Laboratory Effect on Platelet Activity within 24 h of the First 300-mg Oral Dose of Aspirin Given in Hospital during the Acute Phase of Ischemic Cerebral Events

S. Richard^a M. Toussaint-Hacquard^b R. Fay^a J.C. Lacour^a X. Ducrocq^a
T. Lecompte^b

^aCHU Nancy, Service de Neurologie, Hopital Central, et ^bCHU Nancy, Service d'Hématologie Biologique-Hémostase Biologique, Hôpitaux de Brabois, Vandoeuvre-les-Nancy, Faculté de Médecine UHP/Nancy Université, Inserm U961, Nancy, France

Key Words

Aspirin · Ischemic stroke · Platelet

Abstract

Background and Purpose: Aspirin is the most commonly used antiplatelet treatment during the acute phase of cerebral ischemic events. It inhibits the production of thromboxane (TX) A₂, a powerful platelet activator. Despite this protection, early ischemic recurrences are frequent and considered clinical failures of this therapy. Only a few trials have focused on the use of antiplatelet therapy during this phase, and none has described the laboratory effect of the first dose of aspirin given after an ischemic cerebral event. However, this study may help clinicians to understand the mechanisms of early recurrences, and to design new therapeutic strategies, in particular for patients already treated with a daily dose of aspirin. **Method:** We studied laboratory parameters of the first 300-mg oral dose of aspirin given within 48 h after an ischemic cerebral event. Two blood samples were taken from all of the patients: the first during the third hour following aspirin intake (T1) and the second during the twenty-fourth hour (T2). For patients already treated with a daily dose of aspirin, a supplementary sample was taken before aspirin intake (T0).

Platelet reactivity was studied on the basis of serum TXB₂ levels, a metabolite of TXA₂, and light transmission aggregometry after stimulation of platelet-rich plasma by arachidonic acid and by two concentrations of collagen, i.e. 2 µg/ml (Col2), dependent on the TXA₂ pathway, and 20 µg/ml (Col20), independent of the TXA₂ pathway. Results with Col2 were related to results with Col20 (Col2/20 ratio) to limit the impact of variations induced by the effects of preanalytical conditions. **Results:** Fifty patients were included. TXB₂ values ($p < 0.001$) and relative values of the Col2/20 ratio ($p = 0.037$) were significantly higher at T2 compared to T1. For patients already treated with aspirin, TXB₂ levels ($p < 0.001$) were significantly lower at T1 compared to T0, and the Col2/20 ratio tended to decrease ($p = 0.096$). **Conclusion:** Platelet reactivity recovers within 24 h following the first 300-mg oral dose of aspirin during the acute phase of a cerebral ischemic event as demonstrated by an increase in TXB₂ levels, and the Col2/20 ratio, at T2 compared to T1. This would favor early ischemic recurrences. However, for patients already treated with aspirin, this dose is able to decrease TXB₂ levels and to complete the inhibition of the TXA₂ pathway, which shows the utility of this prescription in this case.

Copyright © 2012 S. Karger AG, Basel

Introduction

Aspirin is widely prescribed after ischemic stroke to reduce thrombotic recurrences [1, 2]. It preferentially and irreversibly inactivates the cyclo-oxygenase (COX) 1 [3], thus inhibiting the production of thromboxane (TX) A₂, a powerful platelet activator. However, although numerous studies describe protection against thrombotic recurrences, failures or laboratory aspects of low doses of aspirin in the long term [4] and the effects of the first dose given during the acute phase of ischemic stroke are less known. Only a few trials have focused on this period [5, 6], the most important being IST [7] and CAST [8]. These randomized trials studied the effects of 160–300 mg of once daily aspirin initiated in patients within 48 h after ischemic stroke. They concluded in a reduction of about 7 recurrent ischemic strokes for every 1,000 patients treated with a maximum follow-up of 6 months. This moderate benefit is offset by the immediate high risk of recurrences: 7% of patients present another cerebrovascular event within a week of the first one [9] and 11% within a month [10]. A description of the laboratory effects of aspirin during the acute phase of stroke may help to understand the mechanisms of recurrence and design new therapeutic strategies. This study is all the more important as patients already treated with a 75- to 160-mg daily dose of aspirin were also included to observe the effects of a higher dose given during the acute phase of stroke. The aim of this study was to describe the laboratory effect of the first 300-mg oral dose of aspirin, given in hospital, after an ischemic cerebrovascular event. The effect was measured at the third and twenty-fourth hour after aspirin intake to follow platelet function recovery.

