Message

From: LEVINE, STEVEN L [AG/1000] [/O=MONSANTO/OU=NA-1000-01/CN=RECIPIENTS/CN=594761]
Sent: 3/3/2005 10:02:05 PM
To: FARMER, DONNA R [AG/1000] [/O=MONSANTO/OU=NA-1000-01/CN=RECIPIENTS/CN=180070]; GOLDSTEIN, DANIEL A [AG/1000] [/O=MONSANTO/OU=NA-1000-01/CN=RECIPIENTS/CN=527246]
Subject: RE: seralini article

Donna and Dan,

I had an opportunity to review the paper last evening and wanted to make a few points.

1) I cannot understand why positive controls were not used for either the surfactant or aromatase. This greatly diminishes the validity of these results.

2) The first striking element of Figure 3 is the steepness of RU aromatase inhibition. Even good aromatase inhibitors do not have slopes as steep as what is reported in figure 3. Inhibition for aromatase inhibitors is observed across a log scale, not an arithmetic scale as shown in figure 3. Therefore, this inhibition pattern dose not fit the pattern of characterized aromatase inhibitors.

3) The rate (slope) for inhibition with microsomes is much different in figure 3 compared to figure 5. However, the slope is still extremely steep, reminiscent of a surfactant effect. As you know, aromatase is a microsomal protein. After the aromatase enzyme is translated it becomes anchored in the SER where it is associated reductases (NADPH) which supply it with reducing equivalents that are necessary to catalyze the monooxygenation reaction catalyzed by aromatase. I believe that the surfactant is solubilizing/disturbing the relationship between the reductase and aromatase. Further evidence of this is shown in figure after the 1 h sampling time, where there is a bump in activity. For a short time, the surfactant is improving the association between the reductase and aromatase. Then full solubilization kicks in after 1 h and we see dissociation of the protein and the reductase. This is a well known phenomena that has been known for decades among P450 researchers. After all detergents are used to solubilize and purify these proteins.

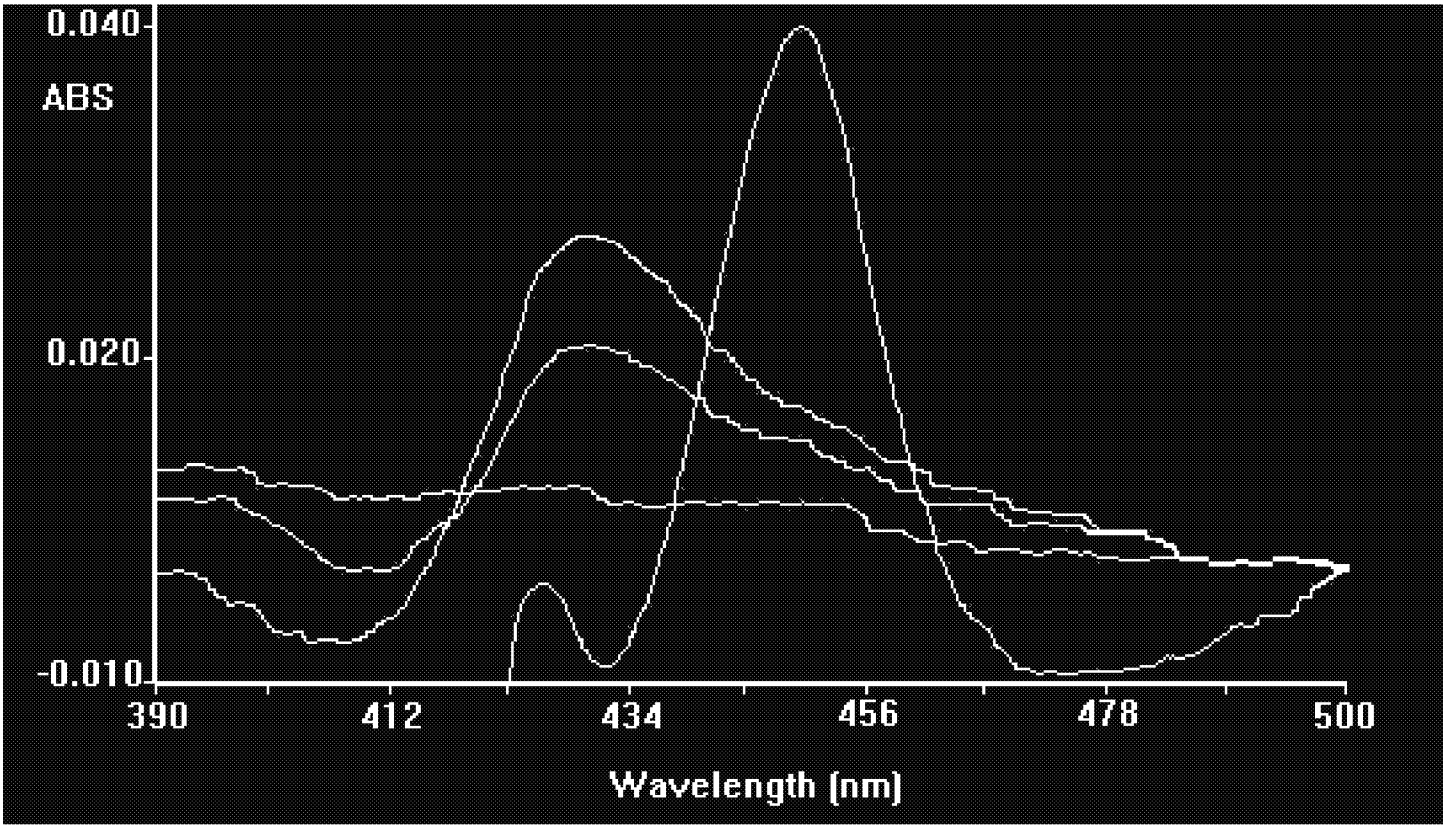
4) To support the findings in figure 5 I would like to see the expression of other house keeping genes. Normalizing to GADPH does not cut it and not showing the PCR gels makes the results in 5B unsupported. Need to show at least one representative gel.

