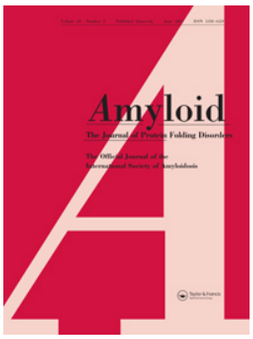

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Amyloid
The Journal of Protein Folding Disorders

ISSN: (Print) (Online) Journal homepage: informahealthcare.com/journals/iamy20

Response

Jeffery Kelly & Evan Powers

To cite this article: Jeffery Kelly & Evan Powers (2021) Response, Amyloid, 28:2, 140-141, DOI: 10.1080/13506129.2020.1853094

To link to this article: https://doi.org/10.1080/13506129.2020.1853094

Published online: 04 Dec 2020.

AMYLOID
2021, VOL. 28, NO. 2, 140–141
https://doi.org/10.1080/13506129.2020.1853094

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LETTER TO THE EDITOR

Response

We thank Dr. Alhamadsheh for his letter, wherein there is no scientific criticism per se of our subunit exchange data shown in Figure 2, the centerpiece of our paper, demonstrating that AG10 is ~4 times more potent than tafamidis at a fixed plasma concentration [1]. This is obvious by inspection of the raw data. Dr. Alhamadsheh prefers kinetic stabilizer comparisons using older assays, which we introduced into the literature [2,3], that we believe lack the clinical applicability and rigor of subunit exchange. Their criticisms to obfuscate the unarguable comparisons in our paper take advantage of an error we made in the penultimate sentence of the discussion section. Unfortunately, we mistakenly used the term 'mean peak plasma concentration' when we meant 'mean or average plasma concentration'.

The 800 mg dose of AG10 every 12 h yields a mean peak plasma concentration of 46.9 μM that drops off to ≈ 10 μM within 4 h, trending towards C_{min} (9.5 μM) at 12 h [4]. Thus, it was reasonable to pick an AG10 plasma concentration of 11 μM for the comparisons made in our paper. That said, there is a more rigorous approach for extracting the average AG10 plasma concentration over the time interval between doses (C_{mean}), where $C_{mean} = AUC_{\tau}/t_{dose}$ (AUC_{τ} = area under the drug concentration vs time curve and t_{dose} = the dosing interval). For AG10, C_{mean} = (47,200 ng h mL^{-1})/12 h = 3933.3 ng mL^{-1} = 13.5 μM [4]. At this concentration, our results indicate that the TTR dissociation rate would be suppressed to between 2.5% and 4.9% of normal (corresponding to 97.5% and 95.1% TTR engagement, respectively), depending on the TTR concentration (the reference range for which is 3.6 μM to 7.3 μM). This is entirely consistent with the measurements of TTR engagement by a fluorescence polarization assay in Figure 7of Fox et al. [4].

The C_{mean} of tafamidis at the 61 mg once daily dose = (174,400 ng h mL^{-1})/24 h = 7266.7 ng mL^{-1}, = 23.6 μM [5]. At this concentration, tafamidis would suppress the TTR dissociation rate to between 4.3% and 5.6% of normal. Importantly, the C_{max} and C_{min} of tafamidis at this dose are 28.5 μM and 18.3 μM, respectively, demonstrating that its concentration drops much less between doses spaced 24 h apart. The main take home of our paper is that tafamidis and AG10 are similarly stabilizing at the plasma concentrations achieved by oral dosing.

Dr. Alhamadsheh also points out tolcapone–TTR binding constants (K_{d1} and K_{d2}) other than the published values we used. Using these alternative binding constants does not change our analysis and does not significantly alter our conclusion that while tolcapone is a potent stabilizer, its short half-life makes it very difficult to use clinically, given the established maximum allowable dose associated with the black box warning.

We dispute the other points Dr. Alhamasheh makes as follows.

- It is claimed that we ignored serum protein binding in comparing the efficacy of tafamidis and AG10. This is incorrect; our assay directly accounts for serum protein binding **because it is performed in blood plasma**. Moreover, our analysis explicitly accounts for binding to albumin, which is responsible for virtually all off-target plasma protein binding.
- It is claimed that we ignored the effect of TTR's second binding site on stabilizer efficacy. This is incorrect. Our analysis explicitly includes K_{d2}.
- It is claimed that our subunit exchange assay has been optimized to measure tafamidis binding and is not more physiologically relevant than other assays used to measure TTR stabilization. This is incorrect. The assay was optimized to measure TTR binding by any kinetic stabilizer and we maintain that adding a small amount (~1 μM) of a tagged serum protein to plasma samples is a much smaller perturbation than acidification to pH 4 or adding 4–5 M urea—assays that are generally less precise than subunit exchange. The author prefers our fluorescent probe exclusion (FPE) assay for kinetic stabilizer comparisons [2], which is strongly influenced by K_{d2}. Binding to the second site in TTR is not necessary to suppress TTR dissociation.
- Finally, it is claimed that our overall conclusions are not correct. This is not true. We set out to test the hypothesis that any arbitrary level of TTR stabilization can be attained by adjusting the kinetic stabilizer oral dose, which determines the plasma concentration. Our results clearly demonstrate that 1.6 g of AG10/day is required to get comparable stabilization to that achieved by 61 mg of tafamidis/day.

Ultimately, the therapeutic efficacy of kinetic stabilisers for TTR can only be determined by clinical trials. The point of our paper is that while AG10 is ≈ 4 times more potent from a purely biochemical perspective, it has to be dosed at 26 times higher levels and more frequently to make up for AG10's pharmacologic deficiencies—the hope is that AG10's high mean peak plasma concentration and prominent metabolite will not create safety issues.

Disclosure statement

JWK and ETP discovered tafamidis and receive sales royalties and sales milestone payments.

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Received 13 November 2020; accepted 16 November 2020