Materials and Methods

This was a single center, prospective study approved by the local institutional ethics committee. Each patient gave written informed consent. (ClinicalTrials.gov, NCT01375400).

Patient Inclusion

Fifty patients hospitalized at the stroke unit of Nancy, Department of Neurology (France), were enrolled during a period of 3 months (December 2010 to March 2011). Eligible patients had presented symptoms of ischemic cerebral events within 48 h, with the diagnosis confirmed by CT or MRI brain scan and a neurologist's exam. They could have been treated with a daily dose of aspirin. Exclusion criteria were: patients under 18 years old, pregnancy, patients at high risk of bleeding, hemorrhagic stroke, treatment by anticoagulants or nonsteroidal anti-inflammatory drugs (NSAID). Age, sex, vascular risk factors, he-

Table 1. Baseline characteristics of patients

	n (%)			
Male	26 (52)			
Female	24 (48)			
TIA	13 (26)			
Stroke	37 (74)			
Hypertension	27 (54)			
Smoking	21 (42)			
Dyslipidemia	19 (38)			
Diabetes mellitus	7 (14)			
Previous aspirin treatment	18 (36)			
		Median	Interquartile range	Extreme values
Age		69	53–78	28–96
Platelet count, g/l		222	183–283	51–565
Reticulated platelets, %		14	10.5–22	5–38
Reticulated platelets count, g/l		34.7	21.5–46.3	2.5–82
Hemoglobin, g/dl		13.7	12.7–15.3	9.8–16.7
Leucocytes, g/l		8	6.4–9.5	5.2–16

moglobin, platelet, and leukocyte counts were noted. Patients received an oral 300-mg dose of aspirin (Kardegic®; Sanofi-Aventis, France), witnessed by the nurse or the neurologist. Two blood samples were taken. The first sampling (T1) was performed during the third hour after aspirin intake, as peak plasma levels occur 30–40 min after aspirin ingestion and platelet inhibition is evident by 1 h. The second sampling (T2) was performed during the twenty-fourth hour, just before the usual second aspirin intake. An additional sampling (T0) was performed before the 300-mg oral dose if the patient had already been treated by a daily dose (75–160 mg) of aspirin. The laboratory tests employed were: light transmission aggregometry (LTA) on platelet-rich plasma stimulated with arachidonic acid (AA) and collagen, and TXB₂ levels.

Light Transmission Aggregometry

Platelet reactivity was measured by LTA (Soderel Medical Aggregometer, Nancy, France). The platelet-rich plasma was stimulated by 1.39 mmol/l of AA (BioData Corporation, Horsham, Pa., USA) and by two concentrations of collagen (Collagen Horm®, Nycomed, Linz, Austria): 2 µg/ml (Col2) and 20 µg/ml (Col20). Col20 induces an aggregation almost independent of the TXA₂ pathway. For collagen-induced platelet aggregation, results with Col2 were related to results with Col20 (Col2/20 ratio) to limit the impact of variations induced by the effects of preanalytical conditions.

TXB₂

As TXA₂ is an unstable component that cannot be directly measured, we estimated the levels by measuring the concentration of its metabolite, TXB₂, by immunoassay (Enzo Life Sciences).

