Physiological Plasma Gas6 Levels Do Not Influence Platelet Aggregation

To the Editor:

Gas6 protein is a vitamin K–dependent protein originally identified as an apoptosis regulator.1 Gas6 binds to Axl, Mer, and Sky, 3 receptors of the tyrosine kinase subfamily, and was recently implicated in platelet aggregation.2 Gas6 knockout mice showed impaired aggregation in response to weak activation with agonists such as 5 μmol/L ADP, 10 μmol/L U46619, and 2 μg/mL collagen. Similarly, loss of any Gas6 receptor impaired the stabilization of platelet aggregates,3 and both Gas6 and Gas6 receptor knockout mice were protected from venous and arterial thrombosis. Gas6 was found to be located in α-granules of murine platelets and to be released during platelet activation.2,4 Data on human platelets are less clear cut. Indeed, preincubation of human platelet-rich plasma (PRP) with an anti-Gas6 antibody inhibited platelet aggregation and secretion responses to both 5 μmol/L ADP and the protease-activated receptor-1 (PAR-1)–activating peptide SFLLRN (20 μmol/L) by >80%.5 Using several approaches, Balogh et al found that Gas6 was contained in human blood plasma but not in human platelets, suggesting that in humans, the putative role of Gas6 in platelet aggregation may be played by Gas6 originating from the circulation.6

Here, we examined whether human platelet aggregation was influenced by physiological Gas6 plasma levels. Samples from 100 healthy male volunteers 18 to 35 years of age (mean 24.3±3.7 years) were used for platelet aggregation studies and plasma Gas6 assay. Gas6 levels were determined by ELISA as described previously7 and were expressed as a percentage of the Gas6 level in a pool of normal plasma. PRP aggregation studies were performed as described previously8 with the following agonists: ADP, collagen, arachidonic acid (AA), U46619, and SFLLRN peptide. The results were expressed as the maximum percentage increase in light transmission over that of the initial platelet suspension, relative to that of autologous platelet-poor plasma (arbitrarily 100%). The collagen lag time was also collected. Each subject was studied twice, at a 1-week interval, and the platelet aggregation results were averaged as described previously.9 There was a good concordance between plasma Gas6 levels in each subject’s paired samples (r=94%; P<0.001), and the mean values between the 2 visits were therefore used. Plasma Gas6 levels ranged from 50.5% to 197.5% (median 81%).

Because the previously reported effects of Gas6 were observed only in conditions of weak activation, we performed platelet aggregation tests with similar or even lower agonist doses.2,5 The Spearman correlation test showed no relationship between the plasma Gas6 level and the platelet response to any of the agonists tested. Plasma Gas6 levels did not correlate with maximal aggregation to 2 μmol/L ADP, 1 μg/mL collagen, 1 mmol/L AA, 1 μmol/L U46619, 10 or 15 μmol/L SFLLRN peptide 7, or the collagen lag time.

These results show that plasma Gas6 levels in healthy subjects do not influence platelet aggregation ex vivo and suggest that they do not reflect platelet susceptibility to activation. The mechanism by which the Gas6 pathway modulates platelet aggregation remains elusive.

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