Antiplatelet Loading Improves Behavioral Outcome in a Rabbit Model of Stroke

Dawn M. Meyer, PhD; Peggy Compton, PhD; Jo-Ann Eastwood, PhD; Karen Gylys, PhD; Justin A. Zivin, MD, PhD

Background and Purpose—No approved acute therapy exists for thousands of patients with ischemic stroke who present ineligible for thrombolitics. The purpose of this proof-of-concept study was to evaluate the efficacy of acute antiplatelet loading on stroke outcome in the rabbit small clot embolic model.

Methods—Sixty male New Zealand white rabbits were embolized via small clots into the middle cerebral artery. Two hours later, animals were treated with (1) aspirin (5 mg/kg; n=20); (2) usual dual antiplatelet loading (aspirin 10 mg/kg+clopidogrel 10 mg/kg; n=20); or (3) high-dose dual antiplatelet loading (aspirin 10 mg/kg+clopidogrel 30 mg/kg; n=20). The coprimary outcomes were as follows: (1) platelet inhibition and (2) behavioral outcome as measured by the P50 (milligrams of clot that leads to neurological dysfunction in 50% of animals in a group).

Results—There was a significant difference in 3-hour arachidonic acid and ADP (P<0.01); 6-hour collagen and ADP (P<0.01, P<0.01); and 24-hour collagen, arachidonic acid, and ADP (P=0.02, P<0.01, P<0.01) platelet inhibition. The behavioral outcome was significantly better in the usual dual antiplatelet loading versus aspirin group (P=0.02).

Conclusions—This study suggests that usual dual antiplatelet loading is clinically beneficial in a validated model of acute stroke. Study of usual dual antiplatelet loading in acute stroke is warranted to provide treatment to stroke victims ineligible for current therapies. (Stroke. 2013;44:3246-3248.)

Key Words: antiplatelet drugs ■ models, animal ■ stroke

Recombinant tissue plasminogen activator remains the only Food and Drug Administration–approved therapy for ischemic stroke.1 Human studies have shown improved stroke outcome with acute antiplatelet loading, with no significant risk of hemorrhage.2–4 The purpose of this proof-of-concept study was to evaluate dose-related efficacy of acute antiplatelet loading on stroke outcome by comparing the following: (1) standard treatment of aspirin (ASA; 5 mg/kg), (2) usual dose dual antiplatelet loading (UD; ASA 10 mg/kg+10 mg/kg clopidogrel), and (3) high-dose dual antiplatelet loading (HD; ASA 10 mg/kg+30 mg/kg clopidogrel). Coprimary end points were (1) platelet inhibition and (2) behavioral outcome as measured by the P50 (milligrams of clot that leads to neurological dysfunction in 50% of animals in a group) 24 hours after stroke in the rabbit small clot embolic stroke model.

Methods

This was a blinded, randomized, controlled study in the rabbit small clot embolic stroke model with male 2 to 4 kg, 1-year old, New Zealand white rabbits (n=60). All sterile surgical, embolization, histological, and behavioral outcome procedures were based on the rabbit small clot embolic stroke model techniques of Zivin and Lapchak.5–7 The University of California Los Angeles and Veterans Administration San Diego Health System Institutional Animal Care and Use Committees approved the surgical and treatment procedures. Animals (20 per group) were randomized to (1) ASA, (2) UD, or (3) HD 2 hours after embolization. The time of drug administration was adjusted to the time at which recombinant tissue-type plasminogen activator administration is no longer Food and Drug Administration approved as a treatment for acute ischemic stroke (3 hours after stroke in humans, which is the equivalent of 2 hours after embolization in rabbits).8 Care was used throughout the study to minimize pain and discomfort. Rabbits were euthanized if they showed extreme discomfort or were unable to reach food or water.

For platelet inhibition, blood was collected at prestroke baseline, hour 3, hour 6, and hour 24 after drug administration and tested in response to ADP, arachidonic acid, and collagen-induced aggregations (2.5 µmol/L, 250 µmol/L, and 12 µg/mL, respectively) via a Chrono-Log aggregometer.9 Platelet inhibition was reported as percent decrease from baseline. The P50 was used as the behavioral outcome. Neurologically normal animals have no deficits, and abnormal animals have ≥1 deficits. Sample sizes were selected to provide 80% power at a 2-tailed significance level of 0.05 with ANOVA and t test. Post hoc multiple comparison used a Dunnett T3 for non-equal variances.

Results

There were no statistically significant differences among the groups with respect to age, weight, surgical time, body temperature during surgery, or clot weight. One animal died.
after embolization but before treatment and was replaced in the study.

