Ferric Chloride-Induced Arterial Thrombosis Model in Pre-Clinical Drug Development

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Abstract

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Despite recent advances in diagnosis and treatment, thromboembolic disorders are a leading cause of mortality worldwide. There is urgent need for development of more rational antithrombotic strategies. Ferric chloride ($FeCl_3$)-induced arterial thrombosis is a useful model for evaluating the efficacy of new antithrombotic drugs. In this study we optimized $FeCl_3$ concentration to better suit preclinical drug development. Carotid artery thrombosis was induced with $FeCl_3$ (2.5, 2.8, 3.0 and 3.5%, 3min) in CD-1 mice. The concentration (3.5%) that consistently induced thrombosis was used to evaluate the effects of anticoagulant and antiplatelet drugs. Mice were dosed intravenously with heparin (200 U/kg) or vehicle (saline 3.33 mL/kg) 1 minute before applying $FeCl_3$. Mice treated with Clopidogrel (3 and 10 mg/kg/dose) or vehicle (water 10 mL/kg) received oral administration 26 and 2 hours prior to $FeCl_3$. Blood flow was monitored for 60min post-FeCl3 using a Doppler flow probe and time to occlusion was defined as the time from the application of $FeCl_3$ to the cessation of blood flow for 30 seconds. Following application of $FeCl_3$, a mean time to occlusion for vehicle was 12.2±1.63 (saline) and 11.4±4.15 (water) min. All animals treated with 200 U/kg heparin and 10mg/kg Clopidogrel retained vascular patency throughout the experimental period. Seven of 9 animals (71%) treated with 3 mg/kg Clopidogrel retained vascular patency throughout the experimental period; two animals had a mean time to occlusion of 7±1.0 min. Our $FeCl_3$-induced arterial thrombosis model is a reliable method to assess the efficacy of potential antithrombotic compounds.

Introduction

Despite recent advances in diagnosis and treatment, thromboembolic disorders are a leading cause of mortality worldwide. There is urgent need for development of more rational antithrombotic strategies, with better efficacy and fewer side effects.

Ferric chloride ($FeCl_3$)-induced arterial thrombosis is a sensitive model previously used in several animal species to induce thrombosis in many sites. $FeCl_3$ applied to the exterior of vessels causes major oxidative stress with the generation of free radicals, which leads to lipid peroxidation and destruction of endothelial cells and results in occlusive thrombus formation.

The mouse carotid artery $FeCl_3$ model is a relatively simple procedure due to easy access of the carotid artery and consistent injury. Despite the large number of published data using this model, the ideal $FeCl_3$ concentration to be used for evaluating anti-thrombotic drugs remains unclear. In this study we optimized $FeCl_3$ concentration to better suit preclinical drug development.

Objectives

In this study we optimized $FeCl_3$ concentration to better suit preclinical drug development.

Methods

Experimental Plan: CD-1 mice (25-30 grams) were randomly divided into 9 groups.

Groups 1, 5, and 6- Mice received an oral dose of vehicle (water, 10 mL/kg – Group 1) or Clopidogrel (3 or 10 mg/kg, Group 5 and 6, respectively) 26 and 2 hours prior to inducing the thrombosis by applying two pieces of filter paper saturated with 3.5% $FeCl_3$ on opposite sides of the carotid artery.

Groups 2-4- Mice received a bolus injection of vehicle (phosphate buffer saline pH 7.4, 4 µL/g) on the left femoral vein 1 minute prior to inducing the thrombosis. Thrombosis was induced by topically applying two pieces of filter paper saturated with 3.0% (Group 2), 2.8% (Group 3) and 2.5% (Group 4) $FeCl_3$ on opposite sides of the carotid artery.

Groups 7-8- Mice received an oral dose of vehicle (water, 10 mL/kg – Group 7) or Ticagrelor (100 mg/kg, Group 8) one hour prior to inducing the thrombosis by applying two pieces of filter paper saturated with 3.5% $FeCl_3$ on opposite sides of the carotid artery.

Surgical Preparation: Animals were anesthetized with isoflurane using a nose cone connected to a vaporizer that delivers 1-2% isoflurane driven by 100% oxygen. Body temperature was monitored throughout the experiment. After anesthesia, a skin incision was made directly on top of the right common carotid artery region, the fascia was dissected and a segment of the left common carotid artery was exposed. A doppler flow probe (Model 0.5PSB, Transonic System) was placed in the carotid artery for blood flow measurements (*Figure 1A*). After approximately 10-15 minutes of equilibration, baseline flow measurements were collected.

