INTERNATIONAL UNIVERSITY OF HEALTH AND WELFARE

RES-247

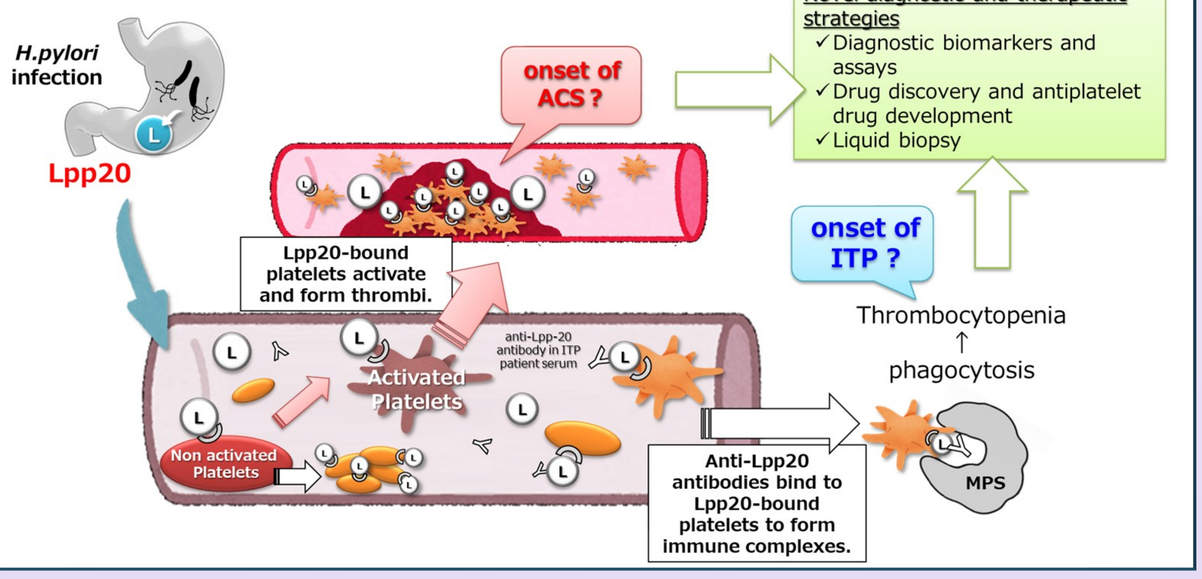
Lpp20 is a Key Player in *Helicobacter pylori* - Induced Platelet Aggregation and Activation.

Asako Kimura ,
Aoi Okamoto, Ryota Fujinuma, Nanaka Morita, Hiroaki Takeuchi

Department of Medical Technology and Science , Narita School of Health Sciences, International University of Health and Welfare

Introduction

- ✓ ***Helicobacter pylori* (*H. pylori*) infection** is implicated in a range of diseases, including **acute coronary syndrome (ACS).**
- ✓ The membrane-associated lipoprotein (**Lpp20**) of *H. pylori* has been detected in the plasma of patients with *H. pylori*-related ITP, where it **directly binds to platelet and induces their aggregation**. (*Takeuchi H et.al, Platelet 2021(5)*)
- ✓ We investigated **whether Lpp20-induced platelet aggregation results from activation rather than adhesion and** aimed to identify **the associated activation pathway.**



Material

His-tagged Lpp20 protein

- ✓ Based on the genomic DNA of *H. pylori* strain 26695, His-tagged Lpp20 protein was over-expressed in *E. coli* BL21
- ✓ LPP20 was obtained by through homogenization, centrifugation, and purification using Nickel Affinity Chromatography.

Platelet rich plasma (PRP)

- ✓ PRP was obtained by centrifuging whole blood anticoagulated with 3.2% sodium citrate (9:1 ratio), collected from healthy Japanese male and female volunteers in their 20s without H. pylori infection or underlying diseases, at 200×g for 10 min at room temperature.

(Approved by the Institutional Review Board of International University of Health and Welfare)

Methods

Lpp-20 induced platelet aggregation assay with/ without anti-platelets agents

- ✓ PRP (3.0×10^7 platelets) was mixed with His-tagged Lpp20 (50μg, 100μg), and platelet aggregation was monitored for 10 minutes using an automatic platelet aggregometer (LMS HemaTracer 712).
- ✓ Prior to Lpp20 addition, PRP was preincubated with aspirin (ASP, 10 mM), ticagrelor (TCG, 20 μM), or U73122 (a phospholipase C inhibitor, 10 μM) for 5 minutes at 37 °C, followed by 10-minute monitoring.

Other assays

- ✓ Platelet activation mediators (Thromboxane B2 and cAMP) were quantified using ELISA.
- ✓ Platelet aggregation was confirmed microscopically.
- ✓ Flow cytometry (FCM) was performed to assess platelet activation markers (CD62P and PAC-1).

- Assays were conducted using the supernatant from post-reaction samples.

