Combination Therapy With Low-Dose Aspirin and Ticlopidine in Cerebral Ischemia

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We compared combination therapy with low-dose aspirin plus ticlopidine to therapy with aspirin alone or ticlopidine alone in patients suffering transient ischemic attack or cerebral infarction. In 17, 24, and 23 patients, respectively, 300 mg/day aspirin, 200 mg/day ticlopidine, and 81 mg/day aspirin plus 100 mg/day ticlopidine were administered orally. Aspirin alone markedly inhibited platelet aggregation induced by arachidonic acid, partially inhibited platelet aggregation induced by adenosine diphosphate, and did not inhibit platelet aggregation induced by platelet activating factor. Ticlopidine alone inhibited platelet aggregation induced by adenosine diphosphate and platelet activating factor, but did not inhibit platelet aggregation induced by arachidonic acid. Combination therapy with aspirin plus ticlopidine markedly inhibited platelet aggregation induced by all three agonists. Plasma concentrations of β-thromboglobulin and platelet factor 4 remained unchanged by aspirin alone, were slightly reduced by ticlopidine alone, and were markedly reduced by aspirin plus ticlopidine. Plasma concentration of thromboxane B2 was reduced by aspirin alone or with ticlopidine, but not by ticlopidine alone. The level of 6-ketoprostaglandin F1α was reduced only by aspirin alone. Bleeding time was significantly prolonged by aspirin alone and by ticlopidine alone, although the greatest prolongation was produced by aspirin plus ticlopidine. Our results indicate that the combination of aspirin plus ticlopidine is a potent antiplatelet strategy, although the clinical importance of the changes observed need to be determined by a properly designed and controlled prospective study. (Stroke 1989;20:1643-1647)

Antiplatelet agents have been used for the prevention of cerebral ischemia,1-6 and aspirin1-4 and ticlopidine5-6 are the agents most widely used in Japan. A mean±SD 22±5% reduction in stroke or vascular death brought about by these agents has been shown in previous randomized trials.4 Conversely, however, the trials show that these agents failed to prevent 73-83% of subsequent strokes and deaths. This partial failure in stroke prophylaxis might be attributable, at least in part, to these agents' incomplete inhibition of the multiple pathways leading to platelet aggregation. It is known that there are three different pathways leading to platelet aggregation.7,8 The chemical mediators for these pathways are adenosine diphosphate (ADP), arachidonic acid (AA), and platelet activating factor (PAF).7,8 Aspirin completely inhibits platelet aggregation induced by AA but inhibits only secondary platelet aggregation induced by ADP and PAF. On the other hand, ticlopidine inhibits both primary and secondary platelet aggregation induced by ADP and PAF, but its inhibition of platelet aggregation induced by AA is minimal.8

We investigated combination therapy with aspirin plus ticlopidine since this combination can block all three pathways leading to platelet aggregation and may have a more effective antiplatelet activity than therapy with either aspirin or ticlopidine alone.

Subjects and Methods

We entered 72 patients who presented to our hospital between January 1986 and March 1988 into this study. The patients were randomly allocated to one of three groups (24 patients in each group). However, seven patients from the first group and one patient from the third group dropped out of the study. Four patients from the first group had to discontinue medication due to gastrointestinal symp-
tomts, three patients from the first group failed to attend the follow-up examination, and the patient from the third group discontinued medication after developing a skin rash.

Patients eventually assessed included 23 with transient ischemic attacks (TIAs) (14 men and 9 women, mean age 60 [range 38–89] years) and 42 with cerebral infarction (28 men and 14 women, mean age 64 [range 39–87] years) who had not taken any antiplatelet agent for at least 2 weeks prior to entry into the study. In the first group, 300 mg aspirin (150 mg aspirin granules b.i.d.) alone was administered orally to 17 patients (six with TIA and 11 with cerebral infarction, 10 men and seven women, mean age 64 [range 40–89] years). In the second group, 200 mg ticlopidine (one 100-µg tablet b.i.d.) alone was administered to 24 patients (nine with TIA and 15 with cerebral infarction, 16 men and eight women, mean age 62 [range 38–87] years). In the third group, both aspirin (one 81-µg Bufferin tablet for children [Bristol-Myers Co., New York, New York] once a day) and ticlopidine (one 100-µg tablet once a day) were administered orally to 23 patients (eight with TIA and 15 with cerebral infarction, 15 men and eight women, mean age 64 [range 41–80] years). The patients were given these agents daily for 3–30 months.

