Short Communication

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Do plasticized polyvinylchloride and polyurethane infusion sets promote infliximab adsorption?

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Abstract

Objectives: Infliximab diluted solutions have been shown to be physicochemically stable for long periods, however the adsorption of infliximab during infusions has not been readily investigated. This study aimed to evaluate potential adsorption phenomena of infliximab during administration through Polyvinylchloride (PVC) and Polyurethane (PU) infusion sets.

Methods: Infliximab (INFLECTRA®) solutions at 0.4 mg/mL and 2 mg/mL were submitted to static (at T0, 24 and 96 h) and dynamic contact (flow rate of 2 mL/min during 2 h with analysis times at T0, 5 min, 30 min, 60 min and 120 min) with three different infusion sets. Two contained PVC plasticized with tris(2-ethylhexyl) trimellitate (TOTM) tubings and one set was in PU tubing. Infliximab was quantified at each analytical time by protein total quantification using UV-spectroscopy according to European Pharmacopeia Monography (2.5.33) and size exclusion chromatography (SEC) which allowed a specific quantification of the monomeric form and was able to highlight potential modification such as aggregation or oligomer formation.

Results: For all analysis times and conditions, infliximab concentrations remained unchanged with a maximum variation of 2.81 and 4.63% from the initial concentrations

assessed by SEC and UV spectroscopy and the percentage of monomeric form remained unaltered.

Conclusions: Our study showed that there was no significant loss of infliximab. According to these results each of the three infusion sets could be used for the administration of infliximab solutions without causing any loss of active substance.

Keywords: adsorption; infliximab; infusion sets; polyurethane; polyvinyl chloride.

Introduction

Infliximab is an IgG1 monoclonal antibody (mAb) that binds and inhibits transmembrane and soluble forms of Tumor Necrosis Factor alpha (TNF- α). This biological medicine is now approved for use in a wide range of diseases from diverse medical disciplines such as rheumatology, gastroenterology and dermatology, such as for example rheumatoid arthritis, spondylitis ankylosing and psoriatic arthritis, Crohn's Disease, ulcerative colitis and psoriasis. The stability of diluted and ready to use infliximab solutions has now been fairly well-studied, for concentrations ranging from 0.4 to 2 mg/mL, generally in polyolefin bags at both refrigerated and room temperature [1–3], and it has shown that it could be stable for up to 90 days, depending on the conditions. However, there is little information about its behaviour during infusions through infusion lines and catheters. Indeed, even though polymeric-based surfaces (polyvinyl chloride, silicone, polyurethane) are always found in medical devices used for infusion, and that they are known for interacting with small peptides, like cyclosporine or insulin [4–6], their potential interactions in clinical situations with mAbs has not been fully studied. As such, adsorption phenomena can be a major concern during the infusion process since the drug lost is not administered to the patient and means a loss of effectiveness of the treatment. Infusion sets are

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medical devices used to connect infusion bags with the catheter, and are generally about 150–200 cm long, and are also known to cause drug loss and potentially release toxic compounds [7, 8]. The objective of this study was therefore to evaluate potential adsorption phenomena of infliximab during administration through Polyvinylchloride (PVC) and Polyurethane (PU) infusion sets.

Materials and methods

To realise the study, the following compounds and devices were used:

- Inflectra® (an infliximab biosimilar) powder for solution for infusion (batch 2485036, expire on 09/30/2020), graciously provided by Pfizer France SAS.
- Sterile water for injection, batch 15496412, expire 11/2018 (Ecoflac®, B Braun).
- 250 mL 0.9% NaCl bags, batch 14KE7321, expire 01/2018 (Freeflex+, Fresenius Kabi).
- Infusion sets:
 - Codan V86 Duo I.V STAR 10, 43.4359, batch n°: H7876-1, expire 05/2019, PVC plasticised with TOTM tubing
 - Doran ref KIS04F, batch n°: 241609 M, expire 08/2020, TOTM plasticised PVC tubing
 - Sendal (Carefusion) ref PERFUSEND A96, batch number TA06157, expire 30/08/2020, PU tubing

All reagents were certified of HPLC grade. Sodium sulphate (Na_2SO_4), disodium phosphate (Na_2HPO_4), and sodium azide (NaN_3) were all purchased for Sigma-Aldrich (Saint Quentin Fallavier Cedex, France).

To sterilely prepare the 10 mg/mL infliximab solutions that were used, Inflectra® powder for solution for infusion was reconstituted by adding 10 mL of water for injection to the medication vial, according to the manufacturer's instructions using a sterile metal needle (Blunt Fill Needle 18 G $\times$ 1 1/2", batch 151014, expire 10/2020, Becton Dickinson, Spain), under the laminar air flow of an ISO 4.8 microbiological safety cabinet. Immediately after reconstitution, 10 mL were removed from a 250 mL 0.9% NaCl bag, then 10 mL of infliximab solution were diluted to a theoretical concentration of 0.4 mg/mL into the bag. For the 2 mg/mL bags, 20 mL were removed from a 100 mL NaCl bag and 20 mL of infliximab solution were added.

