Author's response to reviews

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

Authors:

Edward R Sauter (sautere@health.missouri.edu)
Wenyi Qin (qinw@health.missouri.edu)
John E Hewett (hewettj@health.missouri.edu)
Rachel L Ruhlen (ruhlenr@missouri.edu)
John T Flynn (john.t.flynn@jefferson.edu)
George Rottinghaus (rottinghausg@missouri.edu)
Yin-Chieh Chen (Yinchieh_chen@yahoo.com)

Version: 3 Date: 26 October 2007

Author's response to reviews: see over
October 26, 2007

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.

Referee 1

1. It is required to explain further about the difference in the suppression of PGE2 in NAF by celecoxib between premenopausal and postmenopausal women. For instance, a question may arise that menstruation cycle may influence on the PGE2 levels and its suppression by COX inhibition. In addition, a concern might be raised that assay system might be influenced by menopausal status, which is obviously crucial for this study.

Indeed, one of the aims of the study was to assess if differences in PGE2 response based on menopausal status could be explained by differences in celecoxib levels in the two groups. We discuss at some length (page 11, second paragraph) how postmenopausal women tended to have higher celecoxib levels and a greater PGE2 response, and have added this to the abstract. This is not surprising, as many agents are metabolized and excreted at lower rates with increasing age, although with variability between women of a given age group.

2. The details of NAF collection should be clarified in more depth, because the procedure methodology might affect on the concentration levels.

Greater detail on our NAF collection has been added on page 5.

Referee 2

1. The only glaring missing detail is a description of how you decided which patients would receive the 200mg dose and which the 400mg dose. I initially thought you randomized between doses - assumed- but you clearly had more patients assigned to the 400mg dose arm (see page 8 "Subject section"). Could you clear up for us how you decided which patients got which dose and were these assignments open to any biases?
The first 20 subjects recruited received 20 mg celecoxib twice daily. Analysis of the data from these subjects did not demonstrate a significant downregulation of PGE2 in any subgroup. All subsequent subjects recruited received 40 mg celecoxib twice daily. This has been added to the Methods section, page 5.

Referee 3

1. Please state how the potentially small volumes of NAF were measured as indicated by a statement about the “correction” for aliquot volume.

NAF collected within the capillary tube was measured using a metric ruler, based upon our prior experiments demonstrating that 1 mm within the tube is approximately 1 µL of NAF. This has been added on page 6.

2. Celecoxib concentrations associated with the decrease in NAF/plasma PGE2. Table 2, please clarify the nature of the results in column “After v before”

The results in the column “after v before” reflect median change in PGE2 levels with treatment. A positive value indicates an increase and a negative value a decrease in the median change.

This has been added at the bottom of Table 2.

3. Attention should be drawn to the fact that the levels of PGE2 are higher in premenopausal women post treatment with Celecoxib although this change was not significant. This is a similar result as seen in the previous publication (ref no 16). This observation needs a further comment in the discussion. It would appear that the reduction in PGE2 levels post treatment is driven by the effects of Celecoxib in post menopausal women.

As outlined in the abstract, the purpose of this manuscript was to determine if celecoxib levels influenced PGE2 response, and if the levels were related to menopausal status. Our observations are consistent with the fact that the greater effect in postmenopausal women is due to their generally higher circulating levels of celecoxib. We have added a comment in the abstract that celecoxib levels trended higher in postmenopausal women. In the Discussion on page 11, second paragraph, we review the generally higher levels in postmenopausal women, and that “it is possible that the higher circulating concentrations of drug in postmenopausal women contributed to the greater effect (on PGE2 levels).” We go on to discuss this further on page 13, second paragraph.

4. Final comment before “Toxicity”, statement that no significant differences were found in the PGE2 response in either NAF or plasma of pre vs post menopausal women, but table 2 does show in significant decrease in PGE2 in post but not pre menopausal women

We apologize for this oversight. The sentence has been corrected.

5. Page 11, final sentence paragraph 1, should read we confirmed our original observation that PGE2 levels in NAF, but not in plasma, decreased after Celecoxib treatment in postmenopausal women and that the PGE2 response in NAF correlated with plasma Celecoxib concentration at the 400mg bid dose level

The sentence has been corrected as you suggest.
6. Page 12, paragraph 2, the data do not support the conclusion that the therapeutic threshold concentration is around 500ng/ml, all that can be concluded is that it is between 250 and 850 ng/ml

The sentence which comments on a therapeutic threshold around 500 ng/mL has been removed.

7. Page 12 third paragraph: It cannot be concluded that it is the activity COX2 that is the major contributor to NAF PGE2 levels as there are still appreciable levels of PGE2 in NAF after COX2 inhibition by Celecoxib

PGE2 levels reflect both COX-1 and COX-2 prostaglandin production. Celecoxib is a specific COX-2 inhibitor and its clinical advantage is that it does not inhibit COX-1. COX-1 is assumed to be a constitutively expressed enzyme that is present in almost every cell of the body. COX-2 is assumed to be an inducible enzyme that responds to specific conditions and environments. The degree of reduction of the PGE2 concentration seen in NAF of post-menopausal women following celecoxib treatment is assumed to reflect the action of the drug specifically on COX-2 activity. This is discussed on pages 12-13.

Referee 4

1. As noted above, simply evaluating the relationship of PGE2 change to celecoxib level does not seem to constitute a very large knowledge increment. These results could have been included in the 2006 paper.

We (ref 16) and others (Steinbach et al, NEJM 2000) have observed dose dependent effects of celecoxib. We also observed a greater effect in post than in premenopausal women. Nonetheless, the response to treatment at a given dose varied among women receiving the same dose and of the same menopausal status. It was therefore important to determine the primary reason for response to celecoxib, which was the focus of this manuscript.

