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Salicylate effect on platelets and vascular thrombosis in rheumatoid arthritis

Gilbert B. Bluhm, MD,* Jeanne M. Riddle, PhD,** Donald G. Pica, MD and Gordon D. Langejans, MD***

Platelet surface activation was surveyed by electron microscopy (EM) in 40 patients with classical rheumatoid arthritis (RA) without clinical evidence of recent intravascular coagulation. Increased platelet surface activity (PSA) was noted in 8 of 25 females and 8 of 15 males (16 of 40 or 40%). Abnormal PSA failed to correlate with either the presence of rheumatoid nodules or the titer of rheumatoid factor. Hyperactive platelet populations, however, did tend to correlate with a serum urate level above 5 mg% (7 of 12 vs 9 of 28). Neither “low” nor “anti-inflammatory” levels of serum salicylate appear to afford protection from abnormal PSA.

The authors consecutively reviewed 100 patients with classical RA diagnosed and followed at Henry Ford Hospital (72 females and 28 males) to determine their incidence of thrombotic episodes. The mean age of the group was 59 years and the mean duration of follow-up was 14 years. They found 43 clotting episodes in 30 patients (21 females). There were 14 myocardial infarctions (5 in females); 19 episodes of thrombophlebitis (12 in females); 4 pulmonary embolisms (females); 5 cerebral vascular accidents (4 in females); and one female with peripheral arterial occlusive disease. No significant correlation with rheumatoid nodules, stage of disease progression or functional class of disease was noted when those patients with thromboembolic complications were compared to the others who manifested no clinical thrombosis. This study suggests that in vivo salicylate fails to inhibit PSA as determined by EM and by retrospective review of 100 patients who used prolonged daily dosage of salicylate. The thrombotic episodes were as frequent as in general population surveys, except for data from a cerebral vascular (stroke) survey.
Clinical investigation has documented the ability of acetylsalicylic acid (ASA) to influence hemostasis. ASA prolongs the bleeding time in normal individuals while other studies indicate that platelet aggregation can be inhibited by both ASA and sodium salicylate. Platelet release activity is diminished by ASA in man. Because aspirin has antithrombotic potential, studies are underway to assess its benefit in the prevention of vascular thrombosis.

Accepting the role of the platelet in thrombosis we asked the question, “can the daily use of ASA beneficially influence the patency of blood vessels?” The long term use and consequent benefit of ASA was suggested when the drug’s effect on platelets from patients using from 0.6 to 5.3 grams of aspirin daily for three weeks to two years were contrasted with the platelet data of a control group who had not taken ASA for at least three weeks. Serotonin release from platelets was abolished and secondary aggregation did not occur with epinephrine in those patients using aspirin.

The purpose of this paper is to report the state of platelet activation as determined by electron microscopy (EM) in 40 patients with classical rheumatoid arthritis (RA) who exhibited no clinical evidence of recent intravascular coagulation; and to report the prevalence of thrombotic episodes in 100 patients with classical RA diagnosed and followed at Henry Ford Hospital.

Patient selection

Each patient chosen for a platelet evaluation had classical RA, was ambulatory and participating in a gold injection program, did not require any other anti-inflammatory agent except ASA, and gave no history or clinical evidence for recent intravascular thrombosis. Some patients were deliberately selected who were taking regular daily doses of ASA varying between 2.6 to 4.2 grams, while others were using a smaller dosage or none at all. Also, some patients were selected because they exhibited subcutaneous rheumatoid nodules.

When the specimen for evaluation by electron microscopy was obtained, blood was also taken from the same venipuncture for the following determinations: uric acid, salicylate, blood urea nitrogen, rheumatoid factor titer, complete blood count and platelet count per mm.

For the retrospective review, patients with classical RA were chosen consecutively from a group diagnosed at Henry Ford Hospital (HFH) during the years of 1950-1955. Their selection was based upon prolonged care at Henry Ford Hospital so that a reliable assessment could be made of any other health problems which had occurred since the onset of their rheumatoid arthritis, particularly vascular complications. The details of each medical record were reviewed and data pertinent to the study were tabulated from 100 patients. The regular use of salicylate by these patients was documented especially.

