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Pharmacokinetics, biocompatibility and bioavailability of a controlled release monoclonal antibody formulation

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ABSTRACT

The sustained and localized delivery of monoclonal antibodies has become highly relevant, because of the increasing number of investigated local delivery applications in recent years. As the local delivery of antibodies is associated with high technological hurdles, very few successful approaches have been reported in the literature so far. Alginate-based delivery systems were previously described as promising sustained release formulations for monoclonal antibodies (mAbs). In order to further investigate their applicability, a single-dose animal study was conducted to compare the biocompatibility, the pharmacokinetics and the bioavailability of a human monoclonal antibody liquid formulation with two alginate-based sustained delivery systems after subcutaneous administration in rats. 28 days after injection, the depot systems were still found in the subcutis of the animals. A calcium cross-linked alginate formulation, which was injected as a hydrogel, was present as multiple compartments separated by subcutaneous tissue. An *in situ* forming alginate formulation was recovered as a single compact and cohesive structure. It can be assumed that the multiple compartments of the hydrogel formulation led to almost identical pharmacokinetic profiles for all tested animals, whereas the compact nature of the *in situ* forming system resulted in large interindividual variations in pharmacokinetics. As compared to the liquid formulation the hydrogel formulations led to lower mAb serum levels, and the *in situ* forming system to a shift in the time to reach the maximum mAb serum concentration (T_{max}) from 2 to 4 days. Importantly, it was shown that after 28 days only marginal amounts of residual mAb were present in the alginate matrix and in the tissue at the injection site indicating nearly complete release. In line with this finding, systemic drug bioavailability was not affected by using the controlled release systems. This study successfully demonstrates the suitability and underlines the potential of polyanionic systems for local and controlled mAb delivery.

1. Introduction

The long circulation half-life of the Immunoglobulin G (IgG) molecule allows for reduced frequency of administration as compared to other therapeutic proteins and, therefore, reduces the need for the development of sustained delivery systems for systemic application. However, the increased emergence of local delivery applications have rendered sustained release systems for IgGs more attractive. Antibodies targeting Vascular Endothelial Growth Factor (VEGF) in ophthalmology [1,2], Tumor Necrosis Factor α (TNF α) [3] and Interleukin 1 β (IL-1 β) [4] blocking antibodies in arthritic joint diseases [5,6], or even intrathecally and intratumorally delivered antibodies [7,8] represent suitable candidates for local and sustained-release applications. For such applications, the use of a local sustained release system could reduce systemic complications and improve efficacy of the locally delivered drug due to higher and more constant drug concentration levels at the target site [9,10]. However, the injection to these sites is commonly associated with injection pain, a certain risk of infection and other injection-site reactions [11–13]. Hence, the reduction of dosing frequencies using sustained delivery systems is the key to more patient-friendly drugs, and may decide about future market prospects of a biopharmaceutical product [14]. The development of sustained release formulations for proteins has proven to be associated with major technical obstacles. Protein instability under physiological conditions, or occurring during formulation and manufacturing, caused by degradation products of the delivery matrix, an increased risk for immunogenicity, insufficient drug release, limited injectability and complex manufacturing processes are common challenges for the development of sustained delivery systems for proteins [14–16].

Recently, the electrostatic interactions between polyanions and mAbs have been put to use for the controlled release of mAbs [17]. An

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internal gelation technique with $CaCO_3$ ($CaCO_3$ depot) and $CaHPO_4$ ($CaHPO_4$ depot) as calcium sources was used to physically cross-link the alginate polymers. The slightly acidic pH of the formulation led to the presence of positive charges on the mAb surface and subsequent ionic interactions with the polyanionic alginate matrix. Relatively long release periods *in vitro* and changes in the viscoelastic properties of alginate after mAb addition led to the assumption that the mAbs became part of the alginate network. Despite this firm incorporation, mAb *in vitro* release was almost complete (~96%), the released mAb was bioactive (80–100%) and had a high monomeric content (>94%) [17] rendering the system very promising for its application as local sustained release system.

This study intends to investigate how the *in vitro* data translate into an *in vivo* application and to provide an insight into the systemic pharmacokinetics of mAb, the *in vivo* structure of the depot, the local tolerability and the local drug distribution of the mAb sustained release formulation. These characteristics were directly compared with those of a corresponding mAb liquid formulation. Additionally, as there is only limited theoretical knowledge available about *in vivo* controlled release with mAbs, the study aims to generate a fundamental data set for future studies in this field.

2. Material and methods

2.1. Materials

A human IgG1 monoclonal antibody was provided by Novartis Pharma AG as a >180 mg/mL stock solution. Ultra pure and sterile alginate PRONOVA SLG 100 was purchased from Novamatrix (Sandvika, Norway). All other chemicals were purchased from Sigma-Aldrich (Steinheim, Germany), if not mentioned otherwise.

2.2. Methods

Antibody content determination of non-serum samples using Protein A Chromatography and subsequent UV-detection at 280 nm were conducted as previously described [17].

