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# Thromboembolic Complications Several Days After a Single-Dose Administration of Aspirin

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## Abstract

The antithrombotic properties of acetyl salicylic acid (ASA) used at current doses are largely demonstrated. However, our previous study showed unexpected thrombotic potencies associated with the use of this drug. In this study we investigate the effect of aspirin on an experimental thrombosis induced by laser beams, according to its in vivo plasma concentration. Experiments were done on nine groups of seven Wistar male rats. The groups are defined by the delay between aspirin administration time and the laser-induced thrombosis time. Results from this study showed an enhancement of thromboembolic complications when thrombosis was induced 8 or 10 days after aspirin administration; the number of emboli and the duration of embolization are increased, compared to the control group. The prothrombotic properties of ASA demonstrated in this study, might limit its therapeutic benefit and might explain thromboembolic complications observed in some ASA-treated patients. These results also suggest a biological monitoring several days after aspirin administration to patients. © 1998 Elsevier Science Ltd.

*Key Words:* Aspirin plasma concentration; Platelet aggregability; Induced thrombosis

Aspirin, a well-known antiaggregative agent, is widely used at current doses to prevent arterial thrombosis, cerebral strokes [1], or myocardial infarction [2,3] and to reduce the risk of death in patients. Much investigation has tried to evaluate the possibility that low dose aspirin might be used selectively to inhibit platelet aggregation without interference with vascular prostacyclin production and to inhibit prolongation of the bleeding time.

Aspirin acts pharmacologically as a potent noncompetitive inhibitor of platelet and endothelial cyclooxygenase through permanent acylation [4]. The dose-related selectivity of aspirin has been ascribed to the ability of endothelial cells to synthesize new enzymes in contrast to platelets which are anuclear [5], and also to presystemic inhibition of platelets [6]. Previous studies demonstrated that at high doses, aspirin inhibits either thromboxane or prostacyclin synthesis in view of the $TXA_2$-prostacyclin balance theory [7]. Selective impairment of vascular cyclooxygenase has been attempted on the assumption that intact prostacyclin production might enhance the antithrombotic efficiency of the drug.

Dose selectivity of acetyl salicylic acid (ASA) toward platelet and vascular cyclooxygenase raised the question about the dose which would enhance its beneficial therapeutic effect. Low doses of ASA (1 mg/kg) have been shown to be sufficient to inhibit platelet-cyclooxygenase totally [8].

Thrombogenic properties observed during or after ASA treatment might have practical relevance in clinical practice when ASA is present in the microvasculature at infinitesimal dosage. For example, at

*Abbreviations:* ASA, acetyl salicylic acid; PAF, platelet activator factor; $TXA_2$, thromboxane $A_2$.

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the end of aspirin therapy or after ASA administration in the post-operative period. The potent prothrombotic properties of ASA might limit its therapeutic benefit and might explain thromboembolic complications observed in some ASA treated patients. Since this phenomenon presents a great interest in clinical practice, the aim of the present study is to analyze the effect of aspirin on an experimental thrombosis induced by laser beams in rat mesenteric vessel, several days after its subcutaneous administration at 100 mg/ml/kg, in other words, according to its in vivo plasma concentration.

## 1. Materials and Methods

### *1.1. Animals and Drugs*

The study is carried out on male Wistar rats weighing 200–250 g from Depré breeding center (Saint-Doulchard, France). Rats were anaesthetized with an injection of sodium thiopental (200 mg/kg b.w. Nesdonal, Specia Laboratories, France) into the femoral muscle. The tested products were injected subcutaneously in the thorax region. The time corresponding to the administration of the product is noted as $T_0$. Different groups were defined according to the number of days between aspirin administration and the laser-induced thrombosis (see Figure 1). To induce thrombosis, the small intestine was exteriorized through a median laparotomy and mesentery was spread flat on a glass plate mounted on the microscope table. Investigation was performed in vessels of the same diameter (15–25 μm) of the flat-free portion of the mesentery. At least seven rats were studied in each group. Test and control animals were selected at random.

### *1.2. Microscopy*

Vascular lesions were produced by Argon laser (Stabilite 2016, Spectra-Physics, France). The laser beam was coaxially inserted into the induced light beam of a microscope (Axiovert 10, Zeiss, France). The wavelength used was 514.5 nm and the energy was adjusted to 120 mw. The exposure time for laser shot was 1/15 seconds. Microscopic image was recorded by a videotape recorder through a videocamera (DXL 107, color camera CDD) and monitored on the television screen (Trinitron color video monitor, PVM 1442 QM, Sony, France). The dynamic course of the thrombus formation was continuously monitored and the time of the experiment was recorded automatically.