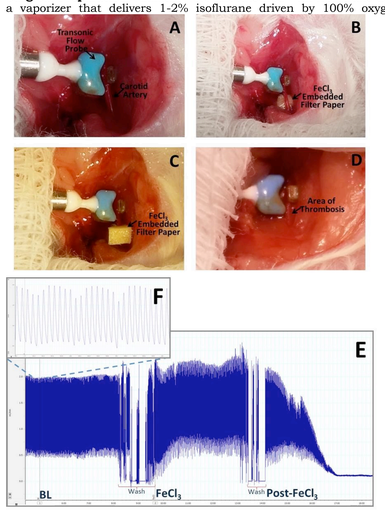

Figure 1. Mouse $FeCl_3$ Model of Carotid Artery Thrombosis. A- Carotid artery was dissected and flow probe was placed beneath the artery. B and C- A filter paper saturated with $FeCl_3$ was placed beneath and on top of the carotid artery for 3 minutes. D- Carotid artery post- $FeCl_3$ exposure showing the area of thrombosis. E- Graphical representation of blood flow waveform (LabChart – ADInstruments). F- Characteristic blood flow wave form.

Thrombosis was induced by applying two pieces of filter paper (1x2 mm - Gel Blot Paper, GB003, Schleicher and Schuell, Keene, NH, USA) saturated with $FeCl_3$ on opposite sides of the carotid artery (one beneath and one above) in contact with the adventitial surface of vessel (*Figure 1B and 1C*). Filter papers were submerged in $FeCl_3$ for 10 minutes prior to applying on the vessel; $FeCl_3$ solution was prepared for each test right before use. The filter paper was applied for 3 minutes and then removed. The vessel was washed with phosphate buffer saline and the carotid blood flow monitored up to 60 minutes following the application of the filter paper (*Figure 1D, 1E and 1F*). The occlusion time was recorded and defined as the time from the application of the filter paper to the cessation of blood flow (0 mL/min) for 30 seconds.

Statistical Methods: Comparisons for the hemodynamic parameters were made between the mean values for each group across each period using analysis of variance (ANOVA). Where ANOVA F values were significant ($p<0.05$) for the comparisons across groups, post hoc t tests were used to determine differences between treatment groups and vehicle. Comparisons were considered significant at the 0.05 level. All statistical analyses were conducted with SAS® version 9.2.

Conclusions

- A concentration-dependent effect of $FeCl_3$ on carotid arterial thrombosis in male CD-1 mice was observed by changes in the incidence of carotid artery occlusion and reflected in the carotid artery average flow.
- $FeCl_3$ concentration at 3.5% was sensitive to commercially available anti-thrombotic therapies, Clopidogrel and Ticagrelor.
- $FeCl_3$ concentration at 3.5% was also sensitive to discriminate between doses ranges.
- $FeCl_3$-induced arterial thrombosis model is a reliable method to assess the efficacy of potential antithrombotic compounds.

Concentration-dependent effects of Ferric Chloride on Carotid Arterial Thrombosis Model in CD-1 Mice

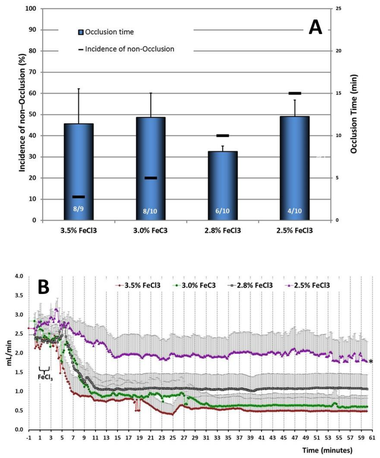

*Figure 2. Carotid artery thrombosis induced by various concentration of $FeCl_3$. A- (A) Occlusion time measurements of ferric chloride–induced thrombosis. Bar graphs represent average occlusion time and horizontal lines represent the percent of non-occlusion. Numbers inside the columns show the number of mice that occluded and the total number of mice. B- Blood flow was continuouslu monitored and data were plotted vs time. Symbols represent the mean, and the bars reflect the standard error (SE). Values represent mean ± SEM; *p=0.0002, 2.5% $FeCl_3$ group compared with 3.5% $FeCl_3$ during the post-FeCl3 period (ANOVA, post hoc t test).*

Effects of Clopidogrel on the Ferric Chloride-Induced Carotid Arterial Thrombosis Model in CD-1 Mouse

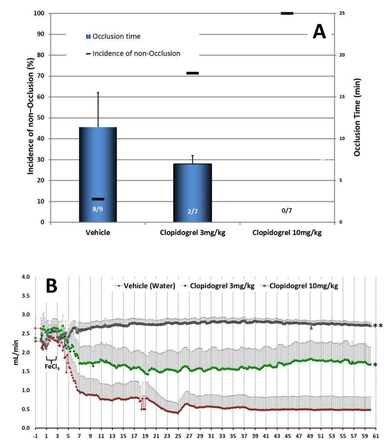

*Figure 3. Effects of Clopidogrel on thrombus formation. Mice was orally administered with Clopidogrel (3 and 10 mg/kg, n=7) or vehicle (deionized water, n=9) at 26 hours and 2 hours before applying the 3.5% $FeCl_3$ for 3 minutes. (A) Occlusion time measurements of ferric chloride–induced thrombosis. Bar graphs represent average occlusion time and horizontal lines represent the incidence of non-occlusion. Numbers inside the columns show the number of mice that occluded and the total number of mice. B- Blood flow was continuously monitored and data were plotted vs time. Symbols represent the mean, and the bars reflect the standard error (SE). Values represent mean ± SEM; * p=0.01, 3 mg/kg Clopidogrel compared with vehicle and ** p <0.0001, 10 mg/kg Clopidogrel compared with vehicle (ANOVA, post hoc t test).*

