Review of: "Targeting Alzheimer's disease hallmarks with the Nrf2 activator Isoeugenol"

Joshua Santos[1]

1 Department of Science and Technology

Potential competing interests: No potential competing interests to declare.

The paper is very good, multiple pathophysiology of A.D. is being addressed or explored but with some issues:

1. Isoeugenol crosses the BBB - How does the authors address or correlate the presence of other factors which makes or prevent drugs from freely entering or passing thru the BBB? The model might replicate the tight juncture of the BBB but how about the other factors that deemed it to be highly specific?
2. inhibits BACE activity in chemico - how does the authors correlate their data with the standard used in the experiment? I would suggest to use IC50 data correlations to prevent confusion of the readers.
3. Isoeugenol activates Nrf2 pathway in an AD cell model – involvement of AKT/GSK3β pathway - provide the whole picture of the western blot and not the cropped portion
4. Isoeugenol reduces pro-inflammatory parameters in a neuroinflammation cell model - provide the whole picture of the western blot and not the cropped portion
5. Isoeugenol decreased the body weight of 6 mo treated animals and no alterations were detected in the fasting glucose and triglycerides: (a) “This preliminary study showed that Iso induced a decrease in the body weight of APP/PS1 treated mice (APP/PS1+Iso compared to APP/PS1 animals; Fig. 6B)” - I am not particularly sure if the Y axis is properly labeled? it is said there that Percent Body Weight loss - so for the APP/PS1 + ISO group -→ the value was negative, does that mean that instead of having a weight loss, the group had increased there weight? Kindly clarify the statement and figure. (b) What could have caused the decreased in the body weight if the adipose tissue were not significantly altered? the other possible scenarios are: shrinking of the organs, or loss of muscle mass. (c) I know that the author would want to establish the metabolic cause to A.D. using the treatment with Iso but it seems that the fasting glucose and adipose level were not changed. Why didn't the authors measured the baseline total cholesterol level and post treatment cholesterol level instead of basing to the adipose tissue value? If the fasting blood glucose hasn't changed, why is HBA1C not utilized instead?
6. Isoeugenol reduced brain Aβ peptides levels in mice with early AD progression - “In contrast, no differences were found in the plasma among APP/PS1 mice (either compared to WT or APP/PS1+Iso; data not shown).” -→ Will the presence of the monomers in the plasma would not cause any rebound formation of the plaque if the monomers were not excreted from the body?
7. Intranasal administration of Isoeugenol is safe for mice: For the pharmacokinetic studies, (a) why is figure 9 shows an Time versus blood concentration of an intravascular route? the Intranasal route should appear as a bell shaped curved because the route used would still need to absorb the drug to the system thus will have an absorption constant. Only

intravenous route can result to a non-absorbed phased in the time versus plasma concentration of the drug. please elaborate on this. (b) I am not sure if it is possible to have the same Tmax for the three compartments or organ, is the author sure about the Tmax values? Especially for the concentration in the brain as the diffusion of the brain is slower compared to other vascular organs. (c) “Accordingly, the brain and lung displayed the lowest and the highest AUC_t values, respectively. Indeed, also C_{max} values and lung-plasma ratios were much higher than in the other matrices, suggesting direct passage of Iso to the lungs, when administered intranasally. Although $t_{1/2\beta}$ values were short for all matrices, $t_{1/2\beta}$ was higher in lung (31.27 min vs. 18.12 e 17,65 min; Table I),” -→ The AUC of the lungs will be greatest since the possibility that the drug administered intranasally would go into the lungs where the drug can be absorbed thus the lungs become the organ of absorption. The large amount of the drug in the lungs cana alter the half life of the drug especially if it exceeds the amount that the body or organ can eliminate or transport. Please comment on this.

8. “As shown in Fig. 10 (A-E), no alterations in glucose (Fig. 10A), triglycerides (TG; Fig.10B), and total cholesterol (Fig. 10C) levels were detected in 11-mo animals after Iso administration, compared to mice administrated with PBS (Vehicle - VEH). Interestingly, Iso significantly reduced LDL cholesterol (Fig. 10E) when compared to the VEH group. These results suggest that Iso is absent of deleterious metabolic effects, and its intranasal administration might have a positive impact on cholesterol metabolism.” -→ in relation to comment number 3, what will be possible scenario that the body weight decreased without altering the other metabolic parameters?
9. FOr 3.11 and 3.12 -→ does this mean that Iso does not have any effect on the disease model even if AD is already present in the disease model?