Aspirin Prolongation of the Template Bleeding Time: Influence of Venostasis and Direction of Incision

By C. Harold Mielke, Jr.

The template bleeding time is a measure of platelet participation in primary hemostasis. Aspirin alters platelet function through interference with prostaglandin biosynthesis. In many individuals, aspirin will consistently prolong the bleeding time. Despite this observation, normal individuals rarely develop a bleeding disorder. This prompted us to investigate the influence of technical variables on the prolongation of the bleeding time by aspirin. Both direction of incision and venostasis influenced the prolongation of the bleeding time by aspirin. A horizontal incision with venostasis produced the most pronounced prolongation, while a vertical incision without venostasis didn’t prolong the bleeding time despite the characteristic changes in platelet aggregation and release. These studies suggest that the influence of aspirin on the template bleeding time is dependent on technical variables and is minimal in the normal subject.

PLATELET PARTICIPATION in small vessel hemostasis is primarily determined by the bleeding time. Since its introduction by Duke1 in 1910, it has been used to identify both quantitative and qualitative disorders in primary hemostasis. Recent improvements in the technique of performing the bleeding time have resulted in a repeatable and reliable test for measuring primary hemostasis.2 The template bleeding time is a disposable device that produces a painless incision of constant length and depth with uniform venostasis.3,4 Since aspirin will prolong the bleeding time,2,5,6 it is commonly assumed that aspirin produces a hemostatic impairment. Despite this prolongation, the normal individual rarely develops a bleeding disorder, as attested by the enormous quantities of aspirin ingested each year by the general population.7,8

This disparity prompted us to investigate the influence of two technical variables, venostasis and direction of incision, on the prolongation of the template bleeding time by aspirin in normal healthy volunteers.

MATERIALS AND METHODS

In this study, 15 healthy normal volunteers, 7 males and 8 females, ranging in age from 20 to 46 yr of age were studied under informed consent. All subjects were free from any form of medication for at least 2 wk prior to entering this study. This was determined by a clinical history and the presence of a normal platelet aggregation and release of 14C-serotonin prior to entry.

Template bleeding times were performed using a disposable device (Hemakit, Inc., Malden, Mass.) that produced a painless incision of 1 mm in depth and 9 mm in length, as previously described. Incisions were performed in both a horizontal (parallel to the antecubital crease) plane on the lateral aspect of the volar surface of the forearm approximately 5 cm below the antecubital crease. The medial aspect of the forearm was avoided since the skin is thin with very little adipose tissue, thus making it easier to cut deeper structures and larger vessels. Venostasis was achieved with a standard blood pressure cuff inflated to 40 mm Hg for 30 sec prior to placing the incision and maintained for the duration of the procedure.

Platelet aggregation in response to serial dilutions of adenosine diphosphate, epinephrine, collagen and the subsequent release of 14C-serotonin was performed as previously described.8 Platelet counts were performed by phase microscopy.9

Upon qualification for entry into the study, each subject had a baseline platelet aggregation and release of 14C-serotonin performed in a fasting state. Next, horizontal template bleeding times were performed on one forearm, with and without venostasis at 40 mm Hg. Next, a vertical incision was placed on the opposite forearm in a similar manner. The subject then ingested two, 5-grain aspirin tablets. Horizontal and vertical bleeding times, as described above, with and without venostasis were performed at 2, 4, 24, 48, 72, and 96 hours after the ingestion of the single dose of aspirin. In addition, blood was drawn for platelet aggregation and release studies at the same time, except the fourth hour post ingestion on the first day was eliminated. In some instances where the studies hadn’t reached baseline values by 96 hr, they were monitored daily until comparable baseline values were reached.

Statistical analysis was performed using standard methods.

RESULTS

The mean template bleeding times ± 1 standard deviation for the 15 subjects under the 4 experimental conditions (horizontal incision/cuff on, horizontal incision/cuff off, vertical incision/cuff on, vertical incision/cuff off) are shown in Table I. Statistical evaluation of the mean changes from baseline were compared by a paired t test in this table. The greatest prolongation in the bleeding time was seen when the incision was made in a horizontal plane using venostasis. There was a doubling of the mean bleeding time from baseline (3.9–8.4 min) at 2 hr, which persisted for 48 hr, starting to correct by 72 hr, and approaching...
baseline by 96 hr. Corresponding with this was a widening of the standard deviation of the mean. This did not normalize until 72 hr and indicated the variability of the individual response to aspirin. When venostasis was eliminated, the mean baseline horizontal incision shortened significantly (3.9–2.6 mm) (p > 0.05). Similarly, the prolongation was not pronounced and reached baseline by 96 hr.

When the vertical incisions were compared to baseline, there was a difference from the findings observed with the horizontal incisions. First, the mean baseline was lower than in either of the horizontal incisions. When venostasis was used, a significant prolongation of the template bleeding time by a single dose of aspirin over a 96-hr period. Corresponding with this was a widening of the standard deviation of the mean. This did not normalize until 72 hr and indicated the variability of the individual response to aspirin. When venostasis was eliminated, the mean baseline horizontal incision shortened significantly (3.9–2.6 min) (p > 0.05). Similarly, the prolongation was not pronounced and reached baseline by 96 hr.

In order to determine whether the proportion of subjects showing an increase in the template bleeding time from baseline was greater than that which would be expected by chance, we tabulated the changes, however small, into either increases, decreases, or no change and compared the numbers in each category by means of the Wilcoxon signed-rank test. As shown in Table 2, these results differ from those in Table 1 only at 96 hr for horizontal incision/cuff on and at 72 hr for horizontal incision/cuff off.

