TITLE OF STUDY

Downstream molecular signals of P2Y12 receptors in hyporeactive patients under clopidogrel treatment

NCT03190005 Unique Protocol ID: TCHIRB-10603117-E

PRINCIPAL INVESTIGATOR

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Methods

This study enrolled 35 patients(subgroup 1 and subgroup 2) who had undergone elective PCI and stent implantation and were receiving routine dual antiplatelet treatment (aspirin + clopidogrel). The study was registered under ClinicalTrials.gov (ID: NCT03190005) and approved by the Institutional Review Board of the Taipei City hospital Reserch Ethics Committee(ID: TCHIRB-10603117-E)

In subgroup 1

Blood samples were obtained from all study patients, and platelets were separated as previously described. The experimental procedure is described in Supplementary Figure A. PRU values were determined for control patients and for patients treated with DAPT after 7 days of treatment. Based on PRU values, patients were classified

as 1) normal reactive (defined as PRU=85-207), 2) hyporeactive (defined as PRU>208), or 3) hyper-reactive groups (defined as PRU < 85). The Western blot responses were determined for each group after treatment with the different drugs, and these values were represented as a bar graph for statistical and comparative purposes. in-vitro experiments were performed using platelet-rich plasma (PRP) samples which were treated with different combinations of drugs. These included 1) ADP (10μM), 2) PGE1 (0.1 μM), 3) aggrastat (glycoprotein IIb/IIIa receptor inhibitor; 0.25μg/ml), 4) AR-C 66096 (P2Y12 receptor antagonist; 1 μM), 5) OPC-13013 (PDE3A tyrosine kinase inhibitor; 10 μM). The control group received no drugs. Reactions were terminated by addition of Indomethacin (50 μM) and EDTA (2 mM) 4 minutes after the drugs were added. Total protein was prepared from untreated and treated samples, and subjected to Western blotting according to standard protocols.

In order to eliminate the effect of liver enzymes, we design subgroup 2:

In this subgroup 2, we developed an ex-vivo system to investigate the role of P2Y12 signaling on HOTPR in patients with different reactivities to DAPT.

PRU measurements

PRU values were determined in 2 ml blood samples using the VerifyNow test (Accumetrics, San Diego, CA, USA) according to the manufacturer's instructions. In this study HOTPR was defined as PRUs ≥208 as recently described [21].

Western blotting

Protein expression was analyzed in PRP samples obtained from patients in the different groups. Briefly, PRP was obtained from 15cc blood after addition of 1.5cc of 3.8% sodium citrate. The samples were centrifuged at 1000 rpm , and complete blood counts were performed on the PRP samples in order to determine the levels of WBC contamination. If the WBC to platelet ratio was > 0.4%, the sample was re-centrifuged at 1000 rpm for 5 minutes repeatedly until the WBC to platelet ratio was < 0.4%. Total protein was prepared from the PRP samples as described. Total proteins were separated by SDS-PAGE, and immunoblotted to evaluate the expression of p-p38, total P38, p-PKCζ, total PKCζ, p-Akt/PKB, total AKT, p-GSK3β, total GSK3β, p-VASP, total VASP, p-Syk, total syk, P2Y12 receptor, PLCβ-1, and Rap1b (Supplementary Table 1). The protein quantifications from western blot images were done using the Image J program (NIH, MD).

CERTIFICATE

Date：May 24, 2017

The project entitled "Downstream molecular signals of P2Y12 receptors in hyporeactive patients under clopidogrel treatment"submitted by investigator Chen Yueh Chung has been approved by Research Ethics Committee of the Taipei City Hospital.
Protocol Version Date: Ver1-1060215
ICF Version Date: Ver 2.1-1060524

Study period: since 01/01/2017 to 12/31/2017
Above project is approved by the TCHREC on 05/24/2017 and valid till 12/31/2017.

※ All protocols should been subject to final endorsement by the board. TCHREC has the right to revoke the approval.
※ No new protocol can be applied if the midterm report or the final report is not handed in.
※ The midterm report should be handed in one month before the expiry date of this certificate.
※ The final report should be handed in before the expiry date of this certificate or project end.
※ The PI should send the amendments to TCHREC at least six weeks before the expiry date of this certificate if there are changes or modification related the protocol. Any change should not be executed until being approved by TCHREC.

Oscar Lee
Chairman
Taipei City Hospital Research Ethics Committee

臺北市立聯合醫院 TPECH 人體研究倫理審查會

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