Results

Platelets: Total platelet counts were performed on each of the 40 patients surveyed and ranged from 160,000 to 457,500/mm. Only two of the platelet counts were under 200,000 and six exceeded 400,000. The blood urea nitrogen was within the normal range (normal up to 25 mg%) with one exception, and it was increased to 30 mg%.

Differential counts by EM: We determined surface activation by employing a technique described in detail elsewhere. The method can be described briefly as follows: A sample of whole blood is drawn using non-wettable equipment. The blood specimen is introduced immediately into a vessel containing a microscope slide coated with Formvar and an appropriate amount of 3.8% sodium citrate to prevent clotting. The specimen is incubated at 37°C for exactly eight hours.
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Figure 1
Round disc shaped platelets and a predominance of dendritic forms, the vast majority of which comprise a normal differential count as determined by electron microscopy. X 9200

Figure 2
Abnormal platelet surface activation is noted by the increased numbers of spread forms and/or aggregates which are illustrated above. X 5700
minutes during which time the platelets adhere to the surface of the Formvar. The slide with adherent platelets is removed, washed in Tyrode’s solution, fixed in buffered osmium and washed in distilled water. Copper grids are then placed on the Formvar surface of the glass slide where the platelets have adhered. Scotch tape is used to strip the copper grids and Formvar film with its adhering platelets from the slide. The specimens are then ready for study by transmission EM.

The platelet morphology (PM) for normal individuals consists of a few round, disc-shaped platelets and a predominance of dendritic type platelets (Figure 1). During the identification and recording of 100 single platelets, an average of 68 ± 50 small platelet aggregates are seen normally. Abnormal platelet surface activation is demonstrated by the occurrence of an increased number of spread form platelets (Figure 2) at a percentage greater than 19 and/or platelet aggregates numbering more than 119. These values are consistent in our experience regardless of age or sex.

Increased platelet surface activation (PSA) was noted in 16 of 40 patients (40%). We found abnormal PSA in 8 of 25 females and 8 of 15 males. There were four patients who manifested abnormal PSA which was expressed by an elevated percent of spread platelet forms alone. Two other patients exhibited an increased number of small platelet aggregates, but a normal number of spread platelets. However, both parameters were increased in the other 10 patients who showed abnormal PSA. Indeed, this has been our experience also in an evaluation of 95 patients with primary gout where 49 (51%) were found to have abnormal PSA and the majority of these exhibited both increased numbers of spread forms and aggregates.

Platelet morphology (PM), Rheumatoid factor (RF) and Rheumatoid nodules: Because RF is a macroglobulin and large molecular molecules have been shown to inhibit PSA, we compared platelet morphology with the titers of RF. Rheumatoid factor titer was determined by the sensitized sheep red blood cell agglutination test after the test serum was absorbed overnight with sheep red blood cells. A titer of 80 or less is negative. In a group of patients with RA, 70% should have a positive titer and our selected group of patients matched well since a positive titer was present in 29 of the 40 (72%). Abnormal PSA was found in 5 of 11 RF negative patients (45%) and in 11 of 29 RF positive patients (38%). Overall, the level of the RF titer did not appear to influence platelet reactivity (Figure 3). However, there is a trend, which is not significant (Chi square 1.67; P=0.2), that the female patients who have no nodules but demonstrate a positive RF titer may be afforded some protection by RF (2/11 vs 4/9). The number of male patients was too few to evaluate in this manner (Figure 3).

Rheumatoid nodules occur in 30% of the patients with RA. Our group of 40 had 16 patients with nodules (40%). The pathophysiology of a nodule in RA is known to be related to local vessel thrombosis. Rheumatoid nodules are usually associated with a positive RF titer and this finding was also true in our group. Therefore, we decided to relate the presence of abnormal PSA in our patients to the occurrence of subcutaneous nodules. Platelet populations from nine patients of 24 (37%) without nodules were activated and six patients of 16 (37%) with nodules; thus, no apparent influence was suggested by the vascular occlusion known to exist in rheumatoid nodules (Figure 4).