Table 2. Laboratory characteristics at T0, T1, and T2

	T0			T1			T2		
	median	interquartile range	extreme values	median	interquartile range	extreme values	median	interquartile range	extreme values
AA, %T	8	2–13	0–82	6	4–10	0–18	8	3–12	0–18
C2, %T	47	30–58	3–90	37	21–48	4–63	38	29–46	5–62
C2/C20, %	61	40–79	6–100	48	32–64	9–94	54	41–61	8–88
TXB ₂ , pg/ml	700	320–2,200	50–20,000	255	110–540	0–1,200	680	320–1,400	85–5,840

Statistical Analysis

All analyses were performed using SAS® Software V9.1.3 (SAS Institute, Cary, N.C., USA). The two-tailed significance level was set at $p < 0.05$. Owing to the small sample size, no adjustment for multiple testing was attempted.

Continuous variables were presented as medians, interquartile ranges, and overall ranges, and categorical variables were presented as frequencies and percents. Within-subject comparisons and correlation analyses were carried out using, respectively, non-parametric paired Wilcoxon and Spearman tests.

Results

Fifty patients were included over a 3-month period. Clinical and laboratory data are shown in table 1.

AA-induced platelet aggregation showed a low median – 6% at T1 and 8% at T2 – with a small interval in extreme values, i.e. 0–18% at T1 and T2. Col2-induced platelet aggregation gave a higher median – 37% at T1 and 38% at T2 – with a larger interval between extreme values, i.e. 4–63% at T1 and 5–62% at T2 (table 2).

At T2, the increase in TXB₂ serum levels as compared to T1 values in absolute (fig. 1a; table 3) and relative values

Table 3. Statistical comparison between T0, T1, and T2 values

	p (T1-T0 absolute values)	p (T2-T1 absolute values)	p (T2-T1 relative values)
AA, %T	<0.95	<0.48	<0.59
C2, %T	<0.26	<0.37	<0.15
C2/C20, %	<0.096	<0.17	<0.037
TXB ₂ , pg/ml	<0.001	<0.001	<0.001

Wilcoxon paired test with the significance level set to $p < 0.05$.

was found to be highly significant ($p < 0.001$). The increase in the Col2/20 ratio was found to be significant only in relative values ($p = 0.037$) (fig. 1b; table 3). No correlation was demonstrated between variations in TXB₂ and Col2/20 ratio values.

Before inclusion in this study, 18 patients were already prescribed a daily dose of aspirin. At T1, TXB₂ serum levels in absolute and relative values showed a significant decrease as compared to T0 values ($p < 0.001$). Absolute Col2/20 ratio values were not significantly lower ($p = 0.096$) (table 3).

Discussion

To the best of our knowledge, this is the first study to describe the effect of an initial oral dose of 300 mg of aspirin, administered in hospital, after an acute ischemic cerebral event.

The first observation of our results is that the first oral intake of 300 mg aspirin inhibits AA-induced platelet aggregation as measured by LTA in all patients. This test seems to be reliable to detect the action of aspirin on the production of TXA₂. Serum TXB₂ levels measured at T1 were low for all of the patients. Thus, there was no 'aspirin resistance' observed in this study. On the other hand, Col2-induced platelet aggregation showed a large variability of response in our study and could reflect interindividual variability. AA-induced platelet aggregation is a good indicator of aspirin taking, but collagen-induced platelet aggregation would be a better indicator of variable responsiveness.

In patients with coronary disease, it has been demonstrated that platelet inhibition is not constant during 24 h after a daily dose of aspirin [11], possibly due to a partial recovery of the platelet TX pathway. We also observed this in our study; there was a significant increase in TXB₂ levels at T2 compared with T1 values. This suggests that

platelets are able to recover and synthesize new TXA₂ over time after aspirin intake. Nevertheless, although COX1 inhibition by aspirin is considered irreversible, residual TXA₂ production could be dependent on newly formed platelets, also called reticulated platelets, associated with diminished antiplatelet effects of aspirin. They could synthesize COX1 and COX2 after exposure to aspirin due to the presence of mRNA [12]. Furthermore, platelet turnover is accelerated in inflammatory states and with extensive atherosclerosis [13], which is a common condition during acute ischemic stroke. There are other sources of TXA₂. COX2, an inducible enzyme present in platelets but also found in nucleated cells, is responsible for the generation of prostaglandin H₂, a precursor of TXA₂ [14, 15]. COX2 is induced by inflammation and could worsen cerebral ischemia [16]. Some mechanisms might prevent the access of aspirin to COX 1: diabetes mellitus by increasing protein glycation [17] and NSAID by a reversible binding to COX 1 [3]. The role of the polymorphism of the COX1 gene in aspirin's effect is still under debate [18–20]. The recovery of platelet function over time was also shown by analysis of collagen-induced platelet aggregation. The significant increase in the Col2/20 ratio would suggest that collagen-induced platelet aggregation would be a good parameter of platelet sensitivity to aspirin.