Platelet aggregation was measured in all patients by adding 2 µM ADP (Niko Bioscience, Tokyo, Japan), 0.4 mM AA (Nakarai Kagaku, Tokyo, Japan), or 0.2 µM PAF (Funakoshi Yakuin, Tokyo, Japan) to platelet-rich plasma with a platelet count of approximately 2×10⁵ cells/µl and using an aggregometer (NKK Hmatracer I, Niko Bioscience) based on turbidimetry as described previously. The extent of platelet aggregation in platelet-rich plasma was expressed as the percent change in light transmission 5 minutes after adding the agonist. Platelet aggregation in whole blood was measured by stimulation with 10 µM ADP, 1 mM AA, or 20 µM PAF using an impedance aggregometer (Chrono-Log C540, Haverton, Pennsylvania). The extent of platelet aggregation in whole blood was expressed as the impedance change (Ω) 5 minutes after adding the agonist. Platelet-aggregation was measured both before and 1 week after starting medication.

Concentrations of β-thromboglobulin (βTG) and platelet factor 4 (PF4) in all patients were quantified by radioimmunoassay (RIA) using kits (Kaken Seiyaku, Tokyo, Japan) and by radioimmunoassay (RIA) using kits (Kaken Seiyaku, Tokyo, Japan) and by radioimmunoassay (RIA) using kits (Kaken Seiyaku, Tokyo, Japan). The results were expressed as the mean±1 standard deviation (SD) and were analyzed by Student’s dependent t test to compare results after medication with those before medication. In addition, one-way analysis of variance was used to compare the three treatment regimens.

Results

There were no significant differences in platelet aggregation or function before medication. In platelet-rich plasma, aspirin alone inhibited platelet aggregation induced by AA significantly more than did ticlopidine alone (p<0.01) but only partially inhibited platelet aggregation induced by ADP (p<0.05) and did not significantly affect platelet aggregation induced by PAF (Table 1). Ticlopidine alone markedly (significantly more than aspirin, p<0.01) inhibited platelet aggregation induced by ADP and PAF but did not significantly affect platelet aggregation induced by AA. Finally, aspirin plus ticlopidine inhibited platelet aggregation induced by all three agonists. No synergism or additive effect of aspirin and ticlopidine was observed for the inhibition of platelet aggregation induced by any of the agonists because there was no significant difference in the inhibition of platelet aggregation between aspirin alone or ticlopidine alone and aspirin plus ticlopidine. Results obtained in whole blood were similar to those obtained in platelet-rich plasma, although the inhibition of platelet aggregation induced by ADP and PAF appeared to be less pronounced in patients treated with aspirin alone and more pronounced in patients treated with ticlopidine alone.

Plasma concentrations of βTG and PF4 remained unchanged by aspirin alone (Table 2), but both were significantly reduced by ticlopidine alone, and an additional effect of aspirin when used concomitantly with ticlopidine was observed for βTG (p<0.05) but not for PF4. Plasma TXB₂ levels were...
greatly reduced by aspirin alone or with ticlopidine but not by ticlopidine alone (Table 2). No additional effect of ticlopidine was observed when used concomitantly with aspirin. Plasma 6-ketoPGF₁α levels were significantly reduced only by aspirin alone. Bleeding time was significantly prolonged in patients treated with aspirin alone or ticlopidine alone, although the greatest prolongation was observed in patients treated with aspirin plus ticlopidine (p<0.05 vs. single agent) (Table 2).

Platelet aggregation induced by ADP was inhibited in all eight patients with complications tested, whereas platelet aggregation induced by AA and PAF was inhibited in seven and six patients, respectively. The amount of blood lost was always small, and there was no significant fall of the hematocrit in any patient. No patient required a blood transfusion. The hemorrhagic complications subsided within a few days after discontinuation of the antiplatelet agents, and there was no evidence of intracranial hemorrhage in any patient.