Conclusions

- ➢ **Lpp-20**, a membrane-associated lipoprotein of *H. pylori*, **induced the platelet aggregation and activation potentially through the arachidonic acid cascade.**
- ➢ **The marked individual variability** in Lpp20-induced platelet activation may reflect **differences in susceptibility to thrombotic conditions** such as acute coronary syndrome.

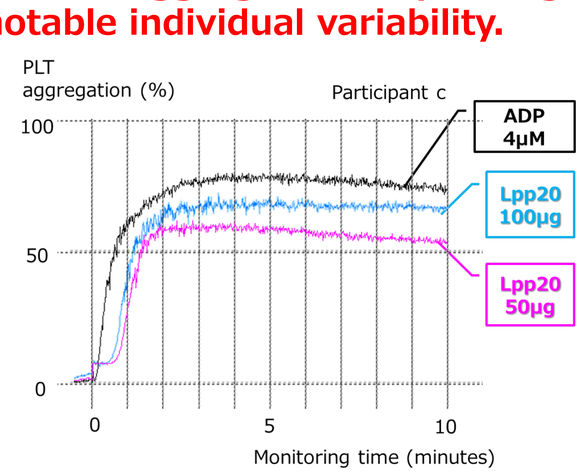
Future Strategy

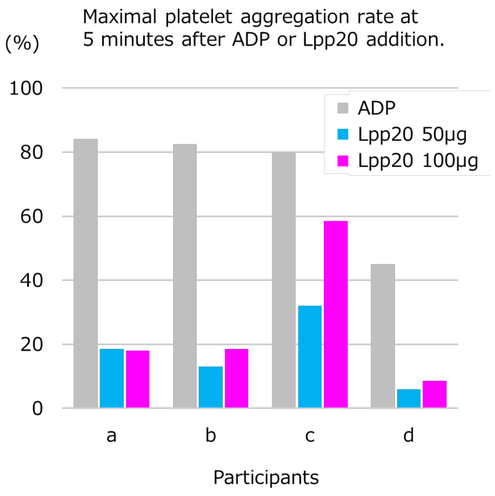
We are currently investigating the following aspects of Lpp20-induced platelet activation:

- ➢ The receptor(s) to which Lpp20 protein binds to trigger platelet activation.
- ➢ The platelet signaling pathways stimulated by Lpp20.
- ➢ The correlation between individual variability in Lpp20-induced platelet activation and plasma factors.

Results

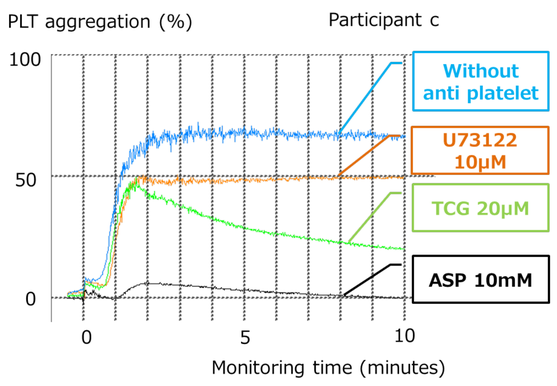
Platelet aggregation assay demonstrated that **Lpp20 induced platelet aggregation depending on its concentration, with notable individual variability.**

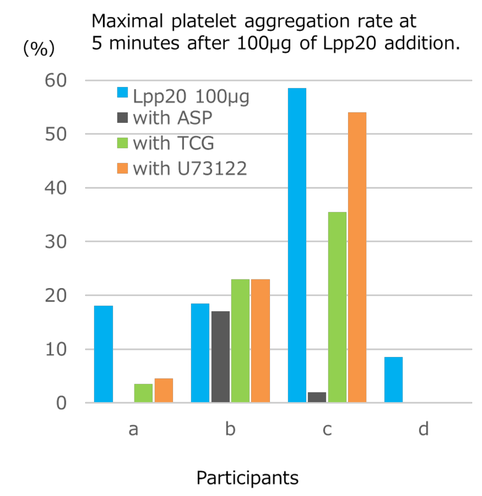




PLT 3.0×10^7 cells
ADP (final conc. 4μM) was used as a positive control.

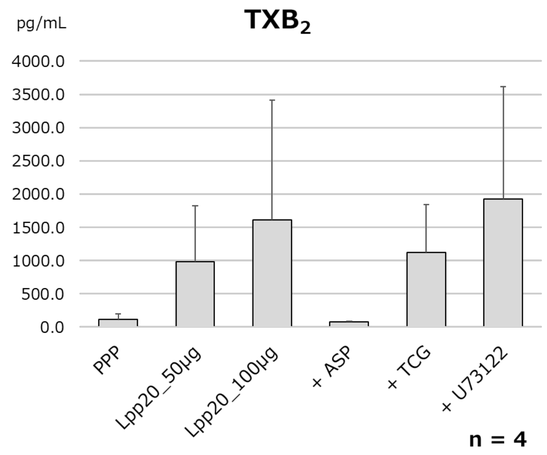
Platelet aggregation assays with antiplatelets agents confirmed that Lpp20-induced aggregation was **strongly inhibited by aspirin preincubation.**