Platelet morphology (PM) and Serum uric acid (SUA): We have previously published data which support the concept that the occurrence of abnormal PM in primary gout may be related to a serum uric acid value greater than 5-6 mg%. This study provided us with an opportunity to study the influence of “non-gouty” uric acid levels on PSA. The uricase method was used for uric acid analysis with a range of normal from 1-7 mg%.
PLATELET MORPHOLOGY IN RA

Female (25)  Male (15)

Solid circles or squares - Abnormal PSA

Negative Titer  Positive Titer

\[ \frac{5}{11} \quad \frac{11}{29} \]

RHEUMATOID FACTOR - ABSORBED TITER

Figure 3

PLATELET MORPHOLOGY IN RA

Female (25)  Male (15)

Solid circles or squares - Abnormal PSA

Negative Titer  Positive Titer

\[ \frac{9}{24} \quad \frac{6}{16} \]

RHEUMATOID FACTOR - ABSORBED TITER

Figure 4
There were five patients with a SUA of 5 mg% and three showed a hyperactive platelet population. Using the remaining 35 patients, 6 of 23 patients (26%) with a SUA less than 5 mg% and 7 of 12 patients (58%) with SUA greater than 5 mg% exhibited an abnormal PSA. There is a trend for a serum uric level of greater than 5 mg% to be associated with hyperactive platelet populations (Chi square 3.51; P > .05, but < .1) (Figure 5).

Platelet morphology (PM) and Serum salicylate levels (SS): The determination of SS has an inherent error of 3 mg%. Some of the patients chosen gave a history of ASA intolerance or denied the recent need of ASA for several weeks. These 10 patients showed SS levels below 3 mg%. Interestingly, four of these patients (40%) had platelets exhibiting abnormal PSA, the same relative percentage as demonstrated for the total group surveyed.

Pharmacological studies on oral ingestion of ASA have shown that two tablets (325 mg each) generally gives a peak SS level in three hours of between 5-10 mg% and by six hours after ingestion the SS is reduced to an insignificant level. In the range of 5-10 mg%, platelet populations from five of 11 patients exhibited abnormal PSA (45%). Therapeutic anti-inflammatory SS levels are maintained between 15-30 mg% and platelets from five of 11 patients (45%) in that range showed abnormal PM. Considering all patients with a SS level of over 10 mg%, the platelets showed increased surface activation in seven of 19 (37%). In this parameter of our study PSA did not seem to be inhibited by either "low" or "anti-inflammatory" levels of SS (Figure 6).

Survey of RA patients and thrombosis

Is there a trend for protection from thrombotic episodes in a group of patients who use ASA daily and regularly either as an analgesic or anti-inflammatory agent? In an attempt to answer this question, we consecutively reviewed 100 patients with classical RA diagnosed and followed at Henry Ford Hospital (HFH) to determine their prevalence of clinical thrombosis. There were 72 females and 28 males. The mean age of the group was 59 years. All but three of the patients were in the fifth through the eighth decades. The average duration of rheumatoid disease was 17 years and the mean duration of patient observation was 14 years (Figure 7).

Nine of 28 males and 21 of 78 females experienced clotting episodes. The males were more prone to MI's and females experienced more frequent thrombophlebitis (Figure 8). The tendency for males to be at a greater risk for arterial thrombosis in our group is not an unexpected finding when one reviews the mortality rates for cardiovascular disease.

Forty-three thrombotic episodes occurred in 30 patients, of which 21 were women. In 14 myocardial infarctions, five female patients were afflicted. Cerebral vascular accidents totaled five, and four of these were female patients. The most common clotting episode was due to thrombophlebitis and these numbered 19; 12 occurred in women. Pulmonary embolism developed in four female patients. One woman exhibited peripheral arterial occlusive disease. The average duration of rheumatoid disease before clinical thrombosis occurred was 16 years. No significant correlation with rheumatoid nodules, stage of disease progression, or functional class was evident when patients with and without thrombosis were compared. Four of the patients who had myocardial infarctions had either diabetes mellitus or hypertension as an identifiable risk factor (Figure 9).

