

Comments, Opinions, and Reviews

Platelet Secretory Products May Contribute to Neuronal Injury

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Background: We do not fully understand the mechanisms for neuronal damage following cerebral arterial occlusion by a thrombus that consists mainly of platelets. The view that certain endogenous substances, such as glutamate, may also contribute to neuronal injury is now reasonably well established. Blood platelets are known to contain and secrete a number of substances that have been associated with neuronal dysfunction. Therefore, we hypothesize that a high concentration (approximately several thousand-fold higher than in plasma, in our estimation) of locally released platelet secretory products derived from the causative thrombus may contribute to neuronal injury and promote reactive gliosis.

Summary of Comment: We have recently been able to report some direct support for this concept. When organotypic spinal cord cultures were exposed to platelet and platelet products, a significant reduction in the number and the size of the surviving neurons occurred in comparison with those in controls. We further observed that serotonin, a major platelet product, has neurotoxic properties. There may be other platelet components with similar effect.

Conclusions: The hypothesis of platelet-mediated neurotoxicity gains some support from these recent in vitro findings. The concept could provide a new area of research in stroke, both at the clinical and basic levels. (*Stroke* 1991;22:1448–1451)

Our understanding of the platelet has evolved from a time when it was considered an artifact in a blood smear,¹ to the present time, when so much is known of its role in hemostasis that some may view this as its only function. In carrying out its hemostatic function, the platelet can also contribute to in vivo propagation of thrombosis, often with devastating clinical consequences such as cerebral thrombosis.² Platelets, despite their small size and anuclear status, have numerous metabolically active functions. Within their cytoplasm are storage organelles, such as dense and α -granules that contain a variety of biologically active substances, including serotonin (5HT) and calcium.³ The organellar contents of the platelet are secreted by an energy-dependent process without loss of other subcellular compartments and with simultaneous generation of thromboxane A₂.³ During the platelet secretory process, a contractile wave of circumferential microtubules centralize the organelles so that their contents are secreted into a canalicular system for release to the exterior.⁴ The granule membrane is

probably recycled by incorporation into the platelet plasma membrane. The platelet products released after stimulation with physiological agonists such as collagen^{5,6} cause local vasoconstriction,^{7–9} endothelial injury,¹⁰ and increased vascular permeability. Furthermore, platelet factor 4, an α -granule protein, has been shown to permeate the de-endothelialized vessel wall.¹¹ Many of the substances secreted by the platelet have either direct actions on cells, including the platelets themselves, or are converted into physiologically more active substances in plasma or on cell surfaces.¹²

We¹³ and others¹⁴ have shown that platelets are the major cellular structure within an acute thrombus. The concentration of platelet secretory products at the site of thrombus formation may be several thousand-fold the concentration in plasma. For example, 50% of an occluding platelet plug is comprised of platelets, and the number of platelets in a unit volume of this plug is estimated to be about 190 times that in the same unit volume of whole blood.¹⁵ We have determined that the concentration of free 5HT in human whole blood before activation of platelets is 36 nM, and the total amount of 5HT after activation of platelets with collagen is 836 nM. Theoretically, the amount of 5HT released by one milliliter of platelet plug, assuming that all the platelets in the plug have been activated, is about 4,000 times the concentration of free 5HT in one milliliter of blood

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[$190 \times (836 - 36) / 36 = 4,222$]. Thus, we suspect an abundant secretion of platelet products at the site of an acute cerebral thrombosis. Conceivably, these products cause further neuron dysfunction upon diffusion into the surrounding neural tissue through a locally disrupted blood-brain barrier. This communication offers evidence in support of this hypothesis. We do recognize that the mechanism(s) of neuronal injury in thrombotic stroke may be more complex and that several other factors may be involved. For instance glutamate, which has been shown to be neurotoxic, is released in large amounts by ischemic neurons. Other factors such as leucocytes, which contain potentially toxic substances, have been shown to concentrate in ischemic regions, although in smaller numbers.¹⁶

