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This Week's Citation Classic®

Kaplan K L, Broekman M J, Chernoff A, Lesznik G R & Drillings M. Platelet α-granule proteins: studies on release and subcellular localization. *Blood* 53:604-18, 1979. [Dept. Medicine, Coll. Physicians & Surgeons, Columbia Univ.; Dept. Medicine, Cornell Univ. Medical Coll.; and Dept. Medicine, New York Veterans Admin. Hosp., New York, NY]

This paper describes the localization of four secreted platelet proteins (platelet factor 4, β-thromboglobulin, fibrinogen, and the platelet-derived growth factor) to the α-granule fraction of platelets. The ability of several agents to induce secretion of α-granule proteins and the effects of cyclooxygenase inhibitors on secretion are also described. [*The SCI® indicates that this paper has* been cited in over 215 publications.]

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My interest in secreted platelet proteins began when I joined the laboratory of the late Hymie Nossel. My task in the laboratory was to develop a radioimmunoassay for platelet factor 4 (PF4),[1] to use together with the radioimmunoassay for fibrinopeptide A (the first peptide cleaved from fibrinogen by thrombin) to try to detect thrombotic events in patients. We felt that a measure of platelet activation might add sensitivity and specificity. When another secreted platelet protein was described, β-thromboglobulin (βTG),[2] this protein was purified in our laboratory as well, and specific and sensitive radioimmunoassays were developed for both PF4 and βTG.[3] Platelet α-granules had recently been distinguished from platelet lysosomes,[4,5] and there was a report that PF4 and fibrinogen were localized in the α-granules.[6] In that report, however, PF4 was assayed by its heparin-neutralizing activity, and it was not clear whether other platelet proteins might contribute to the heparin-neutralizing activity.

Thus, with specific assays for PF4 and βTG available, it was decided to investigate their subcellular localization, using the method of subcellular fractionation developed by Broekman *et al.*[5] Han Broekman agreed to collaborate in such a study, so we proceeded to isolate and fractionate platelets and to assay the various fractions for PF4 and βTG. Additionally, we were able to thrombin-treat the fractions and to assay them for fibrinopeptide A as a marker of fibrinogen. Arthur Chernoff, working in the laboratory of DeWitt Goodman at Columbia, assayed the fractions for platelet-derived growth factor activity. The four proteins were shown to be localized in the fractions that were rich in α-granules, and those fractions were distinct from the dense granule fractions that contained serotonin and from the lysosomes that contained acid hydrolases.

We were also interested in determining the relationship between secretion from the dense granules and the α-granules and in comparing the characteristics of release from these granules with published reports of secretion from lysosomes. Our studies showed that α-granule release induced by thrombin, collagen, and the cyclic endoperoxide analogue (U46619) occurred at lower concentrations of agonist than did dense granule release. Since published studies had demonstrated that lysosomal enzyme release required higher concentrations of agonists than did dense granule release, it appeared that α-granules were most sensitive to release, with dense granules next, and lysosomes least sensitive.

Finally, the effect of cyclooxygenase inhibitors on α-granule release was examined. Indomethacin added to gel-filtered platelets inhibited secretion of α-granule proteins induced by low concentrations of thrombin or collagen but not by high concentrations of these agonists. Similarly, secretion induced by collagen or thrombin was only partially inhibited in platelet-rich plasma prepared from subjects who had ingested aspirin, implying some secretion independent of cyclooxygenase products. Release induced by ADP, epinephrine, and arachidonic acid was completely inhibited by aspirin, indicating complete dependence of α-granule secretion induced by these agents on cyclooxygenase activity. Aspirin ingested by normal volunteers caused a small but significant decrease in the mean plasma βTG level but no significant change in the mean PF4 level, suggesting that *in vivo* release is likely to be induced by agents such as thrombin and collagen.

It is likely that this paper has been cited frequently because it was the first to demonstrate definitively the localization of these important secreted platelet proteins in the platelet α-granules and to begin to characterize the differences between dense granule and α-granule secretion. Subsequently, several papers have confirmed these findings, and recently there have been a number of excellent papers that have used immunoelectronmicroscopy to visualize individual proteins within α-granules.[7]

1. **Kaser-Glanzmann R, Jakabova M & Lüscher E F.** Isolation and some properties of the heparin-neutralizing factor (PF4) released from human blood platelets. *Experientia* 28:1221-3, 1972.
2. **Moore S, Pepper D S & Cash J D.** The isolation and characterization of a platelet-specific β-globulin (β-thromboglobulin) and the detection of anti-urokinase and anti-plasmin released from thrombin-aggregated washed human platelets. *Biochim. Biophys. Acta* 379:360-9, 1975. (Cited 165 times.)
3. **Kaplan K L, Nossel H L, Drillings M & Lesznik G.** Radioimmunoassay of platelet factor 4 and β-thromboglobulin: development and application to studies of platelet release in relation to fibrinopeptide A generation. *Brit. J. Haematol.* 39:129-46, 1978. (Cited 130 times.)
4. **Siegel A & Lüscher E F.** Non-identity of the α-granules of human blood platelets with typical lysosomes. *Nature* 215:745-7, 1967.
5. **Broekman M J, Westmoreland N P & Cohen P.** An improved method for isolating α-granules and mitochondria from human platelets. *J. Cell Biol.* 60:507-19, 1974. (Cited 75 times.)
6. **Broekman M J, Handin R I & Cohen P.** Distribution of fibrinogen and platelet factors 4 and XIII in subcellular fractions of human platelets. *Brit. J. Haematol.* 31:51-5, 1975. (Cited 75 times.)
7. **Kaplan K L.** Platelet α-granule secretion. (Holmsen H, ed.) *Platelet function and metabolism.* Boca Raton, FL: CRC Press, 1986. Vol. I. (In press.)