Certainly one cannot make valid statistical comparisons between epidemiological surveys which include large numbers of individuals and the limited Henry Ford Hospital survey of patients with RA. Nevertheless, a trend is appreciated when comparison is made with other available data. There are conflicting data in findings from one of our
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PLATELET MORPHOLOGY IN RA

○ Female (25)
□ Male (15)

Solid circles or squares – Abnormal PSA

Figure 5

SERUM URIC ACID (MG%)

PLATELET MORPHOLOGY IN RA

○ Female (25)
□ Male (15)

Solid circles or squares – Abnormal PSA

Figure 6

SERUM SALICYLATE (MG%)

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AGE & SEX OF 100 PATIENTS WITH CLASSIC RHEUMATOID ARTHRITIS

NUMBER OF PATIENTS

AGE & SEX OF 100 PATIENTS WITH CLASSIC RHEUMATOID ARTHRITIS

NUMBER OF PATIENTS

Figure 7

CLASSIFICATION of 43 CLOTTING EPISODES in 30 PATIENTS with RA

NUMBER OF PATIENTS

a. Myocardial Infarction
b. Cerebrovascular Accident
c. Thrombophlebitis
d. Peripheral Vascular Occlusive Disease
e. Pulmonary Embolus

Figure 8
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TYPES of THROMBOSIS by AGE GROUP in 30 PATIENTS with CLASSIC RA HAVING 43 CLOTTING EPISODES

- MYOCARDIAL INFARCTION
- CEREBROVASCULAR ACCIDENT
- THROMBOPHLEBITIS
- PULMONARY EMBOLUS
- PERIPHERAL VASCULAR OCCLUSIVE DISEASE

AGE BY DECADE

Figure 9

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previous reported surveys of 280 patients with primary gout, the Framingham and the Tecumseh studies that included the occurrence of coronary heart disease and the report on the prevalence of "completed strokes" reported by Halsey et al in 1968.

Based on our HFH surveys, a protection is only suggested for the patient with RA on aspirin in the seventh decade. In the primary gout patients all but three were males and they had a total mean follow-up period of 1,400 patient years; 244 were in the fifth through the eighth decades. Although the RA patients numbered 100 and only 28 were males, the mean observation period was also 1,400 patients years. Since both groups were surveyed for the same thrombotic episodes, the results are compared. In the sixth and seventh decades there was a sufficient number of patients in both groups to analyze. In the seventh decade (60-69 years) 7 of 48 patients (15%) experienced thrombosis while they were taking ASA; whereas 23 thrombotic episodes occurred in 66 gout patients (35%). The reverse situation was true for the sixth decade (50-59 years) (Table I). However, calculations showed a statistically significant difference between the groups only for the seventh decade (Chi square 9.42; P < .01, but > .001).

By sorting out the data from the Framingham and Tecumseh studies for the incidence of coronary heart disease (CHD) for females, males and by decades, we could compare the prevalence of MI in the female patients with RA. The Framingham data does not extend beyond 62 years. None of our female patients experienced an MI before the sixth decade. In the sixth decade our Henry Ford Hospital RA group experienced almost twice the prevalence of MI as the Framingham or Tecumseh group even though the latter studies included either MI or angina pectoris as evidence of coronary heart disease. The prevalence in the seventh and eighth decades was comparable with the Tecumseh survey (Figure 10). Although the male patients with RA are not charted, they had a higher incidence of MI than the groups used for comparison. While our sample of female patients is small, the data fail to suggest that ASA offers protection against MI in these RA patients.