During the acute phase of ischemic stroke caused by cerebral thrombosis, the neurological deficit is often greater than can be explained by the size of the lesion delineated even by sophisticated imaging techniques.^{2,5} This is particularly true in patients with brain stem infarction and stroke-in-evolution, in which the clinical deficit during the acute phase is alarming although neuroimaging shows little or no abnormality.^{2,5} At present there is no convincing explanation for this discrepancy. With the knowledge that the integrity of the blood-brain barrier is disrupted at the site of cerebral ischemia, it is conceivable that platelet secretory products released from the causative thrombus diffuse into the neighboring brain parenchyma. Experiments in gerbils have shown that during cerebral ischemia, the blood-brain barrier is disrupted at 5 hours (the earliest recorded time period) after induction of cerebral ischemia, is most pronounced at 72 hours, and returns to normal levels at 1 week.¹⁷ Therefore, the time course of blood-brain barrier disruption is compatible with the hypothesis that locally released platelet secretory products may have access to surrounding neurons under acute ischemic conditions. In this study, the authors also show that the blood-brain barrier, under ischemic conditions, becomes permeable to a number of biogenic amines, including 5HT. We have investigated the relation between the extent of platelet activation and acute cerebral infarction in stroke patients and have observed increased platelet activation based on platelet $[Ca_i^{2+}]$ measurement¹⁸; increased platelet secretion, determined by ATP release from dense granules⁶; and a reduced α -granule content.¹⁹ Our finding of maximal platelet activation at 36–72 hours after the onset of stroke in these patients corresponded well with the initial clinical profile observed by others, who saw clinical worsening of stroke over a similar period of time.²⁰

A consideration of thrombotic thrombocytopenic purpura also evokes further evidence for our concepts. This often life-threatening condition is characterized clinically by the presence of thrombocytopenia and neurological dysfunction. Central nervous system abnormalities occur in more than 90% of patients, and nearly half initially present with neuro-

logical complaints.²¹ Adams et al,²² in their neuropathological study of thrombotic thrombocytopenic purpura, observed agglutinated platelets in cerebral microvessels, perivascular foci of nerve cell damage, and, in places, glial proliferation and petechial hemorrhages. Alteration of neurons in small foci around the thrombosed vessels was a common finding. The neurons were usually faintly stained and had pale bluish cytoplasm and nuclei suggestive of degeneration. Disruption of the blood-brain barrier in the cerebral vessels containing the platelet thrombi has also been observed.²³ Although the rarity of cerebral infarction distal to an occluding thrombus in thrombotic thrombocytopenic purpura may be explained by good collateralization, this does not fully explain the perivascular neuronal changes. There remains the possibility that platelet secretory products derived from luminal thrombi cause the damage to the surrounding neurons.

In addition to the observations that platelet aggregates form the major part of an arterial thrombus,^{13–15} there is experimental evidence that platelets actually accumulate at the site of focal cerebral ischemia as a secondary phenomenon.^{24,25} This takes place even at focal areas of ischemia produced by air emboli.²⁴ Thromboxane A_2 , a platelet-specific prostaglandin, is a major product released by platelets upon activation.²⁶ The amount of thromboxane A_2 found at the site of thrombus formation is largely derived from the platelet. There is a specific increase in the amount of thromboxane A_2 , measured as its stable metabolite thromboxane B_2 , at the site of ischemic lesion in the spinal cord.^{27,28} The degree of neurological deficit in the acute phase and the extent of neuropathological damage correlated with the thromboxane concentration at the site of spinal cord ischemia.²⁷ Similar findings of elevated thromboxane levels in models of focal^{29,30} and global^{31–33} cerebral ischemia provide some support for our hypothesis.