In all subjects, aspirin impaired platelet aggregation induced by adenosine diphosphate (8 \( M \) and 3.2 \( M \)), epinephrine (4.1 \( M \)), and collagen (14.8 mg/ml). As expected, this was characterized by reduced maximum aggregation (adenosine diphosphate, epinephrine, collagen), absent secondary wave (adenosine diphosphate, epinephrine), and disaggregation (adenosine diphosphate). Similarly, the release of \(^{14}\)C-serotonin was also abolished. The first to return to baseline was collagen, with a mean time to recovery of 94 hr, followed by epinephrine with a mean recovery time of 113 hr. Adenosine diphosphate 8.0 \( M \) mean recovery was 147 hr, and 148 hr for 3.2 \( M \). In all instances, the horizontal template bleeding time, either with or without venostasis, corrected before the platelet aggregation measurements. Platelet counts were unchanged.

### DISCUSSION

The template bleeding time is a highly repeatable test when there is strict adherence to multiple technical points. The presence of a blood pressure cuff, which creates venostasis to insure adequate venous filling, and the direction of the incision are technical factors that influence the repeatability of the test.

<table>
<thead>
<tr>
<th>Hours Following ASA Ingestion</th>
<th>Cuff on</th>
<th>Cuff off</th>
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<tbody>
<tr>
<td>Horizontal incision</td>
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<td>+</td>
</tr>
<tr>
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<tr>
<td>Vertical incision</td>
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<tr>
<td>96</td>
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*Indicates a significantly greater proportion of subjects increased than decreased at the 5% level (Wilcoxon signed-rank test).
We have studied the influence of these variables in 15 normal subjects and found that not only the direction of the incision, but also the presence or absence of venostasis, has a definite influence, not only on the template bleeding time, but also on the degree of bleeding time prolongation induced by aspirin.

Venostasis was introduced by Ivy in 1935\(^5\) in order to increase the sensitivity of his technique. He experienced difficulty in obtaining consistent bleeding, and hence repeatability, from his forearm punctures. It was felt that the state of capillary tone was the key variable and accounted for this insensitivity and lack of repeatability. Previously, it had been shown that when the capillaries were cut, blood is forced out and the capillary walls collapse, and thus contribute to hemostasis.\(^{11}\) Thus, if capillary tonus could be eliminated and the vessels kept full of blood, sensitivity would increase and a latent bleeding tendency could be detected. He wrote, "One of us (A.C.I.) suggested applying the cuff of a sphygmanometer—40 mm Hg. By increasing the pressure in the capillaries—"tonus" might be eliminated. This was done, with most gratifying results."\(^{10}\) Thus, in order to improve the sensitivity of the Ivy technique over that of the Duke bleeding time, a blood pressure cuff was necessary, and we have continued to use it to the present day. Despite this, there was difficulty in obtaining repeatability, as attested by his remark in 1941: "A relatively reliable mean bleeding time may be obtained by making one puncture during each inflation of the cuff and averaging the time of the first three that bleed." According to this, a patient could receive multiple punctures before three satisfactory measurements could be obtained. Again, this practice persists to some degree to the present day.

Our studies confirm the observations of Ivy;\(^5,10,11\) however, when applied to the template technique, the use of multiple incisions and venostasis isn't necessary in order to achieve a sensitive and repeatable bleeding time test. Previous studies have shown that two horizontal incisions give the same information as three horizontal incisions,\(^2\) and more recently, that one horizontal incision is comparable to the average of two horizontal incisions.\(^7\) The present study shows that sensitivity, as measured by the influence of aspirin, is directly related to venostasis and the direction of the incision. The mean bleeding time is not only greater when venostasis is used, but the prolongation by aspirin is greater and the duration of the influence is longer. This is especially true when the direction of the incision is considered.

Borchgrevink\(^2\) modified the Ivy technique by applying an incision rather than a puncture to the forearm. This was applied in a horizontal direction and this technique persists to the present in most laboratories. As can be seen from this study, the normal bleeding time is longer with a horizontal incision than with a vertical incision. The most likely explanation for this is that the skin edges of the horizontal incision tend to separate more than those of a vertical incision. This would create more of a "tamponade" effect with the vertical incision. In both types of incision, venostasis creates a longer bleeding time. When the influence of aspirin is measured over time, only with a horizontal incision can a statistical difference be detected. The greatest difference is seen when venostasis is used.

It is interesting to note that after the ingestion of aspirin, the bleeding time returned to normal before the in vitro platelet aggregation and release studies. These studies measure the influence of cyclooxygenase on platelet aggregation and the prostaglandin-mediated release reaction.\(^{11}\) This observation suggests an alternate pathway and perhaps provides support for a primary role of thrombin in primary hemostasis, since thrombin is not influenced by aspirin and can initiate platelet aggregation irrespective of cyclooxygenase in the normal subject. Of the agonists tested, collagen was the first to normalize.

From these studies we conclude that the template bleeding time, although a highly repeatable technique, is dependent on the technical variables tested. The prolongation of the bleeding time by aspirin is influenced by the direction of the incision and presence or absence of venostasis. A horizontal incision with venostasis is the most sensitive technique to measure influences on the bleeding time. Finally, the influence of aspirin on primary hemostasis is minimal in the normal subject, supporting the observation that the healthy individual rarely develops a bleeding disorder from it.

REFERENCES

1. Duke WW: The relation of blood platelets to hemorrhagic diseases: Description of a method for determining the bleeding time and coagulation time and report of three cases of hemorrhagic disease relieved by transfusion. JAMA 55:1185, 1910


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