Many clinicians prescribe the first 300-mg oral dose of aspirin during the acute phase of ischemic stroke even if the patient is already taking a lower daily dose. The usefulness of this approach has never been evaluated. Comparison of serum TXB₂ levels and platelet-induced aggregation at T0 and T1 (table 3) in the 18 patients already taking daily aspirin showed that serum TXB₂ levels are significantly decreased at T1 as compared to T0. The Col2/20 ratio is also decreased, though not significantly, probably due to the small number of patients. We conclude that the first oral 300-mg dose of aspirin would be able to complete TXA₂ pathway inhibition in patients with a lower daily dose of aspirin.

Alternative antiplatelet treatment during the acute phase of ischemic stroke should be proposed [21, 22]. For example, increasing the frequency of aspirin administration during first days following cerebral ischemic events could prevent platelet recovery and clinical recurrences. Terutroban, a selective TXA₂ receptor antagonist, could have been a solution to inhibit the effect of residual TXA₂ synthesis but no significant difference was found in secondary prevention of ischemic events when compared with aspirin. However, a significant decrease was noted for patients assigned to terutroban after ischemic stroke despite aspirin treatment [23, 24].

Conclusion

The first oral dose of 300 mg of aspirin administered in hospital after an acute cerebral ischemic event was sufficient to inhibit platelets and to complete TXA₂ pathway inhibition in patients with lower daily dose of aspirin. However, recovery of platelet activity during the 24 h fol-

lowing the treatment was observed and could be responsible for early ischemic recurrences. Easier and faster tests using collagen should be developed to evaluate platelet sensitivity to aspirin for every patient in this situation. New therapeutic strategies during this phase should be proposed and evaluated.