There is also convincing evidence from studies in baboons^{34,35} that 5HT, a major platelet product, can cause neuronal dysfunction. Platelets contain more than 90% of the total amount of 5HT present in blood³⁶ and, like neurons, have 5HT₂ receptors that are blocked by specific antagonists.³⁷ When 5HT was injected into a cerebral circulation with osmotically disrupted blood-brain barrier, there was suppression of neuronal metabolism and electroencephalographic activity.³⁴ In vitro studies have confirmed 5HT suppression of neuronal activity by neuronal membrane hyperpolarization.^{38–40}

Furthermore, there is evidence that platelets contain other substances that are toxic to neurons. Glutamate has been convincingly shown to produce damage to neurons in culture.^{41,42} This damage for the most part occurs through an interaction with *N*-methyl-D-aspartate receptors on neurons. It is believed that at least part of the neuronal damage seen in anoxia is due to the effect of glutamate released from ischemic neurons.^{43,44} It is probable that the major portion of glutamate that causes

neuronal injury comes from ischemic neurons, but intravascular infusion of glutamate apparently can result in similar damage.⁴⁵ As an extension of this latter finding, one may speculate on the significance of glutamate in platelets.⁴⁶ However, the calculated concentration that may be achieved by platelets in the vicinity of a thrombus appears small when compared with the concentration measured in ischemic brain tissue.⁴⁷

Recent studies indicate that platelets may also play a role in neurodegenerative conditions. Several groups of investigators have shown that platelet α -granules contain and secrete peptide fragments of the β -amyloid precursor protein, similar to that seen in Alzheimer's neuritic plaque.^{48–52} Furthermore, Yankner et al report that β -amyloid is directly toxic to differentiated neurons.^{53,54} Although, the source and the mechanism of β -amyloid deposition in Alzheimer's neuritic plaque are unknown, it is possible that the platelet α -granule is the source. Whether the β -amyloid in platelets has any role in the neuronal injury from ischemia is a question that deserves study.

In addition to possibly contributing to acute neuronal injury, platelet secretory products may be involved in stimulating gliosis. Platelet α -granules are known to contain several growth-promoting peptides such as platelet-derived growth factor^{55,56} and transforming growth factor- β ⁵⁷ that regulate mitosis in connective tissue cells. Of these factors, platelet-derived growth factor has been studied most extensively.⁵⁸ This peptide is released from platelets during thrombus formation and has been convincingly shown to cause proliferation of fibroblasts and smooth muscle cells.⁵⁸ In fact, specific receptors for platelet-derived growth factor are known to be present on connective tissue cells.⁵⁸ In the central nervous system, platelet-derived growth factor has been shown to stimulate metabolism⁵⁹ and proliferation of human glial cells⁶⁰ but not of neurons.⁶⁰ Therefore, it is possible that a platelet-derived growth factor released at the time of thrombosis may trigger gliosis in thrombotic stroke.

To test our hypothesis, we have recently studied the direct effect of platelets and their secretory products on central nervous system neurons.^{61,62} We used the roller-tube method to maintain explants of rat spinal cord in culture and identified their neurons by acetylcholinesterase staining. The changes in neuron number and size in the ventral horns were morphometrically quantified. Compared with the control cultures, there was a significant decrease in the number of neurons per ventral horn in the platelet-treated explants, and there was a shift toward smaller-sized surviving neurons. This effect was quantitatively and qualitatively similar with the platelet secretory product-treated explants. The results suggest that the neuronal damage from platelets is through their secretory products. Further, the results of our initial experiments with 5HT suggest that it may be one of the neurotoxic constituents of platelet secretion.⁶³ These studies may constitute the

first demonstration of a toxic effect of platelet secretory products on neurons.

In summary, we have presented clinical and experimental evidence to support the hypothesis that platelet products might contribute to neurological dysfunction beyond that attributed to vessel occlusion. Considering the large number of platelets involved in thrombus formation, it is possible that their secretory products may enhance ischemic neuronal damage.

