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Original Article | Effect of pneumatic tube transport on monoclonal antibody stability

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KEY WORDS
monoclonal antibodies, antibody structure, stability, pneumatic tube system, cleanroom, logistics

ABSTRACT

BACKGROUND AND AIM
Pneumatic tube transport (PTT) is often used to conveniently transport drugs from the pharmacy to the clinical ward. Proteins like monoclonal antibodies are inherently sensitive to mechanical disruption. Despite their increased use, little data is available on the stability of monoclonal antibodies when they are transported via such systems. The effect of PTT on monoclonal antibody stability will be studied.

METHODS
Samples of ready-to-infuse preparations of monoclonal antibodies (bevacizumab, vedolizumab, cetuximab, trastuzumab, trastuzumab emtansine, pembrolizumab, rituximab, nivolumab, pertuzumab, infliximab, panitumumab and infliximab biosimilar) were taken before and after PTT. The structural stability of the antibodies was measured using size-exclusion chromatography with multiple angle laser light scattering. Before transportation, care was taken to remove all air from the infusion bags.

RESULTS
The mean duration of transport via pneumatic tube was 40 seconds. A total of 65 preparations with monoclonal antibodies in different concentrations was analysed. For all antibodies studied no significant difference was seen in antibody structural stability and protein aggregation between the sample before and after transportation with the pneumatic tube system.

DISCUSSION AND CONCLUSION
Because of lack of data and the inherent fragility of protein drugs, most manufacturers advise against transporting monoclonal antibodies via a pneumatic tube. This study shows that real-life pneumatic tube transport does not affect the stability of prepared infusions of bevacizumab, vedolizumab, cetuximab, trastuzumab, trastuzumab emtansine, pembrolizumab, rituximab, nivolumab, pertuzumab, infliximab, panitumumab and infliximab biosimilar respectiverly.Our results show that for 12 commonly used Mab drugs there was no significant effect of PTT on Mab structure and concentration, and that it is possible to use PTT to dispatch these delicate drugs to the clinical ward.

INTRODUCTION

Over the last 2 decades, cancer treatment evolved from classical cytotoxic chemotherapy to targeted therapies and immunotherapy. Certain monoclonal antibodies (Mabs) bind specifically to growth factor receptors and inhibit tumor growth, whereas checkpoint inhibitor Mabs increase the immune respons against cancer cells. The use of Mabs in the treatment of cancer is still expanding. Mabs are also increasingly used in the treatment of autoimmune, allergic and complement-mediated diseases such as inflammatory bowel disease (IBD), rheumatoid arthritis, psoriasis, eye disease and asthma.The high cost of Mabs requires efficient use with minimal waste. Many Mabs are administered intravenously in the hospital day unit, after aseptic preparation in the hospital pharmacy department.

Mabs are highly complex proteins consisting of two light chains and two heavy chains bound together by disulphide bridges and stabilized by protein-protein interactions. This makes them very sensitive to chemical instability, denaturation and aggregation when manipulated[1-4]. In addition to chemical instability, denaturation reflected by protein aggregation is the greatest stability concern of Mabs[3]. Aggregation can be caused by changes in temperature (cold chain interruption) and mechanical stress, for example during drug preparation when shearing forces by repeated pulling up in a needle, or shaking and foaming could destabilize the protein, or during transport when shaking and consequent foaming might occur[3, 5-7]. Loss off stability and aggregation leads to reduced efficacy or increased toxicity of the product. Aggregated antibodies can cause microemboli and have increased immunogenicity, which can lead to the production of neutralizing anti-drug antibodies (ADA), or anaphylactic reactions upon repeated Mabs administration[8,9]. Daily practice often leads to situations that might compromise drug stability. Yet we have very little practical evidence-based guidance on the stability of Mabs after manipulation in day-to-day use.

Manufacturers of Mabs only provide the minimal mandatory stability information for drug registration. However, before administration of the Mabs to the patient, mechanical forces can affect their stability in every step of the preparation and distribution process[3,5]. For example, many hospitals use a pneumatic tube transport (PTT) system to transport medicines to the end user. High accelaration speed and significant impaction can occur in a PTT dispatch system. Given the inherent sensitivity of protein drugs to mechanical forces this transportation method is often not advised. Simulated laboratory settings demonstrate air-liquid interfaces and foaming resulting in aggregation[2, 10, 11], but objective and real-life data on stability of Mabs after PTT are scarse[12]. The aim of this study is to determine if a PTT can be used for transportation of Mabs preparations in an 850 bed hospital with over 4500 Mabs treatments on a yearly basis.

