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

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Potential competing interests: No potential competing interests to declare.

The study explored the potential of Isoeugenol, a skin allergen with electrophilic properties, to activate Nrf2 and reverse some AD hallmarks, which is very interesting and worthy of publication

Comments are as follows:

1. Section 2.2.3. Cell culture and treatment and experimental animal age groups

a. The study design involved both in vitro and in vivo experiments, which is commendable. However, the rationale for selecting different cell types and animal age groups needs clarification, particularly regarding how these models represent distinct pathology and stages of AD.
b. AD mouse models often involve genetic modifications to express human genes associated with Alzheimer's, such as APP (amyloid precursor protein) and PSEN1 or PSEN2 (presenilin genes). But why did the investigators choose to verify the AD model through amyloid beta expression, a pathological feature, rather than relying on genetic confirmation such as the presence and expression of the PSEN1 gene through PCR, RT-PCR, or other genetic analysis methods?
c. Specify why mice microglia cells exposed to LPS and neuronal cells overexpressing the human APP with Swedish mutation (N2aAPPswe) were chosen for in vitro experiments.
d. Provide a clear justification for selecting the AD double transgenic mice (APP/PS1) for in vivo experiments, explaining how these mice accurately reflect different stages of AD, especially at the chosen ages of 6 months and 11 months with the expression of amyloid beta (AB_{40} and AB_{42}) in cortex, hippocampus, and plasma.
e. Regarding Isoeugenol, the authors have rightly emphasized its potential therapeutic effects. To enhance transparency and reproducibility, detailed information on the compound's preparation, extraction, or synthesis method should be included in the methodology section.

2. Results

While the study underscores the potential of Nrt2 activation in mitigating Alzheimer's disease (AD), the absence of evidence in AD mice raises questions. Demonstrating this activation in both N2a cells and AD mice would provide a more robust foundation.

a. In section 3.3. Isoeugenol activates Nrf2 pathway in an AD cell model –involvement of AKT/GSK3β pathway but why

similar result could not be replicated in the AD mice (APP/PS1) ?

b. In Figure 3 (E), a discrepancy is observed in the western blot results for Nrf2 in the cytoplasm between the AD neuronal cells treated with ISO and those without treatment. Upon observing the western blot bands, Nrf2 appears to be highly expressed in untreated cells when referenced against the internal control (Tubulin) compared to the treated cells. How can this observation be explained despite inconsistent protein loading?

3. Discussion

Authors should discuss any known limitations or potential differences between the experimental models used and human AD pathology.