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Comment

The influence of pulmonary surfactant on nanoparticulate drug delivery systems

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ABSTRACT

The pulmonary route is very attractive for drug delivery by inhalation. In this regard, nanoparticulate drug delivery systems, designed as multifunctional engineered nanoparticles, are very promising since they combine several opportunities like a rather uniform distribution of drug dose among all ventilated alveoli allowing for uniform cellular drug internalization. However, although the field of nanomedicine offers multiple opportunities, it still is in its infancy and the research has to proceed in order to obtain a specific targeting of the drug combined with minimum side effects. If inhaled nanoparticulate drug delivery systems are deposited on the pulmonary surfactant, they come into contact with phospholipids and surfactant proteins. It is highly likely that the interaction of nanoparticulate drug delivery systems with surfactant phospholipids and proteins will be able to mediate/modulate the further fate of this specific drug delivery system. In the present comment, we discuss the potential interactions of nanoparticulate drug delivery systems with pulmonary surfactant as well as the potential consequences of this interaction.

1. Comment

The pulmonary route is very attractive for drug delivery by inhalation. This results mainly from a very thin air-blood barrier (<1 μm) and a huge surface area (>150 m^2), which both result in a high systemic bioavailability [1,2]. In this regard, nanoparticulate drug delivery systems, designed as multifunctional engineered nanoparticles (ENP), are very promising since they combine several opportunities like a rather uniform distribution of drug dose among all ventilated alveoli allowing for uniform cellular drug internalization [3]. However, although the field of nanomedicine offers multiple opportunities, it still is in its infancy and the research has to proceed in order to obtain a specific targeting of the drug combined with minimum side effects.

If inhaled nanoparticulate drug delivery systems are deposited on the pulmonary surfactant (**surf**ace **act**ive **a**g**ent**), they are most probably displaced by wetting forces into the aqueous hypophase in the peripheral lung that could be previously shown for microparticles [4]. Afterwards, they may interact with pulmonary cells. The pulmonary surfactant layer consists of phospholipids (~90%) and 4 specific surfactant proteins (SP)-A, B, C, and D (~10%). The phospholipids together with the lipophilic SP-B (~8 kD) and SP-C (~4 kD) reduce the surface tension at the air–liquid interface to prevent alveolar collapse. The hydrophilic SP-A (~28–36 kD) and SP-D (~43 kD) play an important role in the immune system due to enhanced clearance of pathogens as well as specific modulation of the inflammatory immune response [5].

Although biodegradable ENP are considered currently to be the best choice for targeted drug delivery, there is an opportunity for the use of versatile biopersistent ENP. These ENP have to be directed to the target where the drug should be released. In addition, a minimized body exposure is necessary to reduce toxic side effects. Therefore, the ENP should leave the body for instance by renal clearance after releasing their drug load. In this regard, e.g., gold nanoparticles are discussed [6], and it seems possible to specifically target for example cancer tissue [7]. It is known that SP-A and SP-D are able to bind various particulates like bacteria, viruses or allergen particles modulating the interaction of the particles with lung cells as well as with the immune response for example by an increased/decreased mediator secretion [5,8]. Therefore, a possible interaction of these surfactant proteins with nanoparticulate drug delivery system is likely to determine the biodistribution, toxic effects and thereby the success of the ENP drug delivery. Besides specific binding, which may happen by means of the carbohydrate recognition domain of SP-A and SP-D to selected surface groups of ENP [9], unspecific coating is also probable [10–12].