Discussion

It has been shown by American,1 Canadian,2 and French3 studies that aspirin can reduce the incidence of subsequent stroke and death in patients with TIA1-3 or even cerebral infarction.3 The overview analysis of nine randomized trials (including those above) of aspirin for patients with ischemic stroke performed by the Antiplatelet Trialists' Collaboration showed that the risk reduction in the incidence of stroke, myocardial infarction, or vascular death produced by aspirin was 22% (SD 5%). This limited efficacy of aspirin appears to be at least in part attributable to its limited inhibition of the multiple pathways leading to platelet aggregation.7,8 We demonstrate that aspirin completely blocks platelet aggregation induced by AA, while it only partially inhibits platelet aggregation induced by ADP and has very little effect on platelet aggregation induced by PAF.

| Table 2. Effects of Aspirin and/or Ticlopidine on Platelet Function |
|---------------------------|---------------------------|---------------------------|
| Platelet function test    | Aspirin alone (300 mg)    | Ticlopidine alone (200 mg) | Aspirin plus ticlopidine (81 mg plus 100 mg) |
|                          | n  | Before | After | n  | Before | After | n  | Before | After |
| β-Thromboglobulin (ng/ml) | 17 | 89±80  | 70±64 | 24 | 107±77 | 68±49* | 23 | 91±63 | 30±19* |
| Platelet factor 4 (ng/ml) | 17 | 48±43  | 38±39 | 24 | 61±52 | 31±27* | 23 | 61±52 | 31±27* |
| Thromboxane B₂ (pg/ml)   | 8  | 246±143| 82±76†| 15 | 233±183| 218±145| 17 | 215±149| 78±65†|
| 6-Ketoprostaglandin F₁α (pg/ml) | 8  | 40±17  | 24±15†| 15 | 30±16  | 32±14 | 17 | 32±15  | 28±15  |
| Bleeding time (sec)       | 9  | 247±60 | 399±112*| 16 | 278±100| 459±92*| 18 | 260±101| 615±271†|

Data are mean±SD.

*p<0.05, 0.01, respectively, different from before by Student's dependent t test.
Ticlopidine has been reported to activate adenylate cyclase, increasing the concentration of cyclic adenine-3',5'-monophosphate (cAMP). This effect is independent of the ability to inhibit platelet aggregation induced by ADP or AA. The correction of platelet aggregation induced by ADP was inhibited in patients with multiple episodes of TIA or reversible ischemic neurologic deficit (RIND). That pilot study was followed by a double-blind multicenter trial with ticlopidine and aspirin in patients with single or multiple TIA. The reduction in the incidence of subsequent stroke and myocardial infarction was not different between patients treated with 200 mg ticlopidine and those treated with 500 mg aspirin until 6 months after entry into the trial, but the reduction was significantly greater in patients treated with ticlopidine than in those treated with aspirin 12 and 36 months after entry. Two very large multicenter studies on the efficacy of ticlopidine in patients with cerebral ischemia were recently performed in North America. One study involved >3,000 patients with TIA or RIND (Ticlopidine-Aspirin Stroke Study, TASS). 500 mg/day ticlopidine or 1,300 mg/day aspirin was administered in a double-blind trial for 2–6 (mean 3.3) years. The cumulative event rate for 3 years was significantly lower in the ticlopidine-treated group than in the aspirin-treated group. In the other large trial, 1,000 patients with a completed thromboembolic stroke received 500 mg/day ticlopidine or placebo for up to 3 (average 2) years (Canadian-American Stroke Study, CATS). 19-20 A 30% risk reduction of stroke, myocardial infarction, and vascular death was observed in the ticlopidine-treated group compared with the placebo-treated group.

The mode of action of ticlopidine in inhibiting platelet aggregation is not as clearly understood as that of aspirin, which is known to inhibit cyclooxygenase, thereby inhibiting the synthesis of thromboxane A2, a potent platelet-aggregating substance. Ticlopidine has been reported to activate adenylyl cyclase, increasing the concentration of cyclic adenosine monophosphate and leading to the inhibition of platelet aggregation or to produce a thrombocytopenic state by inhibiting the binding of fibrinogen to platelets. Ticlopidine can inhibit both primary and secondary platelet aggregation in response to ADP and PAF, but its inhibition of platelet aggregation in response to AA is not prominent since ticlopidine has no effect on cyclooxygenase. This was confirmed by our present study.