The potential sorption of infliximab to the tubings was evaluated in static and dynamic conditions (using a 2 mL/min flowrate) with the three different infusion sets at using diluted infliximab at 0.4 and 2 mg/mL. Static contact was performed in a climatic chamber at 25 °C and 60% residual humidity and dynamic contact was done at room temperature (22 ± 2 °C). At determined times (0, 5, 30, 60 and 120 min in dynamic study and 0, 1, 2 and 24 h in static study) the infliximab solutions were analysed by total protein quantification and size-exclusion chromatography.

For each condition, infliximab was also quantified in the bag before infusion through the infusion set and each result was expressed as the percentage of infliximab concentration after infusion set compared to infliximab concentration in the bag.

For both conditions (static and dynamic contact), three tubings were studied and one sample was withdrawn from each tubing at all analytical times.

The analyses were performed as follows:

- Size exclusion chromatography (SEC) allowed the separation and specific quantification of the infliximab monomeric form, as well as low and high molecular weight products (respectively LMWP and HMWP) resulting from fragmentation or aggregation. LMWP and HMWP were quantified as a percentage of total area under curve of the different species detected on the chromatograms. The chromatographic separation was performed by liquid chromatography (LC), using an LC-2010-A HT with integrated controller, pump, autosampler, oven and UV–VIS detector (Shimadzu Corporation, Marne la Vallée, France). Two LC separation columns TSK – GEL® G3000SWXL columns (7.8 mm $\times$ 30 cm $\times$ 5 μm , TOSOH Bioscience), purchased from Interchim (Montluçon, France) were used, mounted in series. A sample volume of 100 μL was injected into the system and then eluted using an isocratic flow (Na_2SO_4 0.1 M, Na_2HPO_4 0.1 M and 0.05% m/w NaN_3 in water, buffered at pH 7) at a flowrate of 0.6 mL/min, at a set temperature of 25 °C. The detection wavelength was set up at 280 nm. Validation of the quantification method was performed by preparing one calibration curve daily for three days using five concentrations of infliximab from 0.10 to 3.33 mg/mL diluted in 0.9% sodium chloride. Each calibration curve should have a determination coefficient R^2 equal or higher than 0.999. Homogeneity of the curves was verified using a Cochran test. ANOVA tests were applied to determine applicability. Method accuracy was verified by evaluating recovery of theoretical concentrations to experimental values found using mean curve equation, and results should be found within the range of 95–105%.
- Protein total quantification in each vial was estimated by measuring the absorbance at 280 nm of infliximab® solutions, following European Pharmacopeia Monography 2.5.33 [9] instructions. All the data was acquired on a Jasco V-670 UV/VIS/NIR spectrophotometer (Jasco France, Bouguenais, France), using 1 cm pathlength quartz cuvettes. Protein content was calculated using the Beer–Lambert law, and the results were expressed as a percentage of absorbance variation of each analysed solution from the initial bag.

Data analysis – acceptability criteria

The study was conducted following the methodological guidelines issued by the International Conference on Harmonisation for stability studies (ICH guidelines for stability) [10] and specifications for Biotechnological/Biological products [11], Bardin et al. [12], the European Pharmacopeia [13] and recommendations issued by the French Society of Clinical Pharmacy (SFPC) and the Evaluation and Research Group on Protection in Controlled Atmosphere (GERPAC) [14]. Infliximab concentration after the infusion set was considered acceptable if the monomeric form did not vary by more than 5% from the initial concentration in the bag, when quantified by SEC. For total protein quantification, the absorbance of the solution after perfusion was compared to the absorbance of the infliximab solution in the initial bag. A variation lower than 5% was considered to be acceptable.

Results

Static contact with the three infusion set was studied during 24 h. The evolution of infliximab concentrations as

quantified by SEC is presented Figure 1 for respectively the 0.4 mg/mL (A) and 2 mg/mL (B) infliximab concentrations. No loss of monomeric infliximab was noted by SEC for either studied concentration or infusion tubing. Throughout the study, total protein content did not vary by more than 4.63% for both concentrations and all three infusion sets. Overall, for both studied static conditions and with all infusion set, infliximab concentrations remained within acceptability criteria limits.

Dynamic contact with the three infusion set was studied during 2 h at a 2 mL/min flow rate, following the administration recommendation of infliximab solutions. Evolution of infliximab concentration by SEC was studied at 0.4 mg/mL Figures 2A and 2 mg/mL Figure 2B. During dynamic infusion, concentrations varied by less than 2.21% from reference concentrations, for all conditions and infusion devices. Throughout the study, total protein content did not vary by more than 3.24%, for both concentrations. For both studied dynamic conditions and with all infusion set, infliximab concentration remained within acceptability criteria.

Additionally, for both static and dynamic contact situations, no additional low or high molecular weight species were detected, and present species did not vary in concentration after infusion when compared to before (see Figure 3 as an example chromatogram).

