



Letter

Author's response to “platelet antioxidants: A conundrum in aging”



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We appreciate Iyer and Dayal's insightful comments, and interest in our recent study [1]. They raise thoughtful and important points about the documented higher thrombotic risk in the very old age. However, the increase in platelet reactivity with age in our 60–79 age cohort, and its relative decline in the 80 and above aged elderly cohort, does not contradict the known increase in risk of thrombosis with age [2]. Hemostasis, is a complex interplay between platelet reactivity, tissue injury/damage, and coagulation/fibrinolysis. Higher levels of FVIIIa, FIXa and fibrinogen have been documented in the elderly [3,4]. Moreover, centenarians have been shown to have higher levels of factor X activation peptide, prothrombin fragment 1 + 2, thrombin/antithrombin complex and fibrinopeptide A, all of which promote thrombosis, with no increase in the anticoagulant proteins such as Protein C, S, TFPI [5]. Thus, decreased platelet reactivity in advanced age (both human and mice) [1] is likely a beneficial adaptive response, important in counterbalancing the hypercoagulation state (due to the increased procoagulant proteins) in the elderly. Further follow up studies are needed to dissect the relative contributions of platelets and other hemostasis components in the elderly.

As far as we are aware, our study is the first longitudinal mouse study with regular blood sampling and analysis of platelet redox biology from an early age to death. As suggested by Iyer and Dayal, the changes in platelet antioxidants with age are likely adaptive. Our data from the platelets of a randomly selected group of mice, followed over a period of 12 months, supports an interplay of both adaptive as well as selective mechanisms, which may also play a role in survival. Consistent with the cross-sectional mice (and human) results, we observed that platelets from individual mice showed a progressive drop in antioxidants as they aged to 12 months. However, as their age advanced over 14 months of age, there was a notable increase in catalase and SOD activity, along with a concomitant decline in ROS and sP-selectin levels (a likely mechanism to counter the increasing oxidative stress). However, consistent with the earlier reports [6], our results also show that ROS and sP-selectin levels are elevated in 18 month mice as compared to the 6-month old mice, when considered at the single respective time points. As suggested by Iyer and Dayal, there could very well be

an interplay of the cellular antioxidants to counterbalance the activity of each other and restore platelet redox homeostasis. Interestingly, the ability to mount this adaptive switch at the 12–14 month of age, also appears to be associated with survival. As described [1], when traced over consecutive months, the mice who survived longer had relatively higher platelet antioxidants (measured at 18 months of age) when compared with the mice that died.

Regarding the concerns about ROS measurement, the assay using the cell permeable DCFH-DA dye is a recognized method used by many groups [6–8]. Although the assay itself forms a comparative rather than an absolute measurement for ROS levels, concurrent measurements of protein carbonylation, lipid peroxidation and the altered GSH/GSSG ratio confirmed the increased oxidative stress in our samples. Also highlighted by Iyer and Dayal, while the average lifespan of the C57BL/6 J mice has been documented to be 24–28 months [9], the median survival for the mice in our longitudinal studies was 18–22 months. This could indeed be due to the stress of repetitive bleeding, and/or age-related inflammation, which was consistent for the all the mice in our cohort. Moreover, the impact of environmental conditions in an independent animal facility, especially in the highly inbred strains, on mice lifespan cannot be overlooked.

The consistency of our core observations between mice and human platelets in aging, emphasizes common fundamental principles governing the finely controlled hemostatic pathways. While our recently published study provides new insights to better understand the conundrum of platelet antioxidants in aging, more studies are clearly needed to further explore the underlying mechanism for such adaptive responses.

Disclosures

The authors declare no conflicts of interest.

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