2.3. Preparation of mAb formulations for the animal study

For the animal study each stock solution was sterile filtered using a 0.22 μM filter (Millipore, Molsheim, France), the calcium salts were gamma sterilized and compounding was conducted under aseptic conditions. The mAb liquid formulation contained 25 mg/mL mAb and 20 mM Histidine buffer at pH 6.5. The depots were prepared by suspending 30 mM of the calcium salts $CaCO_3$ or $CaHPO_4$ in a solution containing 20 mg/mL alginate, 25 mg/mL mAb and 20 mM Tris–HCl buffer at pH 8.0. The appropriate amount of a freshly prepared 1 M D-glucono-δ-lactone (GDL) solution was added to obtain a GDL concentration of 60 mM to initiate gelation. The detailed mechanism of the internal gelation process and the impact of mAb incorporation in the alginate matrix can be found in the literature [17–19]. For the placebo formulation no mAb was added and $CaCO_3$ was used to cross-link the alginate. The depot formulations were filled into 1 mL plastic syringes (BD, Franklin Lakes, USA) directly after initiation of cross-linking. A relatively high elasticity for both depot formulations at the injection point was selected in order to prevent dissipation of non-cross-linked polymers directly after injection. The $CaCO_3$ depot was stored over night (approximately 16 h) at room temperature. The rapid gelation of the $CaCO_3$-depot results in a short time interval between preparation and administration of the system in a liquid or semi-solid state. Thus, the injection of this system at the gel point was barely feasible. An additional consideration is the relatively constant pH of the $CaCO_3$ depot enabling long storage periods of the formulation prior to administration. As a consequence, the $CaCO_3$-depot was administrated fully cross-linked as a hydrogel and cross-linking occurred before administration in the syringe. The $CaHPO_4$ depot was stored 90 min at room temperature before injection. At this moment the viscoelasticity of the formulation was at the gel point, the threshold between liquid and solid ($\tan \delta = 1$). This allowed the investigation of the "*in situ* gelation" effect on the *in vivo* depot structure and *in vivo* drug release. The avoidance of a pH below 5.0 prior to administration (Supplemental Fig. S1C) was an additional consideration. This pH-level has been associated with the formation of precipitates and irreversible protein complexation for this protein-alginate combination [17]. At the time of injection the pH of the formulation was 5.2. The free diffusion of ions inside alginate hydrogels can be assumed to cause a rapid increase of pH in the physiological environment post administration. Nevertheless, the low pH inside the $CaHPO_4$ depot enhances drug-matrix interactions, which is expected to improve drug retardation.

2.4. Local tolerability and pharmacokinetic study in rats

The animal study was carried out in accordance with the guidelines of the local animal welfare committee under a license (license number: 425) of the cantonal veterinary office Basel-City, Switzerland. Three adult male Wistar rats were used per formulation; a single animal was used for the placebo formulation. The $CaCO_3$ depot and the placebo formulation were injected fully cross-linked as a hydrogel, whilst the $CaHPO_4$ depot was injected at the gel point (storage modulus (G') = loss modulus (G'')) (Supplemetal Fig. S1B). A sample volume of approximately 0.2 mL was injected subcutaneously into the interscapular region of the animals targeting a mAb dose of 20 mg/kg using a 23 gauge needle. The syringe was weighed before and after injection to determine the amount of injected sample. Sublingual blood samples of approximately 100 μL were collected under deep anesthesia before injection, 3 and 6 h, 1, 2, 4, 8, 16 and 28 days after administration. During the study, animals were regularly checked for general health and signs of irritation or reaction at the injection site. At termination of the study on day 28 the animals were exsanguinated under deep anesthesia. The injection site and the surrounding tissues were inspected macroscopically, sampled and divided into two equal pieces. One piece was processed for histopathology and immunohistochemistry, the other piece was used to determine the residual mAb content at the injection site. The mAb content of all samples was analyzed using a Meso Scale Discovery (MSD; Gaithersburg, Maryland) -based immunoassay, all samples were analyzed in duplicates.

2.5. Pharmacokinetic statistical analysis

From the plasma concentration-time profiles, the maximum plasma concentration (C_{max}) and the time to reach C_{max} (T_{max}) were obtained. Non-compartmental analysis was used for estimation of the basic PK parameters (terminal half-life ($T_{1/2}$) and the dose normalized infinity extrapolated area under the concentration-time curve (AUC∞)). The analysis was performed in Phoenix WinNonlin PK evaluation software package. The relative bioavailability (F_{rel}) of the depot formulations was determined by normalizing the AUC∞ of the mAb depot formulations to the AUC∞ of the mAb liquid formulation.

2.6. Tissue preparation for histology

One part of the sampled injection site was fixed for 48 h in neutral-buffered formalin, processed to paraffin block, sectioned, and stained with hematoxylin and eosin (H&E) for histopathological assessment.

2.7. Evaluation of mAb concentration in serum

The MSD electrochemiluminescence assay platform was used to quantify mAb in rat serum. In between each step the plates were washed using assay diluent (1% BSA, 0.05% Tween 20). Streptavidin labeled MSD plates (MSD; Gaithersburg, Maryland) were blocked