Author’s response to reviews

Title: Tamoxifen Stimulates Arachidonic Acid Release from Rat Liver Cells by an Estrogen Receptor-Independent, Non-Genomic Mechanism

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PDF covering letter
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Emma Vaitch
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BMC Journals
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Dear Ms. Vaitch,

I am uploading the revised manuscript of the original manuscript ID 1927399934175833 entitled “Tamoxifen Stimulates Arachidonic Acid Release from Rat Liver cells by an Estrogen Receptor-Independent, Non-Genomic Mechanism”.

In this cover letter I am listing the revisions to the manuscript and the answers to the reviewers’ critiques.

Sincerely,

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REVISIONS THAT HAVE BEEN MADE

A) I suggest that AA release from cells may be a part of a mechanism by which tamoxifen prevents cancer. (Background – line 7).

B) The induction of apoptosis probably contributes to the effectiveness of tamoxifen in cancer prevention. (Discussion – line 6)

C) ….could, in addition to its effects on the ER, mediate cancer prevention. (Conclusions – line 2).

D) , a concentration that does not affect AA release [11] and would bind to ER with high affinity [12]. (Results – last sentence).

E) Tamoxifen (8 µM) also stimulates significantly AA release from rat glial cells (data not shown) (Results– 2nd line).

F) (2nd paragraph of Discussion).
(Added to discussion): The AA release by tamoxifen and other reagents studied in my laboratory occurs with µM concentrations [5,9,11]. Thus, the possibility that general necrotic cell death may cause AA release must be considered. Lactacystin (5.4 µM), phenylmethylsulphonyl fluoride (1mM), carboxbenzoxyleucyleucyleucinal (1 µM) and carboxbenzoxynyleulyleucynorvalinal (0.5µM) were tested for cell viability by a tetrazolium-based assay. They were not toxic after a 6-h incubation with rat liver cells at these concentrations [16]. Proteosome inhibitors are not toxic to several other cells in culture [17]. No toxicity of tamoxifen, at concentrations of 10 to 20 µM for A549 human lung adenocarcinoma (ER-negative) cells was reported [17]; nor was 10 µM tamoxifen toxic when tested on rat glial cells and breast cancer MCF-7 cells [18]. Even when cell viability of three different breast cell lines (ER-positive MCF-7; ER-negative MDA-MB 239 and ER-negative BT-20 cells) was measured after incubation with as much as 25 µM for 24-h, the loss in viability was due to apoptosis [10]. The concentration of tamoxifen for clinical effectiveness in the treatment off breast cancer is 5 µM [20]. Concentrations of tamoxifen used in this report, are comparable to those that induce apoptosis, not necrotic cell death.

Answers to critiques:

1) Jordan-critique: “tamoxifen is generally only effective in ER positive disease and does not control cancers of any other origin but breast”

A) Jordan-critique: “General toxicity of the compounds”.
Levine-answer: All of the studies on AA release and PGI₂ production use µM concentrations of reagents.

FASEB Journal. 2003;17:800-802
Lipids in Health and Disease. 2003;2: (1) 1.

Several of the proteolytic and proteosome inhibitors have been tested for their effects on rat liver cell viability by the tetrazolium-based assay (in vitro toxicology
assay kit MTT based). After a 6-h incubation in the presence of 1mM PMSF, 92% of the cells are viable (not significantly different from controls). Cells incubated with 5.4 µM lactacystin, 1.0 µM ZLLnV and 0.5 µM ZLLL for 6-h are 80% viable. This 20% loss in viability does not appear to be related to the amplification observed, because lactacystin, ZLLnV, and ZLLL does not result in loss of viability but still amplifies PGI₂ production in the presence of TPA. As judged by microscopic examination, incubation of the inhibitors for 6-h does not result in gross morphological changes. No floating cells are observed. Lactacystin and the peptide aldehydes, at the concentrations used, are not toxic to several other cells in culture.

BBRC. 1996;224:74 79

3) Jordan-critique: Specific tamoxifen toxicity
Levine-answer: No toxicity (necrotic cell death) at concentrations of 10 to 12 µM for a 549 human lung adenocarcinoma (ER-negative) cells is reported (BBA. 1997;1349:275-284). At 10 µM tamoxifen, no necrotic cell death on rat glial cells (C-6) and MCF-7 breast cancer cells is reported (Cancer Res. 2000;60:5395-5400). Cell viability is lost by incubation of ER-positive MCF-7, ER-negative MDA-MB 231 and ER-negative BT-20 cells. However the loss in viability, is a result of apoptosis (Cancer Res. 2000;60:5955-6000). Lastly, the clinical effectiveness of tamoxifen in the treatment of breast cancer is 5µM (Clin. Pharmacol. Ther. 1996;59:401-410).

4) Rigas-critique: “Too strong a conclusion”
Levine-answer: I have followed his suggestions.