Author's response to reviews

Title: Celecoxib Concentration Predicts Decrease In Prostaglandin E2 Concentrations In Nipple Aspirate Fluid From High Risk Women

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RE: MS: 7344945631441188 - Celecoxib Concentration Predicts Decrease In Prostaglandin E2 Concentrations In Nipple Aspirate Fluid From High Risk Women

Dear Dr. Edmunds:

Below find my responses to each reviewer’s questions. Please feel free to contact me if there are further questions.

Sincerely,

Edward Sauter

RESPONSES TO REFEREES’ COMMENTS

Changes in the revised text are in CAPITAL LETTERS.

RESPONSES TO REFEREES’ COMMENTS

Referee 3

1. the observation that the NAF PGE2 levels increase in premenopausal women are clearly demonstrated in fig 1. If the underlying hypothesis is that NAF PGE2 levels somehow reflect the chemprotective effect of celecoxib on breast cancer,
then this observation implies that the breast cancer risk could increase in premenopausal women using celecoxib. Simply concluding that no reduction in PGE2 levels in premenopausal women was observed in contrast to the reduction seen in postmenopausal women because postmenopausal women have higher serum levels of celecoxib post treatment is not a sufficient discussion of these observations.

Thank you for your thoughtful comments. Our conclusions are based on the results of statistical analysis, as well as our review of Figure 1, which illustrates the raw data. Briefly,

There were five premenopausal women who received 200 mg bid celecoxib (Figure 1A). Of these five, three had an increase and two a decrease after treatment. In all five, the plasma celecoxib level was less than 1000 ng/mL. Among the 14 premenopausal women who received the higher dose (Figure 1C), there was a variable PGE2 response to treatment in women with celecoxib levels below 1000 ng/mL, whereas the two women with celecoxib levels well above 1000 ng/mL had a decrease in NAF PGE2 after treatment.

Among postmenopausal women receiving 200 mg bid (Figure 1B), the NAF PGE2 response was variable up to a circulating celecoxib level of approximately 1200 ng/mL. The single individual with a higher level has a minor decrease in PGE2. Among postmenopausal women receiving 400 mg bid (Figure 1D), the five women with circulating celecoxib levels above 1000 ng/mL all had a decrease in NAF PGE2 after treatment.

As we note in the Abstract and main text, celecoxib levels trended higher in post-compared to premenopausal women, and the decrease in PGE2 was noted at higher celecoxib levels. Nonetheless, there was a strong inverse correlation between celecoxib level and NAF PGE2 both in pre- (r= -0.52) and postmenopausal (r = -0.49) women (page 10) receiving 400 mg bid celecoxib. It therefore appears that, regardless of menopausal status, it is the circulating level of celecoxib that is important, with low levels having a variable influence on PGE2, and higher doses decreasing PGE2. We cannot exclude the possibility that low celecoxib levels increase PGE2, although changes at lower celecoxib levels were not significant in our study. We have provided additional explanation of our findings on page 12 of the manuscript.

2. the role of celecoxib as a chemopreventive agent. a. in premenopausal women as outlined above; b. due to the cardiovascular risks. This study was started before these effects were appreciated and they probably mean that these NSAIDS are unlikely to be used as a chemopreventive agent for breast cancer, particularly as there are other proven alternatives (tamoxifen and raloxifene +/- aromatise inhibitors). A greater emphasis has to be given to this state of affairs, which means that even if the results presented in this study are in support of a breast cancer chemopreventive effect, it probably will not support further efforts
being expended in developing this class of drugs as a chemopreventive option.

We have provided a paragraph on page 13 which addresses the concerns you mention.

3. Background: Para 1 line 6, should read ‘Women with breast cancer with TUMOR PGE2 levels above etc.’

Thank you for noting this. This word tumor has been added.

Referee 4

1. The data are still rather sparse and there is a great degree of variability in both the PGE2 and celecoxib assays. The variability in plasma celecoxib (>100x) in particular still seems excessive. Chow HH, et al (2004) reported a range of 82-1700 ng/ml in 68 subjects after a 400 mg dose;

As you mention, the range was 82-1700 ng/ml between the 68 subjects in the Chow study who received celecoxib 400 mg once daily for two weeks. Among women in our study who received a total daily dose of 400 mg, the range was similar, 117.6 to 2281.2 ng/mL. The Chow manuscript does not outline the number of females in the study, nor their menopausal status.

2. it is not clear what "steady-state" refers to in Table 4.

We apologize for any confusion and have removed the term.

3. The HPLC assays for celecoxib were probably done in small batches and inter-batch variability could conceivably produce results like those shown here. Absorption of the drug is affected by food and metabolism by a number of concomitant agents not excluded. The only result relatively inconsistent with chance is in a single subgroup (400 mg, post-menopausal women), and this result really hinges on 2 or 3 datapoints. This work adds marginally to previous work published by these authors and seems better suited as a short note or letter.

At higher circulating celecoxib levels, there was a consistent association between celecoxib dose and PGE2 response both in pre- and postmenopausal women, with a variable effect at lower circulating levels. Among the five premenopausal women who received 200 mg bid celecoxib (Figure 1A), three had an increase and two a decrease in NAF PGE2 after celecoxib treatment. In all five, plasma celecoxib level was less than 1000 ng/mL. Among the 14 premenopausal women who received the higher dose (Figure 1C), there was a variable PGE2 response to treatment in women with celecoxib levels below 1000 ng/mL, but the two with
celecoxib levels well above 1000 ng/mL had a decrease in NAF PGE2 after treatment.

Among postmenopausal women receiving 200 mg bid (Figure 1B), the NAF PGE2 response was variable up to a circulating celecoxib level of approximately 1200 ng/mL. The single individual with a higher level has a minor decrease in PGE2. Among postmenopausal women receiving 400 mg bid (Figure 1D), the five women with circulating celecoxib levels above 1000 ng/mL all had a decrease in NAF PGE2 after treatment.

Each sample batch that we ran included a serum sample spiked with 200 ng/mL celecoxib to assess the recovery rate of the assay. The recovery rate was 99.5% +/- 3.4%. In addition to assessing the recovery rate, we randomly selected 11 post treatment samples for duplicate analyses. We also analyzed six serum samples in duplicate that were collected before the subject started celecoxib. Each of the six baseline serum samples demonstrated zero values at both runs. The 11 post treatment samples each had measurable celecoxib levels, with the deviation within each set of these 11 samples having a CV < 10%.