Summary Report for the MHRA and REC

1. Study Details

Title: Study of the Effect of Ticagrelor and Clopidogrel on the Immune Response of Healthy Volunteers

Chief Investigator: Professor Robert F. Storey

ClinicalTrials.gov number: NCT01846559

CTA number: 21304/0246/0001-0001

EudraCT: 2012-00514-18

STH ref: STH17062 Protocol version 12.2

2. Introduction

Platelets are the main type of blood cell involved in the formation of blood clots that cause heart attacks. We give antiplatelet drugs (aspirin, for example) to reduce the risk of another clot forming in the future and causing another heart attack. Platelets are known to have a role in inflammation and infection as well as clotting. In a recent large clinical trial, known as the PLATO study, it was also shown that patients treated with a new antiplatelet medication (ticagrelor) developed fewer lung infections, as well as fewer heart attacks, compared to the current standard treatment (clopidogrel). We therefore investigated the reasons behind this and looked at the effect of these medications on immune responses.

3. Study hypothesis

Ticagrelor and clopidogrel differ in their effects on immune responses.

4. Aim

To identify the effects of ticagrelor and clopidogrel on the immune responses of healthy volunteers.

5. Study Design

Single-centre randomized, open-label, parallel group study assessing the modulatory effect of ticagrelor on the immune response of healthy volunteers and comparing it to that of clopidogrel and control. An immune response was induced using the well-established method of intravenous injection of *E. coli* endotoxin (lipopolysaccharide, LPS). 30 participants were recruited in total. First participant, first visit: 1/5/13. Last participant, last visit: 10/1/14. REC approval: 7/2/14. MHRA approval: 30/1/13.

5.1 Study interventions

Medications used: ticagrelor (180 mg loading dose followed by 90 mg twice daily maintenance dose) and clopidogrel (300 mg loading dose, followed by 75 mg once daily maintenance dose)

Randomization groups: ticagrelor, clopidogrel, no antiplatelet medication

Study drug accountability log maintained by Sheffield Teaching Hospitals (STH) Foundation Trust pharmacy.

5.2 Study assessments

Assessments of platelet function, including platelet aggregation, platelet P-selectin expression and platelet-leukocyte aggregate formation.

Assessment of inflammatory markers, including pro-inflammatory cytokines and hsCRP.

Assessment of coagulation, including D-dimer and measurements of fibrin clot structure.

Assessment of leukocyte expression of adhesion molecules and chemokine receptors.

5.3 Safety assessments

The administration of endotoxin (lipopolysaccharide, LPS) induced expected adverse events, including malaise, headache, tiredness, muscle aches, fevers, chills, nausea, vomiting, photophobia, shivering, yawning, neutrophillia and neutropaenia. Administration of endotoxin was not associated with any unexpected adverse events or any serious adverse events. One participant in the ticagrelor group suffered trauma during the study (performing manual work and a wall collapsed, causing a fracture of his tibia – reported as an SAE during the study). There were no other adverse events caused by clopidogrel or ticagrelor.

5.4 Study monitoring, audit and inspection

The study was monitored and audited by the sponsor STH Research and Development. STH underwent routine inspection by MHRA after the completion of the study in February 2014. The study was selected for inspection and there were no critical findings. All other findings were addressed as appropriate.

5.5 Interim and final analyses

Interim analyses did not demonstrate a requirement to terminate the study prematurely. Analyses of platelet function, inflammatory markers and coagulation were performed after completion of the study.

6. Summary of results

Lay summary of findings

After endotoxin administration, platelets joined together with white blood cells, forming clumps, called platelet-leukocyte aggregates. This process causes the white blood cells to become particularly activated and this process was reduced by antiplatelet medication.

After endotoxin administration, levels of cytokines (chemicals used by the immune system to co-ordinate responses) increased. Antiplatelet medication reduced the release of cytokines that stimulate the immune system.

After endotoxin administration, there was an increased tendency towards blood clotting, which was reduced by the antiplatelet medications.

During severe infections, the immune system is often excessively stimulated, which can cause collateral damage to host tissue and organs. Antiplatelet medications appear to reduce this excessive stimulation, which may provide benefit in severe infections.

Technical abstract

In the PLATO study, the novel platelet $P2Y_{12}$ inhibitor ticagrelor was unexpectedly associated with fewer deaths following pulmonary infections and sepsis than clopidogrel. Using an experimental human model, we therefore sought to determine the effect of ticagrelor and clopidogrel on innate

