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Letters to the Editor


2. Tsikas D, Zoerner AA, Mitschke A, Gutzi K, Morin LM, Dufort M, Liu G, et al. Nitro-fatty acid nitroalkenes,1 including the comment from Tsikas et al. This study is based on a high-fat diet (HFD)-induced obesity murine model that displays the canonical features of metabolic syndrome, including glucose tolerance, vascular dysfunction, increased inflammation, adipokine dysfunction, and most notably development of pulmonary hypertension. We tested the pharmacological actions of synthetic 11-nitro-octadec-9-enoic acid (OA-NO2), and reported potent protective actions of OA-NO2 in this murine model of HFD-induced obesity and pulmonary hypertension. First and foremost, we stress that under no circumstance did we state or imply the existence of any link between obesity and decreased OA-NO2 biosynthesis. This is an incorrect and misleading interpretation of the data presented in our manuscript.

The letter from Tsikas et al. presents new data indicating no difference in endogenous levels of OA-NO2 in lean, obese individuals, and obese Type 2 diabetics. We read this letter with interest as multiple groups including our own are striving to determine the mechanism(s) of endogenous nitro-fatty acid (NO2-FA) formation, to define the metabolism of these species and their products, and to understand how these mediators modulate signalling pathways in vivo. While the data provided by Tsikas et al. are intriguing, little analytical information was provided which greatly limits interpretation. Considering these concerns, we view this work should be judged on its merit and in its entirety in a peer-reviewed process.

In further clarification of points raised by Tsikas et al. regarding our recent report, we disagree with Tsikas et al.’s opinion that OA-NO2 is a ‘weak’ drug. This pharmacological term was used inappropriately, and in the peer-reviewed literature our group and others have reported that (i) the nitroalkene substituent confers electrophilic reactivity to a broad array of molecules and (ii) low concentrations of OA-NO2 exhibit significant pleiotropic anti-inflammatory actions in a wide range of animal models of metabolic and inflammatory diseases. Moreover, we observed no signs of toxicity at the concentration used (8 mg/kg/day) in the HFD-fed mice. These concentrations are significantly less than those considered safe in rodent, canine, and primate models, from recent preclinical pharmacokinetic and toxicology studies.

Notably, Tsikas et al. claim that OA-NO2 has an inert vinyl functionality2 and suggest a minor or no reactivity towards nucleophiles such as cysteine. Reaction kinetics studies refute this unsubstantiated view and support that OA-NO2 rapidly reacts with biological thiols (ε(C-SH) ≈ 183 ± 6 M⁻¹ s⁻¹) compared with 1 M⁻¹ s⁻¹ for 4-hydroxy-2-nonenal and 15-deoxy-a12,14-prostaglandin J2.3 For the example, the concentration of reduced Cys34 in the albumin component of human serum is 0.45 mM, and OA-NO2 has an equilibrium constant for the reaction with cysteine (Kd) of 7.5 × 10⁻⁶ M.5 These physical properties are consistent with the observation that OA-NO2 rapidly forms protein adducts via Michael addition when added to plasma samples and when present in the vascular compartment. In this regard, we express concern that the analytical method used by Tsikas et al.1,6 does not consider this Michael addition reaction, additional nitroalkene reactions, and acidic nitration, all of which can lead to inaccuracies in the determination of NO2-FA species. Overall, we view that the quantification of reactive (thus metastable) fatty acid nitroalkenes should be reported in a way that benefits the scientific community, with documented methods and controls and not via methodologically incomplete letters.

In summary, the goal of our study was to test the therapeutic efficacy of the exemplary fatty acid nitroalkene OA-NO2 in a model of HFD-induced obesity and pulmonary hypertension. Ongoing studies explore endogenous formation of this and other more predominant NO2-FA species, their protein adducts, complex lipid species, and their metabolites—in both murine models and clinical samples. We are grateful that other laboratories are also pursuing a fruitful area of cell signalling and pharmacological research.

References


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Nitro-oleic acid and epoxoyleic acid are not altered in obesity and Type 2 diabetes: reply

We appreciate the interest in our recent study of the in vivo signalling actions of electrophilic fatty acid nitroalkenes,1 including the comment from Tsikas et al. This study is based on a high-fat diet (HFD)-induced obesity...