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Editorial

Injection of nanoparticles into cloven-hoof animals: Asking for trouble

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Abstract

This article aims at alerting scientists working with nanoparticles or microparticles about the specific adverse reactions due to the intravascular pulmonary macrophages present in pigs and other cloven-hoof animals, but not in humans. The history of a 25-year old study of an ultrasound contrast agent is used to illustrate these differences.

Introduction

An interesting article entitled "Bypassing adverse injection reactions to nanoparticles through shape modification and attachment to erythrocytes" was recently published in Nature Nanotechnology by Wibroe *et al*. [1]. In addition to what is stated in the title, this article contains interesting data about pulmonary reactions in the pigs. This is especially important since pigs during the last decades have been used as a model to study complement activation, and Wibroe *et al*. [1] demonstrate that the pulmonary reactions occur irrespectively of complement activation.

The observation that pigs get pulmonary reactions to injected particles is not new, however, as such reactions were described more than 25 years ago. An old study demonstrates how careful investigators should be in using pigs when testing for toxic effects following intravenous injection of nanoparticles or microparticles to be used for imaging, therapy or as theranostics [2].

In the early 1990s I was involved in the development of an ultrasound contrast agent based upon air-filled albumin microparticles (Albunex®; developed by Nycomed AS, Oslo). The mean bubble size was 4 µm, and the size range was 1-10 µm. A researcher outside the company asked for and received a sample to calibrate the ultrasound signals observed when he studied air bubbles formed as a result of decompression sickness in divers. When the particles were injected in a pig, a massive increase in pulmonary pressure (PAP) and a decline in the systemic arterial pressure (SAP) were observed, and after repeating the injection the pig died. This led to a lot of discussions in Norwegian media; they asked "How can a pharmaceutical company inject a substance in humans, when such injections are lethal to pigs?"

Albunex® had at that time been injected in several hundred people in clinical trials without any cardiopulmonary changes. Scientists in the company obviously had to change their focus from ongoing studies to this "pig effect". A circulation physiologist proposed to test if the reactions could be explained by the presence of the intravascular pulmonary macrophages (PIMs) previously shown to be present in pigs, other cloven-hoof animals such as sheep, goat, cows, and even in cats [3]. It was shown that injection of only 0.005-0.014 ml Albunex® per kg resulted in a dose-dependent haemodynamic effect in pigs, and that this was completely blocked by indomethacin and by the thromboxane A2 receptor antagonist HN-11500 [2]. Furthermore, no haemodynamic effect was observed in rabbits or cynomolgus monkeys following the injection of 0.12-0.48 ml Albunex® per

kg. Thus, it was concluded that the effect was due to release of thromboxane A2 from the PIMs [2].

At the time the haemodynamic study with Albunex® was performed, it had been reported that there was nearly a complete recovery of the intravenously injected iron oxide particles and some other substances in lungs of the cows, sheep and pigs [2]. Biodistribution studies performed later with ^{125}I-labelled Albunex® showed that in pigs more than 90% of the intravenously injected radioactivity was recovered in their lungs. In rats, 80% of the radioactivity was cleared from the blood within 2 min; nearly 60% of the injected dose was recovered in the liver (mainly in the Kupffer cells, i.e. the liver macrophages), only 5% in the lungs, 9% in the spleen and negligible quantities in the kidneys, heart and brain [4]. For further discussion of the effects of uptake of particles of different sizes by PIMs in various species, please see refs [3, 5, 6].

Many research groups are now focusing on the possibility of making new particle-based products for intravenous injection in humans. Not only is the focus of such research on the use of nanoparticles, but also on microbubbles to be used for medical imaging or for ultrasound-guided drug delivery [7, 8]. Based upon the Albunex® story, the following should be a good advice: Do not perform safety studies of such particles by intravenous injection in cloven-hoof animals if you do not have a very specific reason for doing that. The effects one can expect to see in e.g. pigs are most likely not representative for what one would observe with humans. Albunex® was later approved for marketing, but one can easily see that the haemodynamic effect in pigs could have halted commercial development of such an agent.

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