5) Figure 6. The authors make an argument for *purely* competitive inhibition with sparse data and using the antiquated Linvweaver-Burk plot. This is inconsistent with what is illustrated in two scans for the difference spectroscopy. The scan to the left shows what is purported to be a type I and type II binding spectra. The type I spectra (if it was any good) would be used to support the competitive inhibition model and the type II spectra would be used to support a noncompetitive inhibition model. The scan to the left is for glyphosate alone and demonstrates what they are calling a type II binding spectra. I used to consider myself a CYP450 binding spectra aficionado and these type II binding spectra do not look like type II binding spectra. I may have been convinced if the sample cuvette was bubbled with carbon monoxide and the "type II' spectra disappeared and the classic P450 spectra appeared. I am amazed that this was not caught in the review but this is an "old" time method that most folks do not use anymore. Nevertheless, a knowledgeable P450 researcher would have picked this up.

Type I = binding at or near the active site
Type II = covalent coordination with the heme atom of the P450 molecule, which blocks binding of oxygen to the heme and thus inhibits the monooxygenation reaction catalyzed by the aromatase

enzyme.

Below is what the spectrum should have looked like. The more or less flat line is the baseline (scan 1) showing equal absorbance between the sample and reference cuvettes in the dual beam spec. The two peaks that have a trough at about 410 nm and a peak at 430 nm are the type II spectra scanned after addition of 0.1 and 1 uM clotrimazole. The scan with a peak at 450nM was made after bubbling the sample cuvette with CO to demonstrate reversible clotrimazole binding to the heme. This scan was made with microsomes isolated from fish a liver. One of the nitrogens in the triazole ring has free electrons that are able to coordinate with the heme of the CYP450. As you will see, the Type II spectra in the paper look nothing like this and they should. Also, where is their baseline! Additionally, the type I spectra is so marginal I don't buy it. It is not even 3x to 5x the baseline, which I would consider a rock bottom minimum for presenting a scan of this type.



These are my initial thoughts without rereading the paper and doing the necessary additional research to address these concerns. Clearly, these results are an artifact of the test system and this is exacerbated by poor mechanistic data that is does not support the initial observations.

Steve

-----Original Message-----
From: FARMER, DONNA R [AG/1000]
Sent: Wednesday, March 02, 2005 12:28 PM
To: LEVINE, STEVEN L [AG/1000]
Cc: GOLDSTEIN, DANIEL A [AG/1000]
Subject: FW:

Steve,

I have to go catch a plane take a look at this -this is a high level response to go out on the AGBIO cite and give Dan any feed back please...asap

Donna

-----Original Message-----

From: FARMER, DONNA R [AG/1000]
Sent: Wednesday, March 02, 2005 12:26 PM
To: GOLDSTEIN, DANIEL A [AG/1000]
Subject:

<< File: Richard et al.doc >>