Induction of diabetes attenuates the antithrombotic effect of clopidogrel in apolipoprotein E-deficient mice

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Background
Several studies have demonstrated that patients with diabetes mellitus (DM) exhibit an impaired platelet inhibitory response to clopidogrel. This impaired response may contribute to their increased risk of recurrent atherosclerotic events, despite the use of dual antiplatelet therapy. However, the mechanisms for the impaired response have not been fully elucidated.

 Aim
To compare the effects of clopidogrel on thrombus formation in wild type (WT), apolipoprotein E (apoE)-deficient and diabetic apoE-deficient mice.

 Methods
• DM was induced by injection of streptozotocin (STZ) at 55 mg/kg/day (ip, x 5 days) in 9-week old mice (n=10/each group). Anti-thrombotic effects of clopidogrel at 10 mg/kg/day (p.o., x 5 days) were determined in a mouse model of carotid artery thrombosis induced by FeCl3 at 21 weeks.
• Carotid artery blood flow was monitored and the time in seconds to first occlusion was determined.
• In addition, the FeCl3-injured regions of the carotid arteries were perfused, collected and further fixed in 10% neutral-buffered formalin and the thrombotic areas and lumen stenosis were quantitated.
• Antiplatelet effects on ADP-activated GP Ib/IIa and PAR4 TRAP-induced P-selectin expression were also determined by flow cytometry.

 Results
• Blood biochemistry values revealed a significant increase in glucose and unmeasurable insulin levels in diabetic apoE-deficient mice compared with those of WT and non-diabetic apoE-deficient mice, thus indicating induction of DM. Clopidogrel has no effects on biochemical values in any mouse groups (Table 1).
• Similar anti-thrombotic effects of clopidogrel were observed in WT and apoE-deficient mice. However, in diabetic apoE-deficient mice, clopidogrel’s effects were attenuated compared to WT and non-diabetic apoE-deficient mice:
  1. Percent inhibition of thrombus area (µm²) by clopidogrel was 85.5% for WT, 75.0% for apoE-deficient and 1.9% for diabetic apoE-deficient mice (Fig. 1).
  2. The time to occlusion and lumen stenosis also reflected significant losses of clopidogrel’s anti-thrombotic effects in diabetic apoE-deficient mice (Figs. 1 and 2).
• Ex vivo platelet reactivity, assessed by ADP-induced activated GP Ib/IIa expression, was inhibited similarly and completely by clopidogrel in all three groups (Fig. 3), whereas the effects of clopidogrel on ex vivo platelet P-selectin expression induced by PAR4-TRAP was diminished in diabetic apoE-deficient mice compared to WT and non-diabetic apoE-deficient mice (Fig. 4).

 Table 1: Effects of clopidogrel on blood biochemistry values in wild type, apoE-deficient, and diabetic apoE-deficient mice.

<table>
<thead>
<tr>
<th>Mice</th>
<th>Compounds</th>
<th>n</th>
<th>Insulin (mg/mL)</th>
<th>Glucose (mg/dL)</th>
<th>Total cholesterol (mg/dL)</th>
<th>Triglyceride (mg/dL)</th>
<th>Free fatty acid (mEq/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wild type</td>
<td>Vehicle</td>
<td>10</td>
<td>1.1 ± 0.1</td>
<td>329 ± 32</td>
<td>102.8 ± 3.1</td>
<td>77.2 ± 11.4</td>
<td>1.03 ± 0.05</td>
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<tr>
<td>Clomipogrel</td>
<td></td>
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<tr>
<td>1.3 ± 0.2</td>
<td>297 ± 15</td>
<td>99.2 ± 2.4</td>
<td>73.3 ± 9.0</td>
<td>0.96 ± 0.03</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>ApoE-deficient</td>
<td>Vehicle</td>
<td>10</td>
<td>0.4 ± 0.1 **</td>
<td>261 ± 25</td>
<td>667.6 ± 37.8 ***</td>
<td>144.1 ± 5.6 ***</td>
<td>1.58 ± 0.12 ***</td>
</tr>
<tr>
<td>Clomipogrel</td>
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<tr>
<td>0.7 ± 0.1 **</td>
<td>279 ± 32</td>
<td>696.6 ± 28.0 ***</td>
<td>143.9 ± 9.9 ***</td>
<td>1.49 ± 0.05 ***</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Diabetic apoE-deficient</td>
<td>Vehicle</td>
<td>10</td>
<td>0.0 ± 0.0 ***</td>
<td>548 ± 15 ***</td>
<td>1671.1 ± 149.7 ***</td>
<td>192.5 ± 18.6 ***</td>
<td>3.54 ± 0.54 ***</td>
</tr>
<tr>
<td>Clomipogrel</td>
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</tr>
<tr>
<td>0.0 ± 0.0 ***</td>
<td>557 ± 20 ***</td>
<td>1714.4 ± 156.2 ***</td>
<td>190.6 ± 20.2 ***</td>
<td>3.39 ± 0.56 ***</td>
<td></td>
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</tbody>
</table>

There was no statistical difference between any vehicle and clopidogrel groups in WT, apoE-deficient and STZ-induced diabetic apoE-deficient mice. *p < 0.01, **p < 0.001 vs. each WT group.

Conclusion
The present study is the first to describe a mouse model for studying diabetes-mediated attenuation of clopidogrel’s antithrombotic activities.
Mechanisms other than those that alter its pharmacokinetic profile may contribute to the impaired response to clopidogrel in diabetic apolipoprotein E-deficient mice.
Apolipoprotein E-deficient mice with STZ-induced diabetes provide a useful model to conduct further studies on the impaired response to clopidogrel found in patients with DM, which may in part reflect a reduction of the effect of clopidogrel on thromb-inhibited platelet activation.

Fig. 1. Effects of clopidogrel on morphometric parameters of injured carotid arteries, thrombus area (A) and lumen stenosis (B). Results are presented as the mean ± S.E. ***p < 0.001 vs. each vehicle group, **p < 0.01 (Student’s t-test), Vehicle: V, Vehicle + clopidogrel: C.

Fig. 2. Effects of clopidogrel on time to first occlusion. The results are presented as the mean ± S.E. ***p < 0.001 vs. each vehicle group; *p < 0.05 vs. the vehicle group of WT mice, *p < 0.05 (Student’s t-test), Vehicle: V, Vehicle + clopidogrel: C.

Fig. 3. Effects of clopidogrel on platelet expression of ADP-induced activated GP Ib/IIa expression. The results are presented as mean ± S.E. *p < 0.05, **p < 0.01 vs. corresponding vehicle group (Student’s t-test), Open symbols: vehicle treated groups, green symbols: clopidogrel-treated groups.

Fig. 4. Effects of clopidogrel on PAR4 TRAP-induced expression of platelet P-selectin. The results are presented as the mean ± S.E. *p < 0.05, **p < 0.01 vs. corresponding vehicle group; *p < 0.05 vs. the vehicle group of diabetic apoE-deficient mice; **p < 0.01 vs. clopidogrel group of diabetic apoE-deficient mice (Student’s t-test).

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