The present paper of the group of Lehr (Schulze et al., this issue) deals with the interaction of ENP with lung proteins. It particularly focuses on SP-A as the most abundant surfactant protein. The authors investigated SP-A besides other lung proteins that absorb non-uniform to the outer surface of eight different ENP. In particular, high binding of SP-A was found to occur on $BaSO_4$ ENP and AlOOH ENP. In contrast, Carbon Black ENP showed very low

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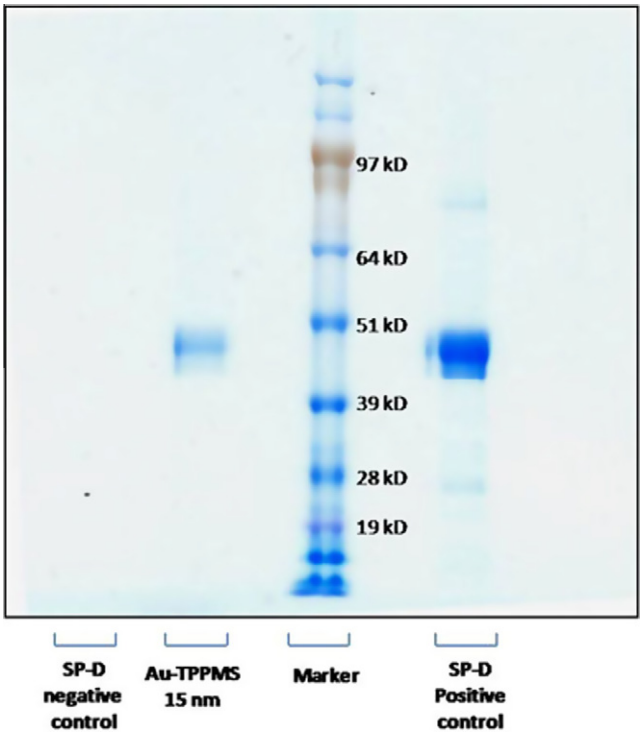

Fig. 1. 125 μg monodisperse mono-sulfonated triphenylphosphine (TPPMS) stabilized Gold NP (core diameter: 15 nm) were incubated with 15 μg recombinant human surfactant protein D (SP-D) for 20 h. Afterwards, Au NP were separated from free SP-D by centrifugation (154,000*g*, 30 min). The Au pellet was purified from free SP-D by several washing steps. Finally, the pellet was resuspended in 30 μl PBS and 10 min sonified. LDS sample loading buffer was added, and the suspension was boiled for 5 min. At the end, proteins were separated by SDS gel electrophoresis. In case for the positive control, 10 μg SP-D was used. The negative control was exactly treated like Au sample but without nanoparticles. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

binding of SP-A. Interestingly, ENP composed of the same material, in particular various TiO_2 ENP as well as various CeO_2 ENP, showed different binding patterns of SP-A. The present paper also describes that ENP, coated with proteins from bronchoalveolar lavage fluid, show a high agglomeration tendency which is in contrast to, e.g., proteins from fetal calf serum that is commonly used as a supplement in cell culture medium.

These are important and relevant results. Agglomerated nanoparticulate drug delivery systems reach a higher size that may change the capability of crossing the air-blood barrier or entering specific cells by endocytotic pathways. However, whether SP-A particularly is able to agglomerate ENP *in vivo* is still unknown. Although it is highly relevant to know the agglomeration capacity of the total alveolar lining fluid, the relevant key proteins that actually cause agglomeration still need to be identified. An estimation of the fate of nanoparticulate drug delivery systems is only possible with detailed knowledge of the conditions upon inhalation and deposition on the pulmonary surfactant layer. Since both SP-A and SP-D are known to agglomerate particles and pathogens, it is probable that also binding to and agglomeration of ENP occurs. In fact, our own *in vitro* studies have shown that SP-D adsorb on the surface of monodisperse mono-sulfonated triphenylphosphine coated 15 nm gold ENP (Fig. 1) which thereby lead to agglomeration of these ENP (Fig. 2).

Importantly, it is not known whether SP-D and SP-A bind specifically to the ENP or just by random coating processes. These issues need to be further addressed, since they may have important impact on the immune response in the lung. Different mechanisms and affinities of specific binding and unspecific adsorption lead to different free protein surface groups that are available for further interaction. The receptor SIRPalpha, mainly present on macrophages and dendritic cells but also on pulmonary epithelial cells, can be for instance bound through the globular heads of SP-D that initiates a signaling pathway that blocks pro-inflammatory mediator production [13]. In contrast, the collagenous tails of SP-D stimulate pro-inflammatory mediator production through binding to calreticulin/CD91 [13]. Thus, the mechanism of binding/adsorption can lead to an opposed modulation of the immune system. These interactions are able to determine success or failure of newly designed delivery systems.

