Flow cytometric assessment of VASP phosphorylation: an index of the efficacy of clopidogrel in patients with atherothrombotic diseases

Aleil B.1, Ravanat C.1, Cazenave J.-P.1, Rochoux G.2, Heitz A.2, Gachet C.1

1 INSERM U.311, Etablissement Français du Sang-Alsace, Strasbourg, France. 2 Service de Cardiologie, Clinique de l’Orangerie, Strasbourg, France.

Introduction
Clopidogrel is an effective antiplatelet agent widely used in the prevention of thrombotic complications in atherosclerotic diseases and interventional cardiology. It is a prodrug which must be metabolised in the liver to acquire antiaggregatory properties. However, since clinical thrombosis still occurs in 5 to 10 % of patients on clopidogrel while platelet aggregation remains unchanged in up to 30 % of such patients, clopidogrel resistance would appear to exist. A reliable laboratory test is therefore needed to identify patients insufficiently protected by clopidogrel. The phosphorylation of vasodilator-stimulated phospho-protein (VASP), an intraplatelet actin regulatory protein, is dependent on the level of activation of the platelet P2Y12 receptor, which is targeted by clopidogrel. The aim of this study was to use a VASP phosphorylation (VASP-P) assay to evaluate the efficacy of clopidogrel therapy for the prevention of platelet activation in patients presenting atherothrombotic diseases.

Methods

**In vitro inhibition of P2Y12 receptors by AR-C69931MX**

Direct inhibition of the P2Y12 receptors by the competitive P2Y12 antagonist AR-C69931MX induced a concentration-dependent decrease in the platelet aggregation whereas the dephosphorylation of VASP in platelets stimulated by ADP decreased as the concentrations of AR-C69931MX increased.

**Conclusion**

The flow cytometric VASP-P state expressed as platelet reactivity index was likewise highly correlated with the inhibition of platelet aggregation induced by the specific P2Y12 antagonist AR-C69931MX.

**Correlation between platelet aggregation and PRI**

The flow cytometric VASP phosphorylation state expressed as platelet reactivity index was likewise highly correlated with the inhibition of platelet aggregation induced by the specific P2Y12 antagonist AR-C69931MX.

**Uni- and multivariate regression analyses of the data for both groups of patients (n=67)**

- **Age**
  - p < 0.0001
  - r = 0.72

- **Sex**
  - p = 0.001
  - r = 0.624

- **Body weight**
  - p = 0.013
  - r = 0.916

- **History of CAD**
  - p = 0.029
  - r = 0.820

- **Diabetes mellitus**
  - p = 0.051
  - r = 0.707

- **Hypertension**
  - p = 0.008
  - r = 0.964

- **Hyperlipidemia**
  - p = 0.217
  - r = 0.095

- **Antiplatelet therapy**
  - p = 0.933
  - r = 0.0001

- **Medication**
  - Aspirin
    - p = 0.122
    - r = 0.328

  - Statins
    - p = 0.018
    - r = 0.886

  - Nitrates
    - p = 0.115
    - r = 0.422

**Response of PRI in study population**

The mean values of PRI were similar in the groups of healthy volunteers and patients not receiving clopidogrel with remarkably small standard deviations. In contrast, the mean PRI in patients receiving clopidogrel was significantly lower than in the other two groups. The extreme values (from 6.6 to 85.8 %) and large standard deviation of PRI in this group nevertheless revealed a wide interindividual variability of the clopidogrel response. As estimated from the mean PRI ± 2 SD, approximately 33 % of patients receiving clopidogrel had a PRI equivalent to values in patients not under clopidogrel.