References

1. Wintrobe MM: Platelets and megakaryocytes, in Wintrobe MM (ed): *Clinical Hematology*, ed 8. Philadelphia, Pa, Lea & Febiger, 1981, pp 355–379
2. Millikan CH, McDowell FH: Treatment of progressing stroke. *Stroke* 1981;12:397–409
3. Holmsen H: Platelet secretion, in Colman RW, Hirsh J, Marder VJ, Salzman EW (eds): *Thrombosis and Hemostasis*, ed 2. Philadelphia, Pa, JB Lippincott Co, 1987, pp 606–617
4. White JW: Anatomy and structural organization of the platelet, in Colman RW, Hirsh J, Marder VJ, Salzman EW (eds): *Thrombosis and Hemostasis*, ed 2. Philadelphia, Pa, JB Lippincott Co, 1987, pp 537–554
5. Joseph R, Welch KMA, Oster SB, Grunfeld S, D'Andrea G: A plasmatic factor may cause platelet activation in acute ischemic stroke. *Circ Res* 1989;65:1679–1687
6. Joseph R, D'Andrea G, Oster SB, Welch KMA: Whole blood platelet function in acute ischemic stroke: Importance of dense body secretion and the effects of antithrombotic agents. *Stroke* 1989;20:38–44
7. Pepper D: Macromolecules released from platelet storage organelles. *Thromb Haemost* 1979;42:1667–1672
8. Gingrich R, Hoak J: Platelet-endothelial cell interactions. *Semin Hematol* 1979;16:208–219
9. Clerck F, Van Nueten J: Platelet-mediated vascular contractions. *Biochem Pharmacol* 1983;32:765–771
10. Fugimoto T, Suzuki H, Tanoue K, Fukushima Y, Yamazaki H: Cerebrovascular injuries induced by activation of platelets in vivo. *Stroke* 1985;16:245–250
11. Goldberg ID, Stemerman MB, Handin RI: Vascular permeation of platelet factor 4 after endothelial injury. *Science* 1980;209:611–612
12. Rapport MM, Green AA, Page IH: Serum vasoconstrictor (serotonin): IV. Isolation and characterization. *J Biol Chem* 1948;174:1243–1251
13. Joseph R, Welch KMA, D'Andrea G, Riddle JM: Evidence for the presence of red and white cells within "platelet" aggregates formed in whole blood. *Thromb Res* 1989;53:485–491
14. Hovig T, Dodds WJ, Rowsell HC, Mustard JF: The transformation of hemostatic platelet plugs in normal and Factor IX deficient dogs. *Am J Pathol* 1968;53:355–374
15. Leung LLK, Harpel PC, Nachman RL, Rabelino EM: Histiocytin-rich glycoprotein is present in human platelets and is released following thrombin stimulation. *Blood* 1983;62:1016–1021
16. Hallenbeck JM, Dutka AJ, Tanishima T, Kochanek PM, Kumaroo KK, Thompson CB, Obrenovitch TP, Contreras TJ: Polymorphonuclear leukocyte accumulation in brain regions with low blood flow during the early postischemic period. *Stroke* 1986;17:246–253
17. Hervonen H, Steinwall O, Spatz M, Klatzo I: Behaviour of the blood-brain barrier toward biogenic amines in experimental cerebral ischemia. *Adv Exp Med Biol* 1980;131:295–305
18. Joseph R, Welch KMA, Grunfeld S, Oster SB, D'Andrea G: Baseline and activated platelet cytoplasmic ionized calcium in acute ischemic stroke: Effect of aspirin. *Stroke* 1988;19:1234–1238
19. Joseph R, Riddle JM, Welch KMA, D'Andrea G: Platelet ultrastructure and secretion in acute ischemic stroke. *Stroke* 1989;20:1316–1319