ABSTRACT

Preparation of the ready-to-administer infusion bags

Upon receipt of a medical prescription the respective Mabs were dissolved and prepared in accordance with the product label using 0.9% w/v NaCl in polyolefin infusion bags (Macoflex® biluer, Macopharma). A closed system transfer device (Tevadaptor®) was used for all preparations studied. Care was taken to remove all air from the infusion bag in order to minimize foaming during transport to the ward via the PTT dispatch system.

Sampling

A volume of 0.5 ml was withdrawn from the prepared infusion bag and transferred into a labelled microtube. The microtubes were stored immediately between 2-8°C until analysis maximum 7 days later. Samples were taken at the pharmacy immediately prior to transportation with the PTT (t0) and at the ward immediately after arrival of the infusion bag (t1). A positive control to mimic the effect of maximal mechanical disturbance on protein stability of the Mab consisted of a sample of pertuzumab that was filled with air, sent over the PTT for five times (t2) and vigourously shaken using a vortex device for 5 minutes (t3). A second positive control consisted of a sample of vedolizmab that was heated at 95°C for 2 hours.

Transport via pneumatic tube transport (PTT) system

The infusion bags were transported via the PTT system Telecom BP Atlas. A separate system was used exclusively for transportation of cytotoxic and potentially hazardous drugs. The Aerocom tubes cover a distance of 62 m through 8 curbes and 3 exchange stations. The speed of the tube is between 2 to 3 m/s and the mean travel time was 40 sec from the pharmacy compounding unit to the hospital infusion center.

Physicochemical analysis using size exclusion chromatography with multiple angle laser light scattering (SEC-MALLS)

Size Exclusion Chromatography (SEC) was used to separate proteins based on size, with larger molecules eluting first. Multi-Angle Laser Light Scattering (MALLS) was used to measure the molecular weight (Mw) of the protein, simultaneously the concentration was determined via UV / refractive index and light scattering intensity. Light scattering has a higher sensitivity for larger molecules and is therefore a sensitive detection method for protein aggregates. The polydispersity index (PDI) is a measure for homogeneity and was used to determine the quality of a solution. It is the ratio of the weight-average molar mass (Mw) to the number-average molar mass (Mn). For monodisperse samples the PDI equals 1.000. The higher the heterogeneity in a sample, the more PDI differs from 1.000. Protein degradation and aggregation will increase the PDI of the sample.To measure the aggregation state of the diluted antibody, SEC-MALLS analysis was performed on the Agilent 1260 HPLC system coupled with a Superdex 200 (Cytiva) size exclusion chromatography column provided with a multiple wavelength UV detector, multi-angle laser light scattering detector (Wyatt) and differential refractive index detector (dRI) (Wyatt). For this, 100 µg of each sample was injected onto the Superdex 200 size exclusion chromatography column using the autosampler. The sample was first separated based on its hydrodynamic volume by SEC followed by detection at UV280 nm, MALLS and dRI. Bovine serum albumin (BSA) was added to the run sequence as a control to check instrument parameters and perform calibrations if needed. From each analysis the % of monomer and aggregates could be determined by peak integration based on dRI. The light scattering was used to make an estimation of the molecular weight in solution while dynamic light scattering (using QELS (Quasi-Elastic Light Scattering) fiber) was used to determine the hydrodynamic radius of the molecules. All samples were analyzed using the same method, flow rate (0.5 ml/min), buffer (PBS + 0.02% sodium azide) and control (BSA).

RESULTS

Over a 1 week time period, 130 samples were collected from 65 infusion bags. Twelve different products in different concentrations were collected. Table 1 illustrates the different products and the concentration ranges used.

