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VIEWPOINT

Aspirin resistance and platelet turnover: A 25-year old issue

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Abstract The evidence of an incomplete inhibition of platelet function by aspirin, despite therapeutic doses of the drug proved to be clinically effective are employed, was first reported in the '80s, in the frame of studies devoted to platelet turnover. Because inhibition of platelet aggregation by aspirin is irreversible, the return after an interval of time of the ability to form thromboxane by platelets in circulating blood should reflect the entry into the circulation of platelets whose cyclooxygenase activity has not been affected by aspirin. Based on this concept, the possibility of monitoring the entry of newly formed platelets into the circulation after aspirin ingestion was documented by measuring the return of thromboxane biosynthesis by platelets challenged *in vitro* by pairs of aggregating agents. The data obtained showed that platelets with intact cyclooxygenase activity could be detected into the circulation of control individuals as early as 4–6 h after aspirin ingestion, and at shorter time intervals in diabetic angiopathy. In the latter setting,the data allowed to conclude that "schedules of aspirin which may suffice in normals are not effective in patients with diabetic angiopathy, presumably because these patients have a high rate of entry of new platelets into the circulation".

To the memory of Scott Murphy and Melvin J.Silver

"Those who cannot remember the past are condemned to repeat it".

George Santayana, The Life of Reason, Volume 1, 1905

"...As you may know, human platelet concentrates stored at room temperature for transfusion purposes, although functionally useful in vivo, progressively lose their aggregation potential in vitro in response to even high concentrations of aggregating agents. The reasons for this are unknown. Recently, a paper by Dr Scrutton's group has appeared in which the Authors imply that, rather than to higher concentrations of single agents, platelet activation in vivo is likely to occur in response to low concentrations of combinations of aggregating

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agents. Mel and I agree that you should explore in detail this area of uncertainty during your research fellowship here at Cardeza."

This was the ending of the invitation letter to join his lab at Cardeza Foundation for Hematological Research in Philadelphia (PA) starting on January 1981, that Scott Murphy sent me when I was a fellow at Mario Negri Institute. I had already worked with Melvin Silver in Milan [1] where he had spent a sabbatical year (1977/8) with Giovanni de Gaetano and Maria Benedetta Donati.

The concept that Scott was alluding to [2] was very fruitful not only with respect to the specific issue: in keeping with the observation that stored platelets retain full aggregation potential in response to low concentrations of aggregating agents in combination [3], the synergistic effects of aggregating agents (e.g. epinephrine and collagen) was documented both *in vitro* in mouse platelets (rodents are indeed very resistant to thrombosis and their platelets do not aggregate *in vitro* in response to epinephrine employed alone), and *in vivo* (mice died from thrombosis following a tail vein injection of 150 μg of collagen plus 1.8 μg of epinephrine) [4]. Of note, aspirin protected mice from the thrombotic challenge, in that model system.

In the frame of their Cardeza project "Thrombokinetics without radioisotopes", Pat Catalano and J. Bryan Smith had reported a discrepancy between the entry of new platelets into the circulation (as determined by monitoring the return of thromboxane in serum, after the ingestion of 100 mg aspirin) and the disappearance of radiolabelled platelets from the circulation (turnover) [5]. Their data implied that the differing patterns observed using these two strategies were largely accounted for by the different experimental conditions employed. Based on the data achieved on stored platelet concentrates as well as in mice, the possibility of monitoring the entry of new platelets into the circulation in platelet-rich plasma (PRP) challenged by combinations of agents was tested [6]. In the course of the study, it was found that, while entirely insensitive to 1 mM arachidonic acid (AA), platelets from some subjects that had ingested 100 mg aspirin 4–6 h before, were able to form thromboxane, to aggregate and to secrete nucleotides in response to lower concentrations of combinations of AA with other aggregating agents. *In vitro* addition of indomethacin (20 μM) to such platelets suppressed thromboxane formation and, in parallel, aggregation and nucleotide secretion. Because inhibition of platelet aggregation by aspirin is irreversible, the return after an interval of time of the ability to form thromboxane by platelets in circulating blood should reflect the entry into the circulation of platelets whose cyclooxygenase activity had not been affected by aspirin. Thus, the data were taken to suggest that in some conditions, platelets with intact cyclooxygenase activity may appear into the circulation as early as 4–6 h after aspirin ingestion. Since platelets turnover in 7–10 days, these data were in keeping with the observations that: a) 10% of platelets (in PRP) with intact cyclooxygenase activity sufficed to overcome the inhibitory effect of aspirin on the aggregation of platelets [7], and b) 10% of normal PRP corrected the lack of aggregation in response to AA of platelets from a patient with cyclooxygenase deficiency, the addition of epinephrine dramatically potentiating such AA-induced aggregation [8]. While helping explain why patients with platelet cyclooxygenase deficiency have mild/absent bleeding symptoms, these data had obvious implications with respect to platelet consumption by injured vessels. A higher than normal rate of entry of platelets into the circulation had been documented in several clinical settings marked by a high risk of atherothrombotic complications e.g. in diabetic patients with angiopathy [9–11].

Shortly after my return to Naples (1984), Mel Silver (who got interested in the pair inducersissue after carefully following the mice experiments) planneda second sabbatical year in Italy, this time in my University Department. In our diabetic Clinic, there was a great interest to define the optimal amount of aspirin to give to these high-risk individuals. The possibility of a trial with aspirin in diabetic angiopathy to be held during Mel's sabbatical was thus planned WITH Pierluigi Mattioli. Aggregation, ATP secretion and thromboxane formation were detected in PRP samples from diabetics collected 2–15 h after ingestion of 100 mg aspirin in single daily dose, following a challenge with AA (1 mmol/L) plus collagen (1 μg/mL). Doses of 330 mg or 1 g aspirin gave similar results [12]. Comparable data were achieved when blood was collected from a group of diabetic subjects 6 h after the ingestion of the last dose of aspirin, in a 4-time-daily regimen (25 mg of aspirin). The return of malondihaldeyde, a reliable index of AA metabolism by cyclooxygenase, after cessation of the regimen of a single daily dose of 100 mg aspirin for 1 month, indicated that the time at which circulating platelets had recovered 50% of their ability to form such metabolite was ≈4.5 days in controls and ≈2 days in diabetic patients. Thus, the data [12] were interpreted to indicate that "schedules of aspirin which may suffice in normals are not effective in patients with diabetic angiopathy, presumably because these patients have a high rate of entry of new platelets into the circulation". In such cases, a long-lasting suppression of thromboxane biosynthesis "may be achieved by low-dose, slow-release preparations of aspirin."

A manuscript, entirely similar to the one that was published in 1986 in the April issue of Blood, had been previously submitted to a more general Journal. One month later, the corresponding Author (Mel) received a non-formal letter by the Editor of the Journal: he regretted that the paper had to be rejected based on a series of considerations summarized in the comments of the Reviewers, and suggested to send it to a more specialized journal that would have been very interested in publishing such important data. While the first Reviewer raised questions on the clinical meaning of the synergistic effects of agonists in human platelet activation, the main comment of the other Reviewer was that, while single low-dose aspirin was becoming the standard treatment in the prevention of the recurrence of ischemic events, our report was likely to bring confusion in the area. In view of this "*I would not publish it*" the Reviewer concluded. Mel's accompanying letter to me from Philadelphia was quite sharp: "*The liner ran down a fishing boat in the dense fog! As you see, rather than by its potential inherent limitations* (the sample size, only a limited number of individuals had concluded this very complex aspirin trial*), the paper and its message have been killed by the one-handed judgment of the Reviewers*