20. Petito CK, Feldmann E, Pulsinelli WA, Plum F: Delayed hippocampal damage in humans following cardiorespiratory arrest. *Neurology* 1987;37:1281-1286
21. Silverstein A: Thrombotic thrombocytopenic purpura: The initial neurologic manifestations. *Arch Neurol* 1968;18:358-362
22. Adams RD, Cammermeyer J, Fitzgerald PJ: The neuropathological aspects of thrombotic acroangiothrombosis: A clinico-anatomical study of generalized platelet thrombosis. *J Neurol Neurosurg Psychiatry* 1948;11:27-43
23. Bornstein B, Boss JH, Casper J, Behar M: Thrombotic thrombocytopenic purpura, report of a case presenting as a chronic neurological disorder and characterized by unusual histological findings. *J Clin Path* 1960;13:124-132
24. Obrenovitch TP, Hallenbeck JM: Platelet accumulation in regions of low blood flow during the postischemic period. *Stroke* 1985;16:224-234
25. Meyer JS: Localized changes in properties of the blood and effects of anticoagulant drugs in experimental cerebral infarction. *N Engl J Med* 1958;258:151-159
26. Moncada S, Vane JP: Pharmacology and endogenous role of prostaglandin, thromboxane A₂ and prostacyclin. *Pharmacol Rev* 1979;30:293-331
27. Jacobs TP, Shohami E, Baze W, Burgard E, Gunderson C, Hallenbeck JM, Feuerstein G: Deteriorating stroke model: Histopathology, edema, and eicosanoid changes following spinal cord ischemia in rabbits. *Stroke* 1987;18:741-750
28. Feuerstein G: Eicosanoids in deteriorating stroke: Review of studies on the rabbit spinal cord ischemia and reperfusion model. *Ann N Y Acad Sci* 1989;559:313-322
29. Gaudet R, Levine L: Transient cerebral ischemia and brain prostaglandins. *Biochem Biophys Res Commun* 1979;86:893-901
30. Gaudet R, Levine L: Effect of unilateral common carotid artery occlusion on levels of prostaglandin D_{2m}, F_{2m}, and 6-keto-PGF_{1α} in gerbil brain. *Stroke* 1980;11:648-652
31. Shohami E, Rosenthal J, Lavy S: The effect of incomplete cerebral ischemia on prostaglandin levels in rat brain. *Stroke* 1982;13:494-498
32. Minamisawa H, Terashi A, Katayama Y, Kanda Y, Shimizu J, Shiratori T, Inamura K, Kaseki H, Yoshino Y: Brain eicosanoid levels in spontaneously hypertensive rats after ischemia with reperfusion: Leukotriene C₄ as a possible cause of cerebral edema. *Stroke* 1988;19:372-377
33. Dempsey RJ, Roy MW, Meyer K, Cowen DE, Tai H-H: Development of cyclooxygenase and lipoxygenase metabolites of arachidonic acid after transient cerebral ischemia. *J Neurosurg* 1986;64:118-124
34. Harper AM, MacKenzie ET: Cerebral circulatory and metabolic effects of 5-hydroxytryptamine in anaesthetized baboons. *J Physiol* 1977;271:721-733
35. Welch KMA, Meyer JS, Teraura T, Hashi K, Shinmaru S: Ischemic anoxia and cerebral serotonin levels. *J Neurol Sci* 1972;16:85-92
36. Hardisty RM, Stacey RS: 5-Hydroxytryptamine in normal human platelets. *J Physiol* 1955;130:711-720
37. Bobker DH, Williams JT: Ion conductances affected by 5-HT receptor subtypes in mammalian neurons. *TINS* 1990;13:169-173
38. Davies MF, Deisz RA, Prince DA, Peroutka SJ: Two distinct effects of 5-hydroxytryptamine on single cortical neurons. *Brain Res* 1987;423:347-352
39. Jahnsen H: The action of 5-hydroxytryptamine on neuronal membranes and synaptic transmission in area CA1 of the hippocampus in vitro. *Brain Res* 1980;197:83-94
40. Colino A, Halliwell JV: Differential modulation of three separate K-conductances in hippocampal CA1 neurons by serotonin. *Nature* 1987;328:73-77
41. Rothman SM, Olney JW: Glutamate and the pathophysiology of hypoxic-ischemic brain damage. *Ann Neurol* 1986;19:105-111
42. Choi DW, Rothman SM: The role of glutamate neurotoxicity in hypoxic-ischemic neuronal death. *Annu Rev Neurosci* 1990;13:171-182