The report of Halsey et al on a prospective study of strokes in an epidemiology survey noted 95 complete strokes (excluded transient ischemic attacks) in 1,802 individuals (47% were males). Of these 891 occurred in patients aged 60-69 years of which 68 experienced complete strokes (7.6%). Two of 48 patients in our RA group (age 60-69) experienced a stroke (4.1%), which is about half the rate found by Halsey et al.

Discussion

Although high hopes are held for aspirin as an effective agent for clinical use in reducing the tendency for thrombosis, it does seem unusual that clinical observations have never reported or suggested a decreased risk for thrombosis associated with ASA ingestion. In fact, a recent controlled study of aspirin effect on postoperative venous thrombosis showed no advantage over the placebo-treated group, while another clinical study which evaluated the ability of aspirin to prevent venous thrombosis and pulmonary embolism after hip surgery suggested an equal benefit for aspirin with those patients receiving warfarin, but a better benefit than dipyridamole. The pharmacological doses required to influence platelet release activity and secondary aggregation in in vitro experiments are 2 to 40 times the therapeutic level of salicylate which one can practically obtain by the ingestion of aspirin. Decreased hearing and tinnitus occur at 25 to 30 mg%; at 35 to 40 mg% hyperpnea begins as a manifestation of salicylate intoxication and acidosis.

A report of the in vivo effect of ASA on platelets measured by serotonin release and platelet aggregation experiments when "long term ingestion" has been used sug-
COMPARISON of INCIDENCE of CHD by DECADE in the HFH STUDY COMPARED with the TECUMSEH and FRAMINGHAM STUDIES

**Figure 10**
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**Table I**

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**NOTE:** Each arthritic group represents about 1400 patient years observation for MI, CVA, PVOD, TP, PE.
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suggested the promise of decreasing thrombosis. Our efforts to relate serum salicylate levels to the degree of platelet surface activation during “long term ingestion” of ASA has yielded less promising data. By the EM evaluation of platelet morphology there were no trends of surface inhibition by “low” or “anti-inflammatory” serum levels of salicylate. The correlation of serum uric acid values above 5 mg% with an abnormal PSA and aggregation suggests a possible harmful effect of ASA (Chi square 3.51; P >.02, but <.1). Low dose salicylate ingestion (less than two grams per day) is known to decrease tubular secretion of uric acid and cause a rise in serum urate. This rise in serum uric acid could actually indirectly serve to increase platelet responsiveness in some individuals.

A rheumatoid nodule develops in association with arteriole and venous occlusion. Where the vessels are patent, the nodular tissue terminates. The occurrence of nodules in RA offers a type of vascular thrombosis for evaluation. Also, rheumatoid factor titers are positive regularly in rheumatoid patients with nodules. In ongoing intravascular clotting abnormal PSA has been demonstrated and has been shown to persist for as long as 18 months. If rheumatoid nodules portend an intravascular thrombotic episode, it was not reflected in the platelet differential counts of our RA patients nor by the number of clotting episodes in the retrospective review of the Henry Ford Hospital patients with rheumatoid arthritis. The presence of rheumatoid factor, a macroglobulin, in significantly positive titers did not appear to inhibit the PSA. Unfortunately, insufficient numbers of male patients exclude assessment of a trend for them.

One cannot make valid statistical comparisons between the limited Henry Ford Hospital survey and the population surveys available as to prevalence of thrombotic episodes because of the great disparity in numbers of individuals in each group. However, there is conflicting evidence of ASA protection from clinical thrombotic episodes in rheumatoid arthritic patients. Because primary hyperuricemia is known to correlate well with abnormal PSA and gout patients have an increased incidence of vascular occlusive disease, it may be an injustice to compare our RA patients with our gout patients. If that be so, we should not conclude a benefit for ASA in the RA group for the seventh decade.

It seems unlikely to us from our data that “a couple of aspirin a day will keep thrombosis away!” However, it is possible that factors exist in the chronically ill patient with RA that override any protection from thrombosis that may be afforded to a “normal” individual regularly ingesting aspirin. Obviously, our study cannot answer that possibility.

Acknowledgements

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