Discussion

In this work, we assessed whether PVC and PU infusion sets could generate clinically relevant adsorption phenomena of diluted infliximab solutions during simulated infusions. Adsorption was the only suspected phenomenon, given the steric hindrance of a mAb, therefore theoretically excluding absorption. As an adsorption phenomena could have decreased the amount of infliximab available in the studied

solutions, the global amount of infliximab was assessed by measuring total protein content. Specific monomeric infliximab was quantified by measuring the AUC of the monomeric form and of the other species (low and high molecular weight species) separated by SEC to provide a more specific information. Neither of those techniques were able to highlight any variation from the initial concentration in the infusion bags, for both low (0.4 mg/mL) and higher (2 mg/mL) concentrations, for any of the infusion sets that we tested. Also, no modifications of infliximab species (low or high weight species) were detected. Overall, our results did not indicate that the chemical nature of the tubing (PVC or PU) has any clinically relevant impact on infliximab adsorption phenomena.

Proteins are surface active molecules, and have a tendency to adsorb to hydrophobic surfaces and interfaces: the adsorption phenomenon is especially relevant when the drug is highly diluted, when the contact surface is important, or both [15, 16]. Interactions with glass, PVC and polyolefin materials have been reported in laboratory or clinical simulating conditions [17–19], but there is scarce literature about the behaviour of mAbs during infusions through medical intravenous tubings. However, it has been reported that, when diluted to 0.01 mg/mL in 0.9% saline solution, mAbs do adsorb to positively charged polyethersulfone and polyamide in-line filters membranes [20]. However, the clinical impact of such findings is limited by the high dilution factor (of 2,500–15,000) applied in that study. It has also been shown that excipients like surfactants (for example polysorbate 20 or polysorbate 80) can greatly reduce adsorption phenomena by limiting the exposure of hydrophobic regions and so decreasing protein-protein interactions and interface-induced aggregation, also prevented by competition for adsorption sites [21]. For example, Blinatumomab (Blinicyto®) is a monoclonal antibody known for his tendency to adsorption onto

Figure 1: Evolution of infliximab concentrations (mean $\pm$ 95% confidence interval, $n=3$) quantified by size exclusion chromatography at 0.4 mg/mL (A) and 2 mg/mL (B) during 24 h of static contact.

Figure 2: Evolution of infliximab concentrations (mean $\pm$ 95% confidence interval, $n=3$) quantified by size exclusion chromatography at 0.4 mg/mL (A) and 2 mg/mL (B) during 2 h of simulated infusion (dynamic condition).

Figure 3: Size exclusion chromatograms of 0.4 mg/mL infliximab solutions before (black curve) and after (blue curve) a 2-h infusion through the studied infusion sets: V86 IV Star (A), KIS04F (B) and Perfusend A96 (C). HMWP: High Molecular Weight Product; LMWP: Low Molecular Weight Product.

surfaces and it is recommended to prepare administration bags and tubings with a polysorbate 80 solution to prevent sorption. In this case, Inflectra® contains polysorbate 80, and in view of our results, it seems therefore quite possible that this excipient does retain some protective capacity, even when diluted with the solution.

Despite the positive information brought by this work about the lack of relevant adsorption phenomena of infliximab solutions to infusion set tubes, interactions between the drug solution and the material surface cannot be totally excluded, as the method used to follow the adsorption (quantification of infliximab) might not be capable of detecting very low levels of adsorption. Indeed, one of the limiting factors when a molecule is adsorbed onto a material is the contact area which could reach a sort of saturation state. In this study, infliximab was infused at two different concentrations (one low at 0.4 mg/mL, and a higher one at 2 mg/mL) respecting the interval of recommended concentrations for clinical use, but which remain relatively high. So even if infliximab had adsorbed onto the infusion sets, its loss could have been too small proportionally (when compared to the initial infliximab quantity) to be significant. Also, modification of the tube's material could occur during infusion, for example leaching out of unwanted chemicals, like plasticizers from the PVC [22, 23]. Therefore, a study of the material's surface would be needed in order to fully rule out any infliximab adsorption.

Overall, this study does validate the use of the three infusion sets that we tested for infliximab infusion, however the impact of the catheter, which could also generate interactions, still needs to be studied, especially as it will have to endure multiple and repeated administrations. Also, as the infusions were performed according to the Inflectra® summary of products characteristics and the stability of the monoclonal antibody was therefore

supposed to be maintained during the infusion. As such, assays evaluating the primary, secondary and tertiary structure were not implemented, nor were tests used for evaluating its physical or chemical stability.

Conclusions

Our study showed that there was no loss of infliximab during the infusion through three different infusion sets, however a sorption phenomenon cannot be excluded and a surface study of the infusion set would be needed to investigate this hypothesis. According to these results each of the three infusion sets could be used for the administration of biosimilar infliximab (Inflectra®) solutions without causing any loss of active substance.

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