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Platelet activation by Porphyromonas gingivalis: Variability of molecular mechanisms

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Introduction

Several clinical studies confirm a positive correlation between the incidence of cardiovascular disease and periodontal inflammation[1,2]. In the course of periodontal disease, periodontal pathogens such as Porphyromonas gingivalis (Pg) are chronically entering the bloodstream and subsequently interact with platelets (activation and aggregation) and the vascular endothelium (invasion, inflammation, and endothelial dysfunction).

Previous work

In a case series of 12 patients with severe chronic periodontitis, the composition of the subgingival microflora before and after periodontal therapy with adjunctive systemic antibiotic therapy over a period of 12 months was examined3. Pg isolates were initially cultured and characterized by amplification of the 16S rRNA cloning and sequencing. Sequencing of cultured Pg isolates revealed a large diversity in the molecular genetic pattern suggesting an intimate adaptation to the host, reflected by the eventual recolonization of periodontal pockets with identical Pg strains after therapy.

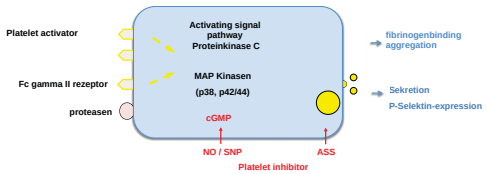
Aim

The aim of the study was to evaluate and compare different Pg isolates regarding their capacity and molecular mechanisms of platelet activation.

Method

After isolation, characterization and cryopreservation of wild-type Pg study isolates (L1, L4, L10), selective culture on Schädler agar plates was carried out under anaerobic conditions. Pg was isolated from colonies and Pg number was adjusted by optical density measurement. Aggregation measurements were performed using a PAP-4 aggregometer. Platelets and Pg isolates were co-incubated and aggregation was recorded continuously. In addition, samples were taken to assess the expression of platelet activation markers (P-selectin) by flow cytometry. Similarly, an analysis of intracellular platelet signaling pathways was performed by Western blot analysis.

Simplified scheme of platelet activation:



Results

Co-incubation of *Pg* isolates (L1, L4 and L10) with human platelets resulted in variable degrees of platelet aggregation:

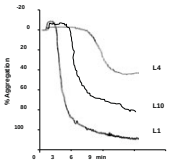


Fig. 1: Variability of platelet aggregation induced by Pg

Consistent with aggregation data, differences in the expression of platelet activation marker P-selectin (measured after 1 min of coincubation) and the phosphorylation of intracellular activating signaling pathways (p38 MAP kinase and protein kinase C, measured after 3 min) were found.
While isolate L1 induced a very high extent of platelet activation, the activation capacity of isolate L4 was significantly lower.

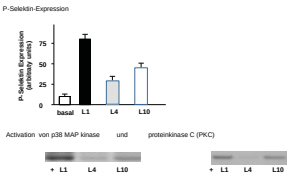


Figure 2: Platelet P-Selektion Expression

As possible mechanisms of platelet activation, the activation of the platelet Fc gamma receptor[4] and activation of exo-proteases[5] was evaluated. For this purpose, blocking antibodies against Fc receptor or protease inhibitors were added to the coincubation experiments:

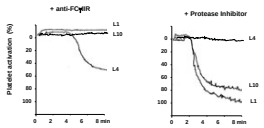


Fig. 3 Coincubation between *Pg* isolates (L1, L4 or L10) and human platelets in the presence of Fc-receptor blocker or protease inhibitors.

The isolate L4 was able to activate platelets despite blockade of Fc receptor, while *Pg* isolates L1 and L10 induced no activation of platelets under these conditions.
In contrast, activation of platelets was completely inhibited by the presence of protease inhibitors in the case of the isolate L4 while these inhibitors did not affect the platelet activation in isolates L1 or L10.

In further experiments, the possibility of inhibition of the *Pg*-induced platelet activation by classical platelet inhibitor (ASS or nitrates) was investigated:

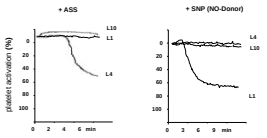


Fig. 4 Coincubation between *Pg* isolates (L1, L4 or L10) and human platelets in the presence of 100 uM ASA or 10 uM sodium nitroprusside (SNP, NO donor)

Pg isolate L4 induces platelet activation despite the presence of ASS. Pg isolate L1 activated platelets despite the presence of an NO donor.

After antibiotic treatment there was a re-colonization of the gingival pockets with *Pg* in 3 out of the 12 patients. These isolates were compared with isolates prior to treatment with antibiotics . As an example, the data for *Pg* isolate L1 are presented:

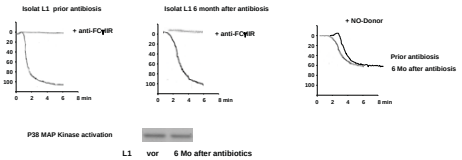


Fig. 5: Coincubation between platelets and *Pg* isolate L1 before and 6 months after antibiotic therapy

There were no differences in the mechanism of platelet activation before and after antibiotic therapy in case of reinfection, suggesting the reestablishment of the identical *Pg* strain.

The following table shows an overview of the aggregation results of all previously tested *Pg* isolates:

	% Aggregation	+ anti-Fc	+ Protease Inh.	+ ASS	+ NO-Donor
L1	94	-	82	-	65
L3	73	-	60	90	-
L4	42	45	-	-	-
L5	65	-	73	99	-
L7	99	-	79	-	-
L10	71	-	66	-	-
L14	100	-	100	-	-
				-	keine Aggregation

Tab. 1: Coincubation between human platelets and *Pg* isolates with or without the addition of the indicated inhibitors. The data represent mean of three individual experiments.

Conclusion and Discussion

In line with previously published data from other groups, we were also able to demonstrate the capcity of *Pg* isolates to activate human platelets. However, our experiments revealed a high variability of individual *Pg* isolates with regard to the degree of platelet activation and major differences in the underlying molecular mechanisms of platelet activation. Therefore, the present study shows, that there is no singular, general molecular mechanism for *Pg* to activate platelets.

Although the composition of the oral microflora in patients suffering from severe periodontal disease may look identical at first glance, the concomitant risk potential for cardiovascular events could be highly different on an individual base and might thus require specific therapeutic interventions for the protection of the cardiovascular system from detrimental *Pg induced* effects.

References

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