43. Bosley TM, Woodhams PL, Gordon RD, Belazs R: Effects of anoxia on the stimulated release of amino acid neurotransmitters in the cerebellum in vitro. *J Neurochem* 1983;40:189-201
44. Rothman S: Synaptic release of excitatory amino acid neurotransmitter mediates anoxic neuronal death. *J Neurosci* 1984;4:1884-1891
45. Lucas DR, Newhouse JP: The toxic effect of sodium-L-glutamate on the inner layers of retina. *Arch Ophthalmol* 1957;58:193-201
46. D'Andrea G, Cananzi AR, Ferro-Milone F, Joseph R, Grunfeld S, Welch KMA: Platelet levels of glutamate and aspartate in normal subjects. *Stroke* 1989;20:299-300
47. Benveniste H, Drejer J, Schousboe A, Diemer NH: Elevation of the extracellular concentrations of glutamate and aspartate in rat hippocampus during transient cerebral ischemia monitored by intracerebral microdialysis. *J Neurochem* 1984;43:1369-1374
48. Van Nostrand WE, Schmaier AH, Farrow JS, Cunningham DD: Protease nexin-II (amyloid β-protein precursor): A platelet α-granule protein. *Science* 1990;248:745-748
49. Smith RP, Higuchi DA, Broze GJ: Platelet coagulation factor XI₂-inhibitor, a form of Alzheimer's amyloid precursor protein. *Science* 1990;248:1126-1128
50. Cole GM, Galasko D, Shapiro IP, Saitoh T: Stimulated platelets release amyloid β-protein precursor. *Biochem Biophys Res Commun* 1990;170:288-295
51. Bush AI, Martins RN, Rumble B, Moir R, Fuller S, Milward E, Currie J, Ames D, Weidemann A, Fischer P, Multhaup G, Beyreuther K, Masters CL: The amyloid precursor protein of Alzheimer's disease is released by human platelets. *J Biol Chem* 1990;265:15977-15983
52. Gardella JE, Ghiso J, Gorgone GA, Marratta D, Kaplan AP, Frangione B, Gorevic PD: Intact Alzheimer amyloid precursor protein (APP) is present in platelet membranes and is encoded by platelet mRNA. *Biochem Biophys Res Commun* 1990;173:1292-1298
53. Yankner BA, Duffy LK, Kirschner DA: Neurotrophic and neurotoxic effects of amyloid β protein: Reversal by tachykinin neuropeptides. *Science* 1990;250:279-282
54. Yankner BA, Caceres A, Duffy LK: Nerve growth factor potentiates the neurotoxicity of β amyloid. *Proc Natl Acad Sci U S A* 1990;87:9020-9023
55. Ross R, Glomset J, Kariya B, Harker L: A platelet-dependent serum factor that stimulates the proliferation of arterial smooth muscle cells in vitro. *Proc Natl Acad Sci U S A* 1974;71:1207-1210
56. Assoian RK, Grotendorst GR, Miller DM, Sporn MB: Cellular transformation by coordinated action of three peptide growth factors from human platelets. *Nature* 1984;309:804-806
57. Assoian RK, Sporn MB: Type β transforming growth factor in human platelets: Release during platelet degranulation and action on vascular smooth muscle cells. *J Cell Biol* 1986;102:1217-1223
58. Ross R, Raines EW, Bowen-Pope DF: The biology of platelet-derived growth factor. *Cell* 1986;46:155-169
59. Westermarck B, Wasteson A: A platelet factor stimulating human normal glial cells. *Exp Cell Res* 1976;98:170-174
60. Besnard F, Perraud F, Sensenbrenner M, Labourdette G: Platelet-derived growth factor is a mitogen for glial but not for neuronal rat brain cells in vitro. *Neurosci Lett* 1987;73:287-292
61. Joseph R, Saroff DM, Delfs JR: Platelet secretory products have damaging effect on neurons (abstract 330). Proceedings of the American Association for the Advancement of Science, Washington, DC, February 18, 1991
62. Joseph R, Saroff DM, Delfs JR: Platelets and their products have damaging effects on neurons (abstract). *Neurology* 1991;41(suppl 1):163
63. Joseph R, Tsering C, Grunfeld S, Welch KMA: Serotonin may mediate platelet neurotoxicity (abstract). *J Neurosci* 1991;17:1093

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