Complement activation of artificial liposomes about 100 nm in diameter

S Matviykiv[1], M Buscema[1], S Bugna[1], T Mészáros[2], J Szebeni[2], A Zumbuehl[3], B Müller[1]

[1] *Biomaterials Science Center, Department of Biomedical Engineering, University of Basel, Basel, CH.* [2] *Nanomedicine Research and Education Center, Semmelweis University, Budapest, HU.* [3] *Department of Chemistry, University of Fribourg, Fribourg, CH.*

INTRODUCTION: Cardiovascular diseases are the number one leading cause of mortality in our society underlining the need to ameliorate patient's treatment. One of the therapeutic approaches focuses on nanotherapeutics. Such platform might base on the mechanical activation of liposomes inside blood vessels by shear forces, where the vesicles locally release a vasodilator at the site of a stenosis. The recently discovered mechano-sensitive lentil-shaped liposomes [1] composed of the artificial Pad-PC-Pad (1,3-palmitoylamido- 1,3-deoxy-*sn*-glycero-2-phosphatidyl-choline) belong to such formulations. In order to advance the technology to the bedside, the *in vivo* hypersensitivity towards Pad-PC-Pad has to be tested and compared to the FDA-approved liposomal formulations Doxil [2,3] and AmBisome [4] that are recognized as foreign by the immune system, giving rise to adverse and even lethal effects at certain doses.

METHODS: Pad-PC-Pad was synthetized [1] and liposomes were prepared using the thin-film method [5]. We hydrated the Pad-PC-Pad film with PBS, and extruded at 65 °C through 100 nm membrane polycarbonate filters at a concentration of 20 mg/mL. Dynamic light scattering (DelsaNano C, Beckman Coulter Inc) did not show any liposome aggregation. We tested three human serum samples, labelled F5, F8, F10 using the MicroVue SC5b-9 (Quidel, USA) ELISA kit. After 0, 5, 15, 30 and 45 minutes of incubation, EDTA was added to stop the reaction. Only serum, PBS, and zymosan we incubated for 45 minutes. Each series contained untreated serum and PBS as negative control and zymosan (Sigma-Aldrich, USA) as positive control, respectively. The optical density was measured at a wavelength of 450 nm.

RESULTS: Figure 1 summarizes the results with human sera using ELISA assay. The relative concentration of the SC5b-9 is shown as a percentage of the baseline, calculated as the average of serum only samples. They indicate the same reactivity between three human serum samples. After a lag phase of 30 minutes, Pad-PC-Pad caused a three-fold increase of the complement activation. In contrast to this moderate reaction, zymosan caused an immediate activation more than 90 times higher than the baseline control.

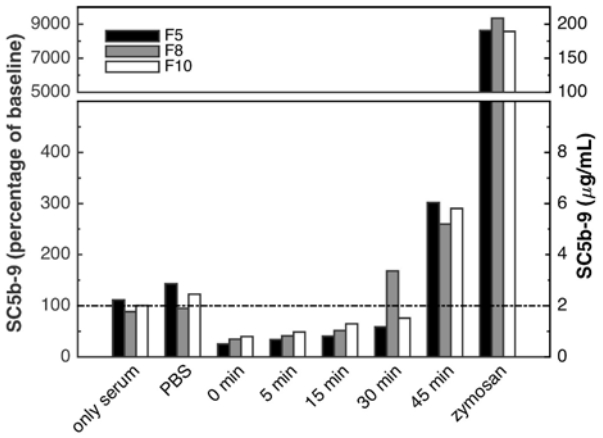

Fig. 1: Surprising lack of complement activation of artificial Pad-PC-Pad liposomes with human sera.

DISCUSSION & CONCLUSIONS: This study explored the *in vitro* complement activation by artificial Pad-PC-Pad phospholipids. Our findings indicate that liposomes do not induce complement activation *in vitro*, unlike the complement activator zymosan that triggered an immediate reaction even at low concentration. Recently, additional studies were conducted [6]. We observed a surprising lack of complement activation both *in vivo* with pigs and *in vitro* with human sera and pig plasma. As a conclusion, Pad-PC-Pad is regarded as a promising phospholipid for future drug delivery applications.

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