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, thrombembolic disorders remain a leading cause of mortality worldwide. There is urgent need for development of more rational antithrombotic strategies. Ferric chloride (FeCl₃) arterial thrombosis model is a reliable model for evaluating the efficacy of new antithrombotic drugs. In this study we optimized FeCl₃ injection volume and time point of administration. Carotid arterial thrombosis model was subcutaneously injected with FeCl₃ at 3.5% for 30 seconds and the results of thrombosis were examined. Seven of 9 animals (71%) treated with FeCl₃ at 3.5% for 30 seconds retained vascular patency throughout the experimental period.

Introduction

Despite recent advances in diagnosis and treatment, thrombembolic disorders remain 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 (FeCl₃)-induced arterial thrombosis is a sensitive model previously used in several animal species to induce thrombosis in many sites. FeCl₃ 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 thrombosis formation.

The mouse carotid artery FeCl₃ 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 FeCl₃ concentration to be used for evaluating antithrombotic drugs remains unclear. In this study we optimized FeCl₃ concentration to better suit preclinical drug development.

Objectives

In this study we optimized FeCl₃ concentration to better suit preclinical drug development.

Conclusions

A concentration-dependent effect of FeCl₃ 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.

FeCl₃ concentration at 3.5% was sensitive to commercially available anti-thrombotic therapies, Clopidogrel and Ticagrelor.

FeCl₃ concentration at 3.5% was also sensitive to discriminate between dose ranges.

FeCl₃-induced arterial thrombosis model is a reliable model to assess the efficacy of potential antithrombotic compounds.

Methods

Experimental Plan: CD-1 mice (20–30 gms) were randomly divided into 9 groups.

Group 1-7: Mice were treated on either side of carotid artery (3 mg/kg, Group 1) or Carotid arterial thrombosis model was induced by subcutaneous injection of FeCl₃ at 3.5% for 30 seconds, followed by applying FeCl₃. Mice were treated with either 10 mg/kg or 15 mg/kg of either variable on the left common carotid artery, respectively, 1 minute before inducing the thrombosis by applying two pieces of filter paper saturated with 3.5% FeCl₃ to opposite sides of the carotid artery.

Group 8-9: Mice were treated on either side of carotid artery (3 mg/kg Group 8 and 5 mg/kg Targapore, 10 mg/kg Group 9) one hour prior to inducing the thrombosis by applying two pieces of filter paper saturated with 3.5% FeCl₃ to opposite sides of the carotid artery.

Results:

Graphs 1 and 2: Bar graphs representing means ± SD of each group. Summary (ANOVA, post hoc t test). Figures 2 and 3: Effects of Ticagrelor on the Ferric Chloride-Induced Carotid Arterial Thrombosis Model in CD-1 Mouse.

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

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

Summary

At the lowest concentration of FeCl₃ (3.5%) tested, there were 7 out of 9 animals that completely occluded within 30 minutes (3.5±0.2 minutes), while all animals retained vascular patency throughout the experimental period (60 minutes).

At the intermediate concentration of FeCl₃ (5.0%) tested, there were 5 and 8 out of 10 animals respectively, that completely occluded within 30 minutes (2.8±0.3 minutes) and 30 minutes (2.8±0.3 minutes), respectively. Ticagrelor significantly increased the protective effect on FeCl₃-induced arterial thrombosis (p<0.0001, 3 mg/kg, p<0.01, 5 mg/kg).

At the highest concentration of FeCl₃ (7.0%) tested, there were 7 out of 10 animals that completely occluded within 30 minutes (3.5±0.3 minutes), while all animals retained vascular patency throughout the experimental period (60 minutes).

The concentration-dependent effect of FeCl₃ on arterial thrombosis was dose-dependent as differences were seen in both the total number of mice and the total number of minutes. Blood flow measurements continued and data were plotted in Figure 2. The blood flow measurements were plotted using Graph Pad Prism version 6 (Graph Pad Software, USA).

Figure 2: Effects of Ticagrelor on thrombus formation. Bar was visually monitored with Targapore (250mg/kg) or water for 30 minutes, then sacrificed and examined. Tickagrelor (100 mg/kg) on FeCl₃-induced arterial thrombosis model. Significantly reduced thrombus formation in Ticagrelor-treated mice (p<0.0001).

Figure 3: Effects of Ticagrelor on arterial thrombosis model in CD-1 mice. Ticagrelor effectively reduced thrombus formation in CD-1 mice (p<0.0001).

Figure 4: Effects of Ticagrelor on thrombus formation. Ticagrelor was administered with Targapore (250mg/kg) or water for 30 minutes, then sacrificed and examined. Ticagrelor (100 mg/kg) on FeCl₃-induced arterial thrombosis model. Significantly reduced thrombus formation in Ticagrelor-treated mice (p<0.0001).