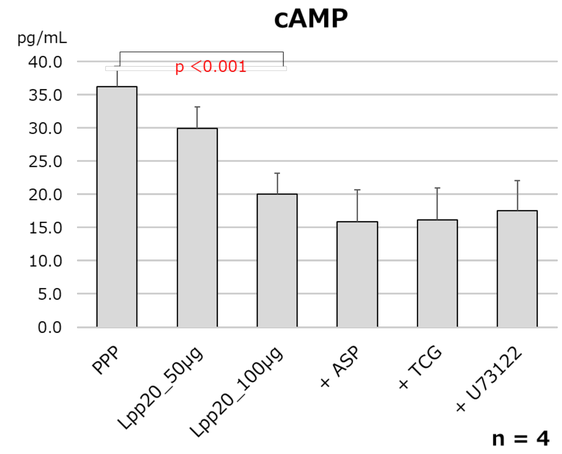




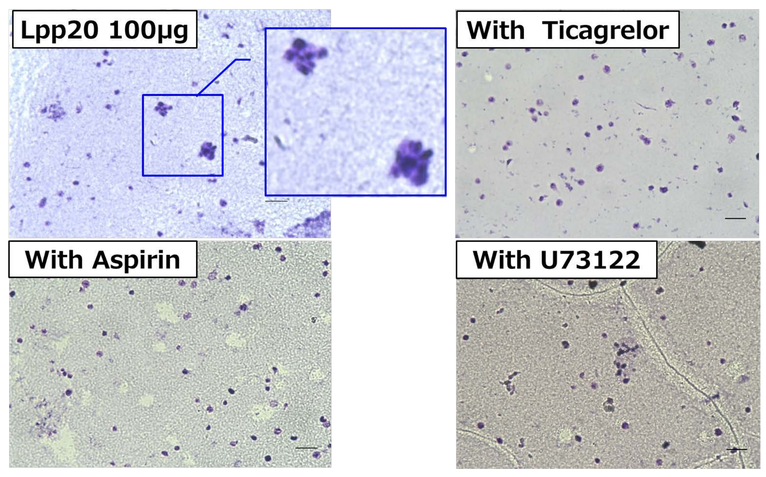
PLT 3.0×10^7 cells
Lpp20 100μg

Measurement of platelet activation mediators suggested that the arachidonic acid cascade may contribute to Lpp20-induced platelet aggregation and activation.

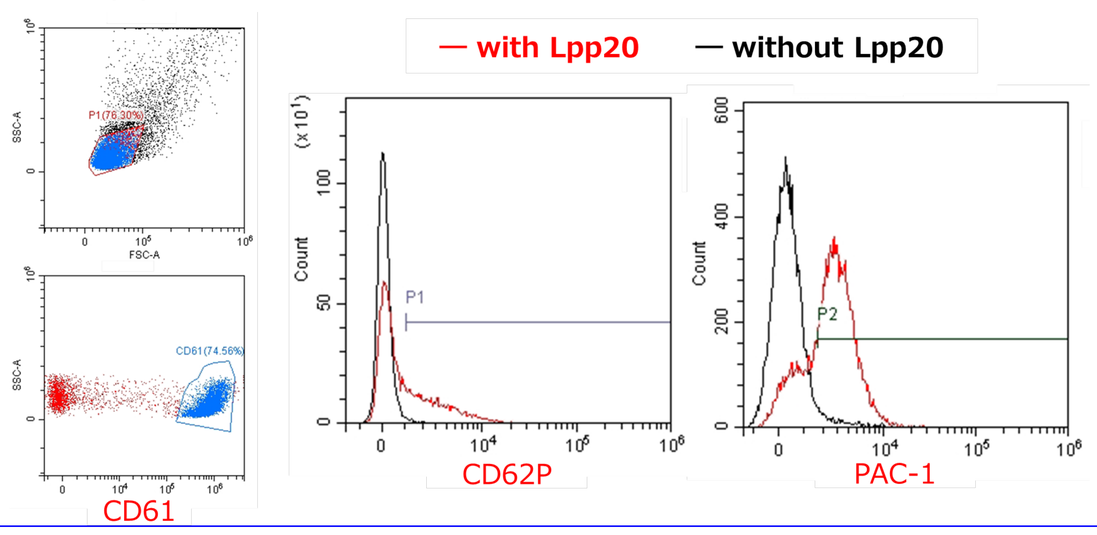




Microscopy confirmed the presence of Lpp20-induced platelet aggregates, consistent with the results of the aggregation assay.



FCM demonstrated that Lpp20-induced platelet aggregation was driven by platelet activation rather than mere adhesion.



In connection with this presentation, there is no COI to be disclosed with any companies.

APCCMI 2025