trade name	generic name	powder/ solution	dilution	n° of samples (t0+t1)	mean conc (mg/ml)	conc range (mg/ml)	class	IgG
Avastin	bevacizumab	solution	NaCl 100 ml	12	5,06	2,69 - 9,38	anti-VEGF	IgG1
Entyvio	vedolizumab	powder	NaCl 250 ml	3	1,18	1,18 - 1,18	α4β7-integrin	IgG1
Erbitux	cetuximab	solution	NA	5	5	5 - 5	anti-EGFR	IgG1
Herceptin	trastuzumab	powder	NaCl 250 ml	6	1,6	1,33 - 2,33	anti-HER2	IgG1
Kadcyla	TDM-1	powder	NaCl 250 ml	1	0,93	0,93 - 0,93	anti-HER2	IgG1
Keytruda	pembrolizumab	solution	NaCl 100 ml	2	1,85	1,85 - 1,85	anti-PD-1	IgG4
Mabthera	rituximab	solution	NaCl 500 ml	1	1,26	1,26 - 1,26	anti-CD20	IgG1
Opdivo	nivolumab	solution	NaCl 100 ml	8	1,93	1,93 - 1,93	anti-PD-1	IgG4
Perjeta	pertuzumab	solution	NaCl 250 ml	4	1,95	1,59 - 3,02	anti-HER2	IgG1
Remicade	infliximab	powder	NaCl 250 ml	21	1,29	0,93 - 1,91	anti-TNF	IgG1
Remsima	infliximab	powder	NaCl 250 ml	1	1,09	1,09 - 1,09	anti-TNF	IgG1
Vectibix	panitumumab	solution	NaCl 250 ml	1	4,1	4,1 - 4,1	anti-EGFR	IgG2

Table 1: Overview of products used for the experiment

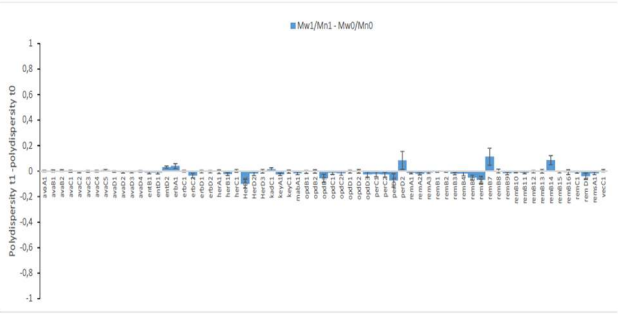

Figure 1: Change in polydispersity of t1 versus t0 per sample (N = 65)

Figure 1 illustrates the difference in polydispersity for all samples between t1 (after transportation via pneumatic tube) versus t0 (before transportation via pneumatic tube). All values are between +0.1 and -0.1, indicating that the samples are monodisperse, in the typical heterotetrameric format (two heavy chains and two light chains) and free of aggregates, even after transportation via the PTT system. Figure 2 shows the PDI's of the Mab preparations, grouped per product. No distinct difference can be seen between t0 and t1.

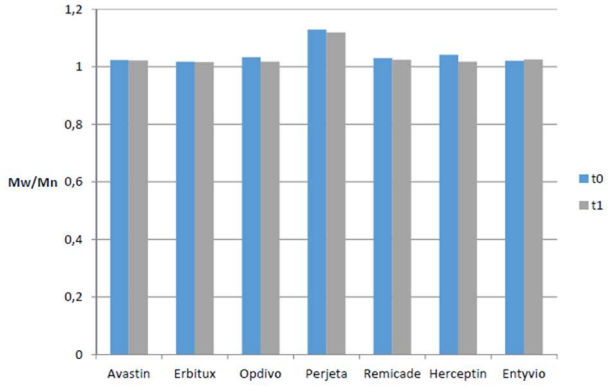

Figure 2: Difference in Polydispersity (Mw/Mn) between different antibody preparations, grouped per product

The highest abberation of the PDI was seen in the positive control with pertuzumab. However, even this sample remained remarkably stable and free of aggregates (Figure 3).

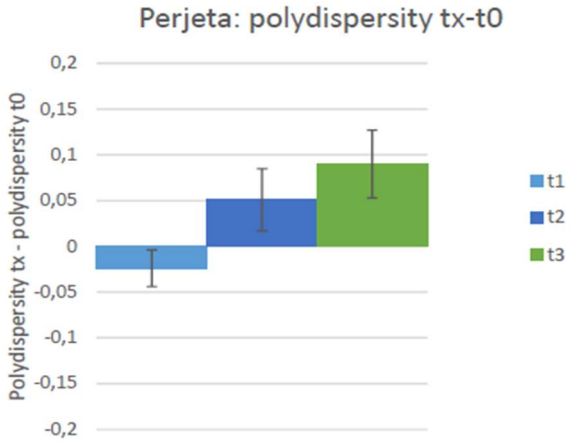

Figure 3: Differences in polydispersity of pertuzumab after 5 x transportation via PTT (t2) and vortexed for 5 min (t3)

The second positive control with vedolizumab showed a clear increase of PDI and protein aggregation, demonstrating the validity of SEC-MALLS to detect Mab destabilisation (Figure 4).

