Immune complement activation by a faceted nano-container in the pig model

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INTRODUCTION: Pharmacotherapy uses a wide range of liposomes as nano-carriers for targeted delivery or controlled release of drugs and diagnostic agents. The nano-carriers can substantially alter the absorption, distribution, metabolism, and excretion of the encapsulated drugs, improving their efficacy and reducing their toxicity. However, besides their unique therapeutic advantages, these carriers share the potential problem of being recognized by the immune system as foreign, which leads to the rise of adverse reactions, loss of efficacy, and risk of anaphylactic shock. The activation of the complement (C) can result in a hypersensitivity reaction, termed C activation-related pseudo-allergy (CARPA).[1] This phenomenon frequently occurs with liposomes or lipid-based drugs and creates adverse effects, in a high percentage of people, leading occasionally to an anaphylactic reaction or even to death.

The goal of the present study was to explore the complement activation induced by artificial, facetted Pad-PC-Pad vesicles[2] both *in vitro* (human serum) and *in vivo*.

METHODS: The *in vivo* study comprised the injection of bolus of 0.5 and 5.0 mg lipid in three pigs. The physiological reaction, i.e. systemic arterial pressure, pulmonary arterial pressure, and heart rate, was monitored during six hours. Blood cells, thromboxane and blood biomarkers were screened for potential toxicity before and six hours after injection. The positive control was induced injecting 0.1 mg/kg of Zymosan A (Sigma-Aldrich Co. LLC.).

RESULTS: The highest physiological reaction we detected was a 30 % elevation (5 mmHg) of the pulmonary arterial pressure with 5 mg/kg of lipids of aggregated liposomes, which represents insignificant changes only. The formulation led to time-dependent liposomal aggregates that could be disrupted through filtration. We registered a decrease of lymphocytes by 20 % to 40 % in all pigs without other changes in blood cells or thromboxane. When the liposomes were filtered prior to the injection no reaction of the complement was found. Only minor changes in the biomarkers were observed, such as the single detection of a 300 % increase of bilirubin and the concomitant increase of 200 % urea.

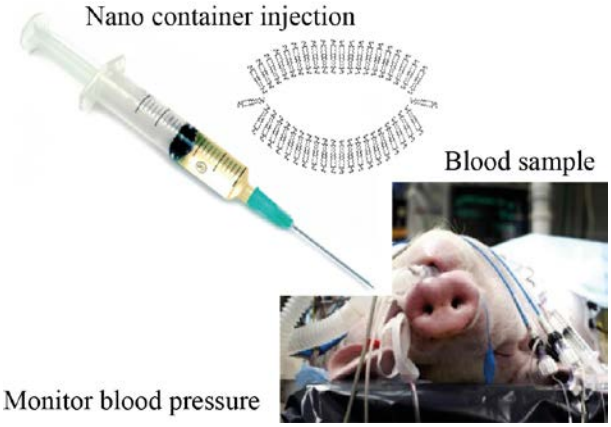

Fig. 1 Scheme of the animal experiment.

DISCUSSION & CONCLUSIONS: The present study indicates that Pad-PC-Pad liposomes extruded to 100 nm in diameter are not inducing a significant CARPA reaction (CAS 1) neither with a dose of 5 mg/kg phospholipids nor with the injection of 110 mg lipids. This result has to be related to published data that show high CARPA reactivity with pharmacologically approved liposomal formulations at a dosage between 0.01 and 0.5 mg/kg phospholipids.[3] The lymphocyte decrease starts after 90 minutes only, which needs to be explored in detail. Such a long period of examination is not yet found in literature. In conclusion, Pad-PC-Pad liposomes are promising nano-containers for drug delivery.[4]

REFERENCES: [1] J. Szebeni et al. (1998) *Crit Rev Ther Drug Carrier Syst.* **15**:57-88. [2] M.N. Holmes (2012) *Nature Nanotechnol* **7**:536–43. [3] J. Szebeni et al. (2012) *Nanomed* **8**:176-84. [4] T. Saxer, A. Zumbuehl, B. Müller (2013) *Cardiovasc Res* **99**:328-33.

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