Not only surfactant proteins might change the surface properties of nanoparticulate drug delivery systems but also the surfactant phospholipids that are known to play important roles for

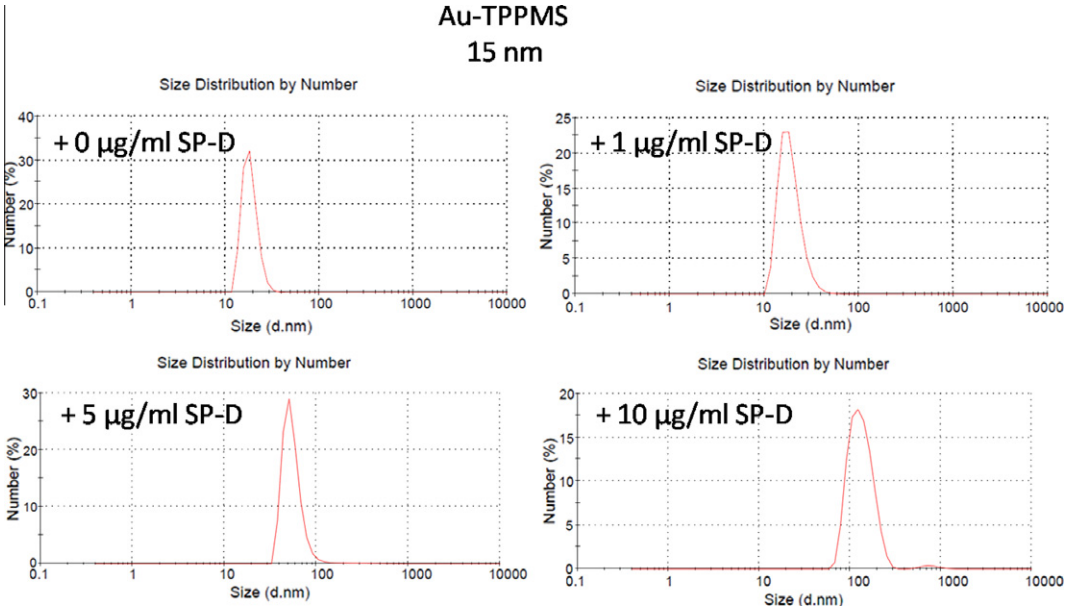

Fig. 2. Hydrodynamic diameter of monodisperse mono-sulfonated triphenylphosphine (TPPMS) stabilized gold NP was measured in a Malvern Zetasizer with or without additional surfactant protein D (SP-D). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

many biological functions. Phosphatidylcholine and phosphatidylinositol for example are well known to be involved in cell signaling, whereas phosphatidylinositol and phosphatidylinositolphosphates regulate the activity of at least a dozen enzymes that control many key cellular functions, including differentiation, proliferation, metabolism, and apoptosis. Adsorption of pulmonary surfactant phospholipids was for instance shown on nano-sized gold particles [14] and on carbon black nano-sized particles [15]. Our own first results show that surfactant lipids bind unspecific to different functionalized multi-walled carbon nanotubes, but it influences the subsequent binding pattern of blood plasma proteins [16]. Further studies need to be done regarding the possible consequences on biological effects.

It is highly likely that the interaction of nanoparticulate drug delivery systems with surfactant phospholipids and proteins will be able to mediate/modulate the further fate of this specific system. Therefore, it is of high importance to investigate these interactions when designing nanoparticulate delivery system for application into the lung. Furthermore, experiments with *in vitro* systems, which investigate the translocation of ENP across the air-blood barrier, should integrate pulmonary surfactant components – in particular SP-A and SP-D – to obtain relevant results that can be used for extrapolation towards the *in vivo* complexity.

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