the discussion by one third would seem reasonable.

We have shortened the last portion of the discussion, as recommended by the reviewer.

4) It should be clearly stated that the specificity of the effect of tamoxifen can be different in the human and rat.

We have added a paragraph in the discussion (pages 15-16) to address potential species differences in the response to tamoxifen.