Effects of Ticagrelor on the Ferric Chloride-Induced Carotid Arterial Thrombosis Model in CD-1 Mouse

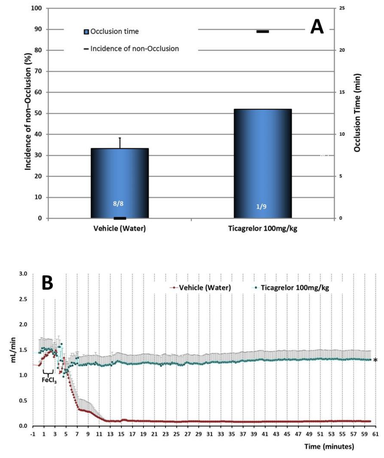

*Figure 4. Effects of Ticagrelor on thrombus formation. Mice was orally administered with Ticagrelor (100mg/kg, n=9) or vehicle (deionized water, n=8) 1 hour before applying the 3.5% $FeCl_3$ for 3 minutes. (A) Occlusion time measurements of ferric chloride–induced thrombosis. Bar graphs represent average occlusion time and horizontal lines represent the incidence of non-occlusion. Numbers inside the columns show the number of mice that occluded and the total number of mice. B- Blood flow was continuously monitored and data were plotted vs time. Symbols represent the mean, and the bars reflect the standard error (SE). Values represent mean ± SEM; * p <0.001, 100 mg/kg Ticagrelor compared with vehicle (t test).*

Summary

- At the highest concentration of $FeCl_3$ (3.5%) tested, there were 7 out of 9 animals that completely occluded within 30 minutes (11±4.2 minutes), while one animal occluded at 40 minutes post-$FeCl_3$ and one animal (11%) retained vascular patency throughout the experimental period (60 minutes).
- At 2.8% and 3.0% $FeCl_3$, there were 6 and 8 out of 10 animals respectively, that completely occluded within 30 minutes (8±0.7 and 12±2.9 minutes respectively). At 2.8% and 3.0% $FeCl_3$, 4 (40%) and 2 (20%) animals respectively, retained vascular patency throughout the experimental period (60 minutes).
- At the lowest concentration of $FeCl_3$ (2.5%) tested there were 4 out of 10 animals that completely occluded within 30 minutes (12±1.9 minutes), while 6 animals (60%) retained vascular patency throughout the experimental period (60 minutes).
- The concentration-dependent effect of $FeCl_3$ on thrombus formation was also shown by reductions in average carotid blood flow throughout the 60 minute monitoring period. Exposure to 2.5%, 2.8%, 3.0% and 3.5% $FeCl_3$ reduced blood flow by 27% (1.9±0.5 mL/min vs 2.6±0.2 mL/min), 53% (1.1±0.4 mL/min vs 2.3±0.1 mL/min), 66% (0.91±0.4 mL/min vs 2.7±0.2 mL/min) and 71% (0.76±0.4 mL/min vs 2.6±0.3 mL/min) respectively, as compared to baseline.
- 10 mg/kg/dose of Clopidogrel protected against $FeCl_3$ (3.5%) induced carotid arterial thrombosis, 7 out of 7 (100%) retained vascular patency throughout the experimental period (60 minutes). The protective effect of Clopidogrel (10 mg/kg/dose) on $FeCl_3$ inducing thrombus formation was confirmed by a constant carotid blood flow during the 60 minutes monitoring period.
- At 3 mg/kg/dose Clopidogrel 5 animals out of 7 (71%) retained vascular patency throughout the experimental period (60 minutes). Carotid blood flow was reduced by 30% at 15 minutes after exposure to 3.5% $FeCl_3$ as compared to baseline. Two animals treated with 3 mg/kg/dose Clopidogrel underwent repeated cycles of gradual reduction in flow and then abrupt increases, but stable thrombi never formed and flow returned to near baseline levels.
- 100 mg/kg/dose of Ticagrelor also protected against carotid arterial thrombosis, 8 out of 9 (89%) retained vascular patency throughout the experimental period (60 minutes). The protective effect of Ticagrelor (100 mg/kg/dose) on $FeCl_3$ inducing thrombus formation was also shown by the average carotid blood flow during the 60 minutes monitoring period. Carotid blood flow was only reduced by 11% at 15 minutes after exposure to 3.5% $FeCl_3$.