2. p. 6. More detail regarding the processing of the NAF samples seems indicated. How were samples cleaned up? Was extraction performed? In addition there should be some information regarding assay quality control procedures (CVs, duplicate measures, QC samples, etc.)

Neither cleaning nor extraction was performed. During our 13 years of experience handling NAF samples, we have found that “cleaning up” the samples leads to variable loss of the agent of interest in addition to the removal of other agents. As a result, we no longer attempt to clean samples up. We have added information on quality control procedures in the Methods section.

For NAF and plasma analyses, a standard curve was prepared using serial dilutions of PGE2. Whenever possible, NAF and plasma samples were run in duplicate and the average of the two values was reported. The goodness of fit ($R^2$) was 0.99 for NAF and plasma samples. This has been added on page 6.

3. p. 9. Absence of any data on pre-treatment celecoxib levels is surprising. These might have been eliminated due to cost, but these data would have assured the reader re: specificity of the assay and lack of contamination by off-study drug use.

We apologize for any confusion. Tables 2 and 3 indicate before treatment values. The before treatment values are all pre-treatment.
4. The range in plasma drug levels in the 400 mg group was over 100-fold. This will create problems in the data analysis (see below) and begs the question as to the explanations for such extreme variability.

There is known variability in circulating celecoxib levels in excess of 100 fold (reference 18), related to variability in the metabolism and excretion of the agent. This has been added to the manuscript, page 12. Concentrations trend higher in postmenopausal women, as our data demonstrate.

5. The use of non-parametric statistics seems appropriate for generating P values given what must be an extremely non-normal data distribution for celecoxib and perhaps PGE2 as well. The paper provides no information regarding the actual magnitude of change within-person (in fact this may be problematic if variability is extreme). Figure 1, which provides rankings only, does not allow the reader to see what these relationships really look like. The authors state that NAF PGE2 and plasma celecoxib were related similarly in pre- and post-menopausal women – but Table 2 shows that the median PGE2 level actually increased after treatment in pre-menopausal women at both dose levels. This anomaly could be explained by an extremely odd distribution of the data, with high variance.

We have prepared plots of the raw data for your review (Figure 1). As you can see from plots C and D, the change in PGE2 from pre to posttreatment was inversely related to celecoxib level in both pre and postmenopausal women, the difference being that most celecoxib levels in premenopausal women were at or below 227 ng/mL, with an increase in PGE2, whereas most postmenopausal celecoxib levels were at or above 860 ng/mL, with a decrease in PGE2 after treatment. Thus, higher dose in the postmenopausal group is the likely reason for the more common decrease in PGE2 after treatment in the postmenopausal group. This has been added, and is discussed on pages 10 and 11.

6. Table 2. It is not clear what delta-PGE2 column heading means – is this a mean or median delta? As a result, it is also not clear what the P value for delta PGE2 means.

Delta PGE2 is the median value of the change (post minus pretreatment) in PGE2 values. This has been added to Table 2.

7. The text states that there was no significant difference in PGE2 response in pre- and post-menopausal women. The basis for this statement is not given, and the power for making this comparison must be extremely low.

We apologize, this statement was incorrect. There was a significant decrease in NAF PGE2 in post- but not premenopausal women (Table 2). This has been corrected.

8. The insertion of toxicity results here seems out of place. Belongs in the Methods section, if anywhere.

As you suggest, the section on Toxicity has been moved to Methods.

9. The median celecoxib level in pre-menopausal women on 200 mg dose changed from 195 to 117 (Table 2 vs Table 3) with the subtraction of just one subject. This indicates that the point estimates for concentrations are very unstable, especially when based on medians involving only 5-6 subjects.
The group you cite was the smallest one in the study and no significant differences were observed. All other groups had twice or more than twice as many subjects.

10. p. 12. The conclusion that there exists a threshold for celecoxib action between given plasma levels does not seem supportable, in view of the study size and data anomalies described above.

At your suggestion, we have removed comments about a threshold for celecoxib action.

11. p. 12. It is not clear how the authors have excluded the possibility that PGE2 levels in NAF (or plasma) reflect COX-1 activity, perhaps to a greater degree than does COX-2 activity.

We apologize if this was not clear. We believe that PGE2 levels in plasma and NAF reflect both COX-1 and COX-2 activity. The significant decrease occurred in the group with the highest overall celecoxib levels, from a median of 8.33 to 6.81 ng/ml. It is our belief that celecoxib decreased the PGE2 contributed by COX-2, but not that contributed by COX-1, which is why levels decreased a median of 18% rather than to a greater extent. This has been added to pages 12-13.

12. p. 4. Paper should give either ng/ml or ?M, or both, but should not switch.

This has been corrected.

13. The statement regarding reference 7 gives the impression that long-term celecoxib use was shown to reduce breast cancer risk in a human trial; in fact, this was an observational study and the statement should reflect that.

Thank you. This has been corrected.

Referee 5

1. Three of the women in each treatment group had prior cancer. While it is unlikely that their data affected the overall results, it would be nice to have the authors confirm this. Women who have already had breast cancer may have very different levels of PGE2 than those who are at high risk without cancer. Although the numbers with cancer are small, readers might want to know whether their values were markedly different from those for women without prior cancer.

The results for the women with prior cancer in the opposite breast did not differ from results from other participants. This has been added to page 10.

2. It is not clear what the p-value reported in the last column of tables 2 and 3 refers to. The column header does not clearly define what hypothesis was tested.

The explanation for what the p value refers to has been added to Tables 2 and 3.