In our previous study we investigated the relation between stroke recurrence and platelet aggregation in patients treated with aspirin or ticlopidine. Platelet aggregation induced by ADP was significantly inhibited after administration of these drugs in the group of patients without recurrence, but aggregation was not significantly inhibited in the group of patients who had a recurrent stroke. However, stroke still recurred despite the inhibition of platelet aggregation induced by ADP in some patients. One possible explanation for this discrepancy might be the presence of intact pathways for platelet aggregation via AA and/or PAF, although this could not be proven since platelet aggregation induced by AA and PAF was not measured in that study.

Therefore, we tried combination therapy with aspirin plus ticlopidine, which can inhibit all the pathways leading to platelet aggregation via ADP, AA, and PAF. The combination of aspirin plus ticlopidine inhibited platelet aggregation by all three agonists. Combination therapy also markedly reduced concentration of β2G and PF4, whereas aspirin alone or ticlopidine alone did not affect or only slightly reduced them. This shows that aspirin combined with ticlopidine can suppress in vivo platelet secretion, which cannot be suppressed when only one or two of the pathways leading to platelet aggregation are inhibited by aspirin alone or ticlopidine alone. In addition, the combination of aspirin plus ticlopidine produced a greater prolongation of bleeding time than did aspirin alone or ticlopidine alone. This is additional evidence that the combination can inhibit in vivo platelet function by inhibiting all pathways leading to platelet aggregation. Moreover, platelet survival was prolonged and platelet lysis was reduced after treatment with aspirin plus ticlopidine despite the fact that neither measure of platelet function was significantly altered after treatment with aspirin alone or ticlopidine alone. The correction of platelet survival and lysis by the combination therapy might reflect inhibition of the consumption and destruction of platelets in vivo. These results indicate that the combination of aspirin plus ticlopidine is a potent antiplatelet strategy, although the clinical importance of the changes that we observed need to be determined by a

We previously performed the first trial of ticlopidine for the secondary prevention of TIA. The dose of ticlopidine used in that trial was 200 mg/day (one 100-mg tablet b.i.d.), which is the usual dose in Japan, enough to suppress platelet aggregation in the average Japanese. In that study we demonstrated that the incidence of ischemic events was reduced by ticlopidine as well as that platelet aggregation induced by ADP was inhibited in patients with multiple episodes of TIA or reversible ischemic neurologic deficit (RIND). That pilot study was followed by a double-blind multicenter trial with ticlopidine and aspirin in patients with single or multiple TIA. The reduction in the incidence of subsequent stroke and myocardial infarction was not different between patients treated with 200 mg ticlopidine and those treated with 500 mg aspirin until 6 months after entry into the trial, but the reduction was significantly greater in patients treated with ticlopidine than in those treated with aspirin 12 and 36 months after entry. Two very large multicenter studies on the efficacy of ticlopidine in patients with cerebral ischemia were recently performed in North America. One study involved >3,000 patients with TIA or RIND (Ticlopidine-Aspirin Stroke Study, TASS). 500 mg/day ticlopidine or 1,300 mg/day aspirin was administered in a double-blind trial for 2–6 (mean 3.3) years. The cumulative event rate for 3 years was significantly lower in the ticlopidine-treated group than in the aspirin-treated group. In the other large trial, 1,000 patients with a completed thromboembolic stroke received 500 mg/day ticlopidine or placebo for up to 3 (average 2) years (Canadian-American Stroke Study, CATS). A 30% risk reduction of stroke, myocardial infarction, and vascular death was observed in the ticlopidine-treated group compared with the placebo-treated group.

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properly designed prospective controlled study for
the secondary prevention of ischemic stroke.

On the other hand, hemorrhagic complications
were observed more frequently among patients
treated with aspirin plus ticlopidine than among
those treated with aspirin alone or ticlopidine alone,
otherwise the smaller doses we used since the combi-
nation of 300 mg/day aspirin plus 200 mg/day ticlo-
pidine had produced frequent hemorrhagic compli-
cations in a preliminary study. Optimal doses of
aspirin and ticlopidine in combination should be
determined by further investigations to minimize
the hemorrhagic complications while preserving the
antithrombotic effect.

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