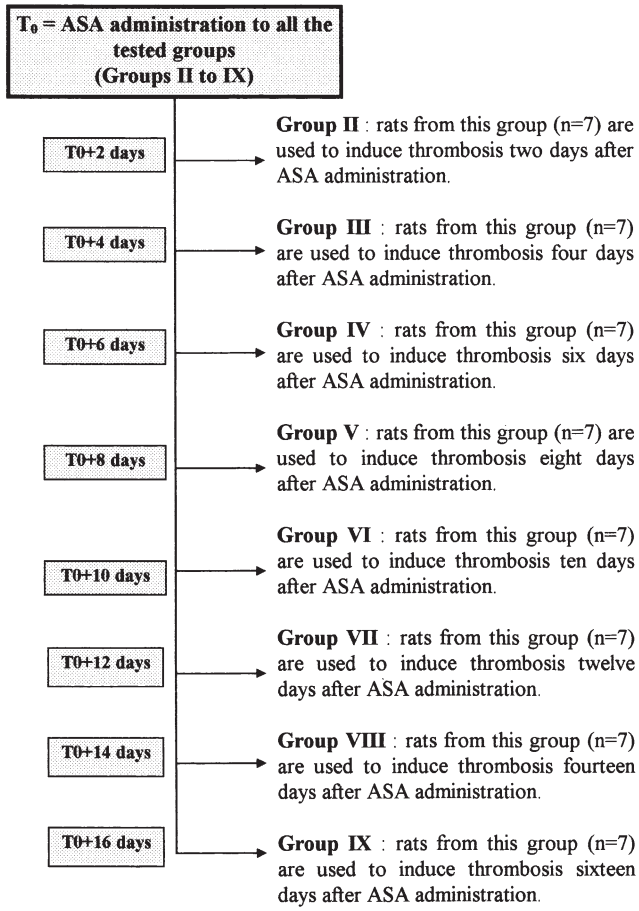


Fig. 1. Different groups are defined in function of the number of days between aspirin administration and laser-induced thrombosis.

### *1.3. Laser-thrombus Formation*

The laser spot for a single shot was positioned on endothelium at the border between the luminal portion of the vessel wall and the lumen of the arteriole. The results were evaluated by direct observation through the microscope and by recording the total sequence of intravascular reaction. At least three parameters were measured: (1) the number of laser injuries required to induce platelet thrombus formation (when no visible thrombus occurs after the first shot, a second, third, and fourth laser shot were applied to the vessel at the same site as the first one,

1, 5, and 8 minutes, respectively; (2) the number of emboli removed by blood stream after thrombus formation; and, (3) the duration of embolization (minutes).

### *1.4. Drugs Tested*

The drugs used were aspirin (Aspegic®, Laboratoires Synthélabo, France) and saline solution of NaCl 0.9% as control.

### *1.5. Statistical Analysis*

The results were treated statistically using a personal computer with PCSM® Software (Deltasoft, France) by non-parametric analysis of variance of the control and experimental animals groups according to the Mann-Whitney method. The statistical confidence of the difference between experimental and control values was $p<0.05$.

### *1.6. Experimental Groups*

Experiments were done on nine groups of seven Wistar male rats each. Rats receiving the NaCl 0.9% were used as the control group (Group I).

## 2. Results

### *2.1. Effects of Aspirin on Laser-induced Thrombosis According to the Delay of Its Administration*

As shown in Table 1, there are significant modifications of the number of laser beams in any experimental groups compared with control. Aspirin, two days after its administration still demonstrated potent antithrombotic activities. In fact, compared with the control group, aspirin administrated at 100 mg/kg, two days before thrombosis induction, decreases the number of emboli and reduces the duration of embolization ($p<0.05$). Table 1 shows an opposite effect of the administration of aspirin (100 mg/kg), 8 and, in lesser extent, 10 days before thrombosis induction, the number of emboli is increased and the duration of embolization is prolonged ($p<0.05$).

No statistically significant differences were observed between the other groups and control. The same trends are shown in the second and the third arterial induced thrombosis (data not shown).