immune responses and the prothrombotic state induced by systemic inflammation. 30 healthy volunteers were randomized to ticagrelor 90 mg bd (n=10), clopidogrel 75 mg od (n=10) or no antiplatelet medication (controls; n=10) for one week. *E. coli* endotoxin was then administered intravenously. Serial blood samples were collected before randomized therapy, before endotoxin and over 24 hours after endotoxin. Platelet aggregation and platelet-monocyte aggregate formation were potently inhibited by ticagrelor. Ticagrelor markedly reduced peak levels of major pro-inflammatory cytokines; TNFα was reduced by 66% ($p<0.001$), IL-6 by 47% ($p<0.001$), IL-8 by 29% ($p=0.001$), CCL2 by 38% ($p<0.001$) and G-CSF by 51% ($p<0.001$) compared to control. Ticagrelor also reduced the peak level of D-dimer by 48% compared to control ($p<0.001$) and significantly reduced inflammation-induced changes in fibrin clot density and ultrastructure. Clopidogrel had less effect overall than ticagrelor and measures of platelet $P2Y_{12}$ reactivity correlated with changes in TNFα, D-dimer and fibrin clot structure, suggesting that the effects of ticagrelor were $P2Y_{12}$-mediated. In conclusion, ticagrelor exhibited marked suppression of multiple components of the inflammatory and prothrombotic responses to bacterial endotoxemia. The greater potency of these effects of ticagrelor compared to clopidogrel provides a potential explanation for the reduced mortality following sepsis observed in the PLATO study.

The full findings of the study are included in the manuscript that is provided as a supplement to this document (the manuscript was submitted to *Blood* journal but unfortunately was not accepted).

7. AEs and SAEs

The administration of endotoxin (lipopolysaccharide, LPS) induced expected adverse events, including malaise, headache, tiredness, muscle aches, fevers, chills, nausea, vomiting, photophobia, shivering, yawning, neutrophillia and neutropaenia. Administration of endotoxin was not associated with any unexpected adverse events or any serious adverse events. In the clopidogrel group, one participant withdrew due to slight worsening of a pre-existing, mild derangement of liver function tests. One participant in the ticagrelor group suffered trauma during the study (performing manual work and a wall collapsed, causing a fracture of his tibia – reported as an SAE during the study). There were no other adverse events caused by clopidogrel or ticagrelor.

8. Withdrawals

44 participants were assessed for eligibility. 3 were excluded as they had significant past medical history. One was excluded due to an abnormal physical examination. 5 were excluded due to abnormal screening lab tests. After randomization, in the no antiplatelet group, one participant withdrew due to the common cold and one withdrew as he no longer wished to take part. In the clopidogrel group, one participant withdrew due to regional clopidogrel supply issues and one participant withdrew due to slight worsening of a pre-existing derangement of liver function tests. In the ticagrelor group, one participant withdrew due to a traumatic injury.

9. Discussion

This study demonstrates for the first time that ticagrelor and clopidogrel markedly reduce inflammatory and prothrombotic responses to bacterial endotoxin in humans *in vivo.* Since ticagrelor and clopidogrel belong to different chemical classes, with their only known shared property being $P2Y_{12}$ receptor inhibition, our data suggest that the effects of ticagrelor and clopidogrel were due to $P2Y_{12}$ inhibition. The findings of this study are supported by previous animal models that have suggested that clopidogrel and prasugrel reduce the release of pro-inflammatory cytokines.

Sepsis is characterised by a detrimental pro-inflammatory cytokine storm that is classically associated with host tissue damage, acute organ dysfunction and increased mortality. Since

ticagrelor is a more potent $P2Y_{12}$ inhibitor than clopidogrel, greater suppression of the cytokine storm of sepsis offers a potential explanation for the improved outcome from sepsis seen in the PLATO study. Bacteraemia also greatly increases the risk of subsequent myocardial infarction and stroke. By attenuating the prothrombotic state associated with infection to a greater degree than clopidogrel, ticagrelor may have decreased the risk of subsequent atherothrombotic events in patients with pulmonary infections and sepsis in PLATO.

10. Conclusion

Ticagrelor exhibited marked suppression of multiple components of the inflammatory and prothrombotic responses to bacterial endotoxemia. The greater potency of these effects of ticagrelor compared to clopidogrel provides a potential explanation for the reduced mortality following sepsis observed in the PLATO study.

11. *Dissemination of study findings*

The findings have been presented to the Cardiovascular Patient Panel at the Northern General Hospital. In addition, the results have been presented at conferences (as detailed below) and are being submitted to high impact factor journals.

12. Conference abstracts

Thomas M, Outteridge S, Ecob R, Sangha G, Faulkner R, West L, Judge H, Dockrell D, Sabroe I and Storey R. Ticagrelor inhibits the release of pro-inflammatory cytokines TNF and IL-6 during human endotoxaemia. *European Heart Journal* 2014; 35: 1170

Mark R Thomas, Ramzi A Ajjan, Fladia Phoenix, Samuel N Outteridge, David H Dockrell, Ian Sabroe, Robert F Storey. Ticagrelor inhibits changes in fibrin clot structure induced by bacterial endotoxemia. *Journal of the American College of Cardiology* 2015; 65:10_S

13. Publication plan

Efforts are currently being made to publish the work in high impact factor journals. Unfortunately the manuscript has been rejected, largely on grounds of insufficient priority. The manuscript is currently being revised and will be submitted to other high impact factor journals.

Date: 7/5/15

Submitted by: Prof Robert Storey