Reviewers report

Title: Tamoxifen and the Rafoxifene Analog LY117018: Their Effects on Arachidonic Acid Release From Cells in Culture and on Prostaglandin I2 Production by Rat Liver Cells

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Reviewer: Francois Blachier

Reviewers report:

General The paper by Levine reported on the in vitro effects of tamoxifen and rafoxifene analog on the arachidonic acid release and prostaglandin I2 production using rat liver and glial cells and cells originating from human colon and breast carcinoma. The data presented suggest that tamoxifen rapidly increase arachidonic acid release in the four cell models used and basal and stimulated 6-keto-PGF1 alpha net production in rat liver cells.

The tamoxifen-stimulated production of 6-keto-PGF1 alpha is inhibited by the COX-2 inhibitor celecoxib and by the COX-1 inhibitor piroxicam although piroxicam was found to be much less effective than celecoxib for such an inhibition. In contrast, actinomycin D and an estrogen antagonist did not affect 6-keto-PGF1 alpha production stimulated by tamoxifen. Lastly, the raloxifene analog was found able to block the tamoxifen-stimulated prostaglandin production without inhibiting the arachidonic acid release.

Although the data are clearly presented, the results obtained appear too preliminary to allow clear-cut conclusions on the effects of tamoxifen and rafoxifene analog on arachidonic acid metabolism in these different cell models. Indeed, some mechanisms proposed by the author to explain the results obtained remain, in my opinion, too much speculative and require further experiments in order to fully validate them.

Major Compulsory Revisions (that the author must respond to before a decision on publication can be reached)

1. According to the material and methods, free radioactive arachidonic acid was measured in the culture media by counting an aliquote by liquid scintillation(page 3, line 25). In my opinion, a chromatographic step would be useful (HPLC, TLC) in order to separate arachidonic acid from other compounds (i.e. arachidonic metabolites).

2. From the numerous metabolites generated by cells from arachidonic acid, only 6-keto-PGF1 alpha was measured. What about the other metabolites (e.g. PGF2 alpha, PGE2, lipoxygenase products etc.)?

3. According to fig 1, the free arachidonic acid released in the medium after tamoxifen treatment can amount to as much as approximately 25-30 % of the radioactivity incorporated by the cells (presumably mostly in phospholipids). What concentration does it represent in the culture medium? Is this concentration cytotoxic against cells? What percentages of released arachidonic acid is converted to 6-keto-PGF1 alpha?

4. As indicated in fig 4, celecoxib and piroxicam can inhibit totally (although at different concentrations) the tamoxifen-stimulated 6-keto-PGF1 alpha production in rat liver cells. The author conclude (p5, line 2) that "COX-2 is the most likely isoform expressed both constitutively and after induction by lactacystin in the presence of TPA". Is there any Western blotting data which would confirm such a conclusion?

5. In the discussion (page 5, line 30), the author propose that tamoxifen and the raloxifene analog can "affect phospholipase activities". The measurement of phospholipase activity as well as arachidonic acid content in phospholipids in the absence and presence of these agents would allow to confirm this mechanism likely responsible for increased arachidonic acid net production.
Minor Essential Revisions (such as missing labels on figures, or the wrong use of a term, which the author can be trusted to correct)

Discretionary Revisions (which the author can choose to ignore)
Please indicate in the fig. 3 to fig. 7 legends the cell type used for the experiments.

What next?: Unable to decide on acceptance or rejection until the authors have responded to the major compulsory revisions

Level of interest: An article of limited interest

Quality of written English: Acceptable

Statistical review: No

Declaration of competing interests:

None