ANOVA showed significant difference between groups in 3-hour arachidonic acid and ADP inhibition ($F_{4,8}=4.8, P=0.01$; $F_{245.2}=245.2, P<0.01$); 6-hour collagen and ADP inhibition ($F_{18.2}=1601.5, P<0.01$); and 24-hour collagen, arachidonic acid, and ADP inhibition ($F_{4.4}=5,014.6, P<0.01$). Variances were found to be unequal among the groups. Post hoc analysis of response to collagen, arachidonic acid, and ADP is displayed in the Table.

The P50 for the incidence of behavioral deficits was significantly higher in the UD versus ASA group ($6.10±1.5$ versus $2.73±1.73$ mg; $P=0.02$) but not in the UD versus HD group ($6.10±1.5$ versus $5.8±1.38$ mg; $P=0.47$) or the ASA versus HD group ($2.73±1.73$ versus $5.8±1.38$ mg; $P=0.24$; Figure). The level of platelet inhibition was not correlated with P50 outcome ($P=0.38$).

**Discussion**

The purpose of this proof-of-concept study was to evaluate the dose-related efficacy of acute antiplatelet loading in an ischemic stroke model. This is the first nonrodent study of acute antiplatelet loading for the treatment of ischemic stroke and showed that antiplatelet loading significantly decreased platelet aggregation and resulted in better behavioral outcome than ASA alone.

HD loading showed a benefit in platelet inhibition but provided no additional behavioral benefit. Perhaps, factors other than platelet inhibition alone limit additional improvements in behavioral outcome. Furthermore, a potential for increased hemorrhage or other toxic effect could have occurred in the HD group. It was not possible to assess for intracranial hemorrhage in this model; however, future work will assess this. Also, a small effect size between the UD and HD could require a larger sample size to detect differences.

Limitations do exist. First, mechanism of action was not assessed. Second, young, healthy animals may not reflect clinical practice. Third, this model examined dual antiplatelet loading in small clot embolic strokes. The recent Clopidogrel in High-risk patients with Acute Non-disabling Cerebrovascular Events (CHANCE) study and ongoing Platelet-Oriented Inhibition in New TIA and minor ischemic stroke (POINT) study examine patient populations reflective of this model. Dual antiplatelet loading treatment must be examined in models of large artery occlusion to warrant translational study in a wider patient population. Finally, the lack of placebo-treated and clopidogrel-only–treated groups did not allow for the comparison with a true control group. These groups were not included, given the proof-of-concept study nature of the study. In clinical practice, patients with stroke receive ASA within 48 hours of transient ischemic attack or ischemic stroke as a standard of care based on American Stroke Association guidelines. Here, ASA alone served as an active control to reflect clinical practice. Evaluation of the clopidogrel-only

<p>| Table. Dunnett T3 Post Hoc Comparison of Inhibition of Platelet Aggregation |
|--------------------------------|----------------|----------------|----------------|----------------|</p>
<table>
<thead>
<tr>
<th>Inhibition of Platelet Aggregation</th>
<th>Index Group</th>
<th>Comparison Group</th>
<th>Mean Difference</th>
<th>Significance</th>
</tr>
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<tr>
<td>3 h Collagen ASA UD</td>
<td>−0.90</td>
<td>0.97</td>
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<tr>
<td>ASA HD</td>
<td>2.50</td>
<td>0.52</td>
<td></td>
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<tr>
<td>UD HD</td>
<td>1.98</td>
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<td></td>
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<tr>
<td>AA ASA UD</td>
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<td>ASA HD</td>
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<tr>
<td>UD HD</td>
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<td>ADP† UD HD</td>
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<tr>
<td>6 h Collagen ASA UD</td>
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<td>&lt;0.01*</td>
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<td>ASA HD</td>
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<td>&lt;0.01*</td>
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<tr>
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<td>AA ASA UD</td>
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<tr>
<td>24 h Collagen ASA UD</td>
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<td>ASA HD</td>
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<tr>
<td>UD HD</td>
<td>4.00</td>
<td>0.01*</td>
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</tr>
<tr>
<td>ADP† UD HD</td>
<td>−15.65</td>
<td>&lt;0.01*</td>
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</tbody>
</table>

ASA indicates aspirin; HD, high-dose dual antiplatelet loading; and UD, usual dose dual antiplatelet loading. *Statistically significant.
†Because the ASA group was not treated with an ADP receptor blocker, there was no ADP platelet inhibition seen from baseline at 3, 6, or 24 h.
groups would allow for testing of the hypothesis that the outcome benefit seen was because of dual antiplatelet loading and not just clopidogrel alone. With feasibility established, future studies will include these groups.

Conclusions
This study provides support for acute antiplatelet therapy treatment of ischemic stroke. This widely available, inexpensive therapy has the potential to improve patient outcome after ischemic stroke in millions of people for whom no approved, acute treatment currently exists.

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Disclosures
None.

References
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