References

- Patrono C, Baigent C, Hirsh J, Roth G: Antiplatelet drugs: American College of Chest Physicians Evidence-Based Clinical Practice Guidelines (8th Edition). *Chest* 2008;133:199–233.
- Bousser MG: Antithrombotic agents in the prevention of ischemic stroke. *Cerebrovasc Dis* 2009;27:12–19.
- Catella-Lawson F: Vascular biology of thrombosis: platelet-vessel wall interactions and aspirin effects. *Neurology* 2001;57:5–7.
- Hohlfeld T, Weber AA, Junghans U, Schumacher M, Boucher M, Schror K, Siebler M: Variable platelet response to aspirin in patients with ischemic stroke. *Cerebrovasc Dis* 2007;24:43–50.
- Sandercock PA, Counsell C, Gubitz GJ, Tseng MC: Antiplatelet therapy for acute ischaemic stroke. *Cochrane Database Syst Rev* 2008, p CD000028.
- Schwammenthal Y, Tsabari R, Shenkman B, Schwartz R, Matetzky S, Lubetsky A, Orion D, Israeli-Korn S, Chapman J, Savion N, Varon D, Tanne D: Aspirin responsiveness in acute brain ischaemia: association with stroke severity and clinical outcome. *Cerebrovasc Dis* 2008;25:355–361.
- International Stroke Trial Collaborative Group: The International Stroke Trial (IST): a randomised trial of aspirin, subcutaneous heparin, both, or neither among 19,435 patients with acute ischaemic stroke. *Lancet* 1997;349:1569–1581.
- CAST (Chinese Acute Stroke Trial) Collaborative Group: CAST: randomised placebo-controlled trial of early aspirin use in 20,000 patients with acute ischaemic stroke. *Lancet* 1997;349:1641–1649.
- Petty GW, Brown RD Jr, Whisnant JP, Sicks JD, O'Fallon WM, Wiebers DO: Survival and recurrence after first cerebral infarction: a population-based study in Rochester, Minnesota, 1975 through 1989. *Neurology* 1998;50:208–216.
- Coull AJ, Lovett JK, Rothwell PM, Oxford Vascular Study: Population based study of early risk of stroke after transient ischaemic attack or minor stroke: implications for public education and organisation of services. *BMJ* 2004;328:326.
- Henry P, Vermillet A, Boval B, Guyetand C, Petroni T, Dillinger JG, Sideris G, Sollier CB, Drouet L: 24-hour time-dependent aspirin efficacy in patients with stable coronary artery disease. *Thromb Haemost* 2011;105:336–344.
- Guthikonda S, Lev EI, Patel R, Delao T, Bergeron AL, Dong JF, Kleiman NS: Reticulated platelets and uninhibited COX-1 and COX-2 decrease the antiplatelet effects of aspirin. *J Thromb Haemost* 2007;5:490–496.
- Lucas AR, Korol R, Pepine CJ: Inflammation in atherosclerosis: some thoughts about acute coronary syndromes. *Circulation* 2006;113:728–732.
- Patrignani P: Aspirin insensitive eicosanoid biosynthesis in cardiovascular disease. *Thromb Res* 2003;110:281–286.
- Cipollone F, Rocca B, Patrono C: Cyclooxygenase-2 expression and inhibition in atherothrombosis. *Arterioscler Thromb Vasc Biol* 2004;24:246–255.
- Dore S, Otsuka T, Mito T, Sugo N, Hand T, Wu L, Hurn PD, Traystman RJ, Andreasson K: Neuronal overexpression of cyclooxygenase-2 increases cerebral infarction. *Ann Neurol* 2003;54:155–162.
- Watala C, Golanski J, Pluta J, Boncler M, Rozalski M, Luzak B, Kropiwnicka A, Drzewoski J: Reduced sensitivity of platelets from type 2 diabetic patients to acetylsalicylic acid (aspirin): its relation to metabolic control. *Thromb Res* 2004;113:101–113.
- Momary KM, Shapiro NL, Brace LD, Shord SS, Grossi E, Viana MA, Helgason CM, Nutescu EA, Cavallari LH: Influence of cyclooxygenase-1 genotype on ex vivo aspirin response in patients at risk for stroke. *Cerebrovasc Dis* 2009;27:509–518.
- Maree AO, Curtin RJ, Chubb A, Dolan C, Cox D, O'Brien J, Crean P, Shields DC, Fitzgerald DJ: Cyclooxygenase-1 haplotype modulates platelet response to aspirin. *J Thromb Haemost* 2005;3:2340–2345.
- Hillarp A, Palmqvist B, Lethagen S, Villoutreix BO, Mattiasson I: Mutations within the cyclooxygenase-1 gene in aspirin non-responders with recurrence of stroke. *Thromb Res* 2003;112:275–283.
- King A, Bath PM, Markus HS: Clopidogrel versus dipyridamole in addition to aspirin in reducing embolization detected with ambulatory transcranial Doppler: a randomized trial. *Stroke* 2011;42:650–655.
- Hankey GJ, Eikelboom JW: Adding aspirin to clopidogrel after TIA and ischemic stroke: benefits do not match risks. *Neurology* 2005;64:1117–1121.
- Bousser MG, Amarenco P, Chamorro A, Fisher M, Ford I, Fox KM, Hennerici MG, Mattle HP, Rothwell PM, de Cordoue A, Fratacci MD, PERFORM Study Investigators: Terutroban versus aspirin in patients with cerebral ischaemic events (PERFORM): a randomised, double-blind, parallel-group trial. *Lancet* 2011;377:2013–2022.
- Bal Dit Sollier C, Crassard I, Simoneau G, Bergmann JF, Boussier MG, Drouet L: Effect of the thromboxane prostaglandin receptor antagonist terutroban on arterial thrombogenesis after repeated administration in patients treated for the prevention of ischemic stroke. *Cerebrovasc Dis* 2009;28:505–513.