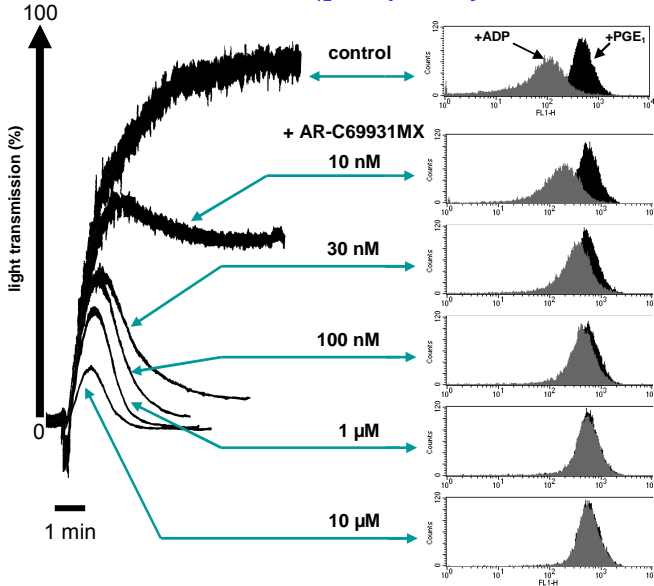


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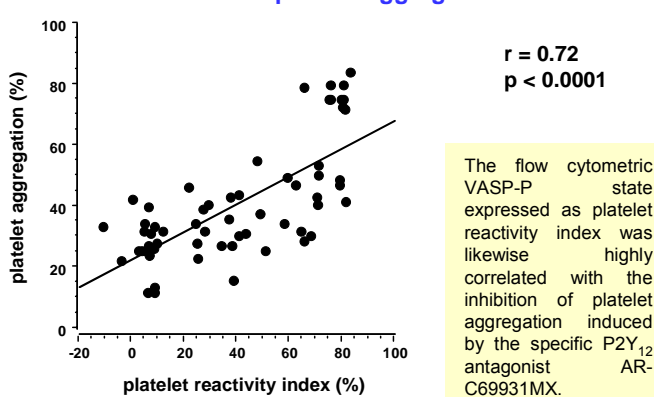
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1. *In vitro* inhibition of P2Y₁₂ receptors by AR-C69931MX



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.

2. Correlation between platelet aggregation and PRI



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

Flow cytometric assay.

VASP-P was measured by quantitative flow cytometry using the commercial kit from Diagnostica Stago / Biocytex according to the method described by Schwarz et al. [Thromb Haemost 1999; 82: 1145-52]. The **platelet reactivity index (PRI)** expressed as a percentage was calculated from the difference in VASP-P median fluorescence intensity (MFI) between resting (+PGE₁) and activated (+ADP) platelets according to the relation :

$$PRI = [(MFI_{PGE1} - MFI_{ADP}) / MFI_{PGE1}] \times 100$$

In vitro inhibition of P2Y₁₂ receptors.

Blood from 10 healthy volunteers was incubated with a range of concentrations (0 to 10 µM) of AR-C69931MX a specific antagonist of the P2Y₁₂ receptor generous gift from AstraZeneca. After that, each blood samples was analysed in flow cytometry for VASP phosphorylation and in platelet aggregation tests (in platelet rich plasma activated by ADP 5 µM).

Study population.

VASP phosphorylation was analysed in three groups of subjects: healthy volunteers (n=47, age (years; mean±SD) 41.6±13.1, male 64%) patients with ischemic cardiovascular diseases (previous history of myocardial infarction, ischemic stroke or peripheral artery disease) who were not receiving clopidogrel or ticlopidine (n=34, 68.4±9.8, 74%) and patients with ischemic cardiovascular diseases who are treated with clopidogrel at a daily dose of 75 mg for more than one week (n=33, 64.9±12.7, 82%).

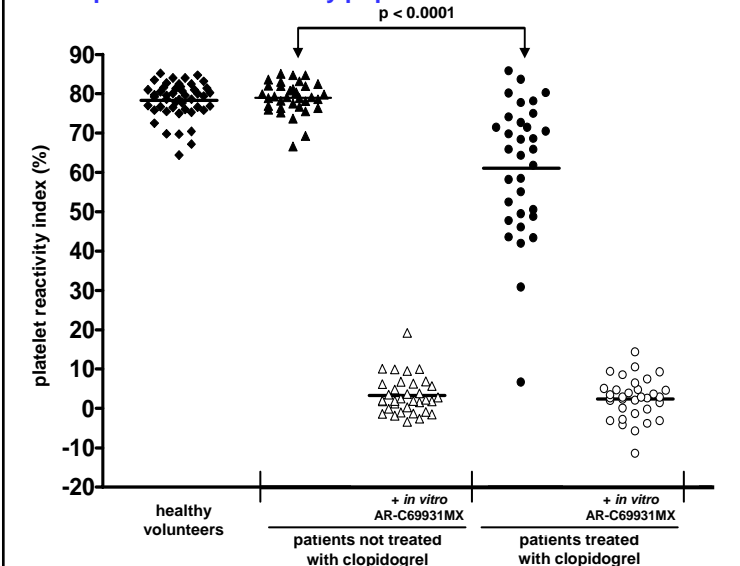
Conclusions

The flow cytometric analysis of VASP phosphorylation showed a strong correlation with the platelet aggregability after specific inhibition of the P2Y₁₂ receptor and seems to be a good test to evaluate the efficacy of clopidogrel therapy on the inhibition of the platelet activation by ADP. This test showed a large heterogeneity of the impact of clopidogrel in patients receiving the drug. One third of our patients under clopidogrel therapy displayed no inhibition of platelet activation. Whether these "unprotected" patients are more susceptible to recurrent ischemic complications will now be investigated in follow-up studies.

3. Determining factors in the results of PRI

Factors	Correlation coefficient (r value)	Univariate analysis (p value)	Multivariate analysis (p value)
Age	-0.022	0.861	—
Sex	0.061	0.624	—
Body weight	-0.013	0.916	—
History of CAD	0.029	0.820	—
Diabetes mellitus	0.051	0.707	—
Hypertension	0.008	0.954	—
Hyperlipidemia	0.217	0.095	0.933
Active smoking	0.115	0.422	—
Clopidogrel	0.594	< 0.0001	< 0.0001
Aspirin	0.122	0.328	—
Statins	0.018	0.886	—
Nitrates	0.060	0.630	—

4. 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 treated with 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.