## 3. Discussion

Kinetic studies have shown that the inhibition of ASA was due to the irreversible acetylation of platelet and endothelial cyclooxygenase [7,9]. The synthesis of new cyclooxygenase cannot occur in platelets that are anuclear and the return of functional cyclooxygenase can only be obtained by the entry into circulation of platelets originating from megakaryocytes in which this mechanism has been reported to occur with a two-day long [10]. In contrast, endothelial cells can rapidly synthesize new functional cyclooxygenase [11]. The turnover of the enzyme seems to be the major single determinant of the efficiency of aspirin.

After oral ingestion, aspirin is rapidly absorbed at the stomach and duodenum [12]. After the ingestion of 600 mg, the plasma level of aspirin reached 65 mg/l in one hour. The absorption is passive. Then, aspirin is rapidly hydrolyzed in the circulation and tissue by esterases to give salicylic acid. In less than 1 hour, 30% only of absorbed aspirin is present on acetyled form. From 50% to 90% binds rapidly to the plasmatic proteins, particularly to the albumin. Only the free form of aspirin possess the therapeutic activities.

This investigation displays three phases of ASA action, according to the delay between its administration and the thrombosis induction by laser beam. First, ASA showed a potent antithrombotic effect, which disappears three days after its administration. The difference observed between the control group and ASA tested group 2 days after its administration reached the level of significance ($p<0.05$). In the second phase, between three and seven days after its administration, ASA did not show any effect. In contrast, 8 and 10 days after its administration, aspirin provoked an enhancement in the number of emboli and in the duration of embolization testifying to prothrombotic properties.

The administration of ASA at 100 mg/ml/kg can, first, prevent thrombus formation induced by endothelial cell damage. But, a long time after ASA treatment, this substance provokes an enhancement of thromboembolic complications. So, the effect of ASA may depend on the drug concentra-

Table 1. Effects of aspirin on laser-induced thrombosis at different times after its subcutaneous administration at 100 mg/kg

| Group | Number of laser beams | Number of emboli | Duration of embolization (minutes) |
|---|---|---|---|
| I (Control) | 1.99±0.84 | 4.97±1.44 | 1.8±0.63 |
| II | 2.37±0.70 | 1.33±0.71[a] | 0.52±0.42[a] |
| III | 1.79±0.38 | 3.39±1.55 | 1.43±0.89 |
| IV | 1.47±0.37 | 5.33±1.96 | 2.16±1.01 |
| V | 1.58±0.39 | 7.97±2.56[a] | 2.97±0.82[a] |
| VI | 1.78±0.69 | 6.80±2.89 | 3.45±1.59[a] |
| VII | 1.63±0.55 | 5.23±1.14 | 2.06±0.76 |
| VIII | 2.05±0.38 | 3.88±0.93 | 1.60±0.64 |
| IX | 1.66±0.00 | 5.63±0.93 | 2.19±0.47 |

Results are expressed as the mean±standard deviation.
[a] $p<0.05$ (statistically different to control).
$T_0$: Aspirin administration (100 mg/kg).

tion in the microvasculature, pharmacokinetics, and availability as acetyl salicylic acid at vascular level. The effect may be related to an "effect curve": small concentrations of ASA may cause large changes in effect on a very steep concentration. This is in accordance with a prolonged presence of the drugs, at least to six hours after oral administration, despite a transient duration of effects. Another mechanism should be the generation or the activation of the synthesis of an active metabolite with prothrombotic activity or the inhibition of the synthesis of an agent with anti-thrombotic activity by ultra low concentration in plasma. Such an effect has been previously reported by Czevionk et al. [13] demonstrating that an augmented platelet adherence to aspirin treated endothelial monolayers and stimulated with thrombin, an effect that they attributed to decrease of prostacyclin release and the resultant abrogation of the direct inhibitory effect of prostacyclin on the platelets. Thus, inhibition of vascular cyclooxygenase by administration of ASA could lead to the generation of PAF which is a potent stimulus for the activation of platelets [14] and polymorphonuclears [15] in vitro, which could cause subsequent endothelium injury.

In conclusion, results from this study showed a potent antithrombotic effect of aspirin three days after a single-dose administration. Aspirin did not show any effect three to seven days after this administration. But, thrombosis induced 8 and 10 days after this administration were more pronounced, compared to the control group ($p<0.05$). The mechanism of this action is unknown, but it could be due to the accumulation of infinitesimal doses of ASA in the microvasculature or by other undefined actions. This prothrombotic activity of ASA seven days after a single-dose administration may explain thromboembolic phenomenon observed in patients when they stopped ASA treatment. These results must be taken into consideration at the end of ASA therapy and suggest further study.

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