Insights into the Local Inflammatory Reaction Induced by Intramuscular Injection of Sustained-Release Drug Microsuspensions

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ABSTRACT SUMMARY

This study presents an in depth investigation of the local in vivo disposition of intramuscularly (IM) administered microcrystalline drug suspensions. Histopathological examination of the injection sites at different time points showed a chronic inflammatory reaction induced by the exotic particles with particle internalization by macrophages. This complex in vivo behavior might drastically alter our current understanding of the IM drug release kinetics.

INTRODUCTION

Sustained-release formulations for intramuscular (IM) administration, especially crystalline microsuspensions of poorly soluble compounds, have emerged during the past decade as viable dosing alternatives to oral drug delivery systems. Nevertheless, the complexity of their in vivo behavior has not been studied thoroughly so far. Preliminary observations made by our research group suggested for example that a notable local inflammatory reaction, mainly consisting of macrophages, occurs in rats after IM injection with a long-acting crystalline prodrug formulation for this longitudinal in vivo study. Male Wistar rats (300-350g) were intramuscularly injected with 20mg/kg eq. (60µL/300g) of this sterile aqueous suspension in the m. biceps femoris. 27 Gauge needles were used for maximal reduction of the structural damage to the muscle tissue. For comparison and ease of imaging, a second group of rats was injected with equal volumes of a sterile yellow-green fluorescent polystyrene suspension, having comparable particle sizes, suspension concentration and medium composition. A vehicle control solution was administered into the contralateral hind legs of all rats.

The immune response induced by polymeric or engineered nanoparticles and the interplay with macrophages are subject to extensive investigation. However, no data on the immune response generated by drug microcrystals has been reported yet to our knowledge. The likely intracellular uptake of dissolving (pro)drug crystals via phagocytosis might greatly influence the particle residence time or result in the modulation of the drug release rate and extent. This lack of understanding of the biological factors governing the in vivo crystal and drug disposition is currently hampering a rational formulation design.

The aim of the present study was therefore to obtain insights into the local inflammatory reaction induced by IM injection of sustained-release drug microcrystals in rats. Qualitative aspects of the inflammation, such as the onset, the morphology and especially the temporal and spatial characterization of the inflammatory and phagocytosis processes, were systematically investigated. A deeper understanding of the in vivo disposition of IM injectable microcrystals and their possible interactions with immunological cells will undoubtedly contribute to the further optimization of this successful drug delivery strategy and might be applicable to other particle-based drug formulations in the future.

EXPERIMENTAL METHODS

A long-acting injectable crystalline drug suspension (d50 = 1.2µm) of a poorly water-soluble ester prodrug ("Compound A") was used as model formulation for this longitudinal in vivo study. Male Wistar rats (300-350g) were intramuscularly injected with 20mg/kg eq. (60µL/300g) of this sterile aqueous suspension in the m. biceps femoris. 27 Gauge needles were used for maximal reduction of the structural damage to the muscle tissue. For comparison and ease of imaging, a second group of rats was injected with equal volumes of a sterile yellow-green fluorescent polystyrene suspension, having comparable particle sizes, suspension concentration and medium composition. A vehicle control solution was administered into the contralateral hind legs of all rats.

Histopathological examination of the injection sites and the draining lymph nodes was performed 2h, 4h, 8h, 24h, 48h, 72h, 1 week, 2 weeks, 4 weeks and 8 weeks after injection (n = 3 for each time point) with the Compound A and the fluorescent polystyrene suspensions to examine the morphology of the administration site and the time-dependence of the local inflammatory reaction induced by the microparticles. The local disposition at the IM injection site and the draining lymph nodes of polystyrene beads were investigated by fluorescence microscopy. The intracellular partitioning of drug microcrystals and polystyrene beads were investigated by means of polarized light microscopy and fluorescence microscopy, respectively.
RESULTS AND DISCUSSION
The intact rat hind legs showed no signs of inflammation. Upon dissection, minimal to slight erythema surrounding the suspension boluses could be observed depending on the time of administration (Figure 1A).

![Figure 1A](image1.jpg)

**Figure 1** – Macroscopic (A) and microscopic (B) (HE staining; 1.5x) overview of rat IM administration site with chronic inflammation 2 weeks after injection with Compound A suspension. Scale bar represents 1mm.

Histopathological assessment of the IM administration site 1 week or more after injection with the Compound A suspension showed a central cell-free region (often with additional small peripheral foci) consisting of drug microsuspension, surrounded with a pronounced granulomatous inflammatory reaction mainly composed of a migrating front of epitheloid macrophages (Figure 1B and Figure 2). The band of inflammatory macrophages was in turn lined by a mixture of fibroblasts, histiocytes, lymphocytic aggregates, capillary sprouts (neovascularization) and some granulocytes and plasma cells (Figure 2). The macrophages were loaded with microparticles as evidenced by their swollen and granulated appearance under bright field microscopy and the birefringence observed with polarized light microscopy.

![Figure 2](image2.jpg)

**Figure 2** – Detail (HE staining; 20x) of the rat IM administration site 2 weeks after injection showing chronic inflammatory reaction (mainly composed of epitheloid macrophages) surrounding and invading the microcrystalline suspension. Scale bar represents 100μm.

Evaluation of the administration sites of polystyrene microspheres with bright field and fluorescence microscopy showed a similar evolution and intensity of the inflammatory reaction, suggesting that these characteristics might be intrinsic to particle-based formulations. Fluorescence microscopy confirmed the intracellular accumulation of microparticles into macrophages as early as 72h after injection (Figure 3).

![Figure 3](image3.jpg)

**Figure 3** – Bright field and fluorescence micrograph (40x) overlay of IM administration site 72h after injection with polystyrene microspheres showing macrophages with intracellular polystyrene beads. Scale bar: 50μm.

CONCLUSION
This study revealed an extensive chronic inflammatory reaction with particle internalization in epitheloid macrophages after IM injection of a long-acting crystalline microsuspension in the rat. This complex in vivo behavior might drastically alter the understanding of the in vivo drug release kinetics. In a later stage of research, quantitative information extracted from the data obtained in this study will be correlated to drug plasma concentration profiles and will help to extrapolate in vitro macrophage enzyme kinetics to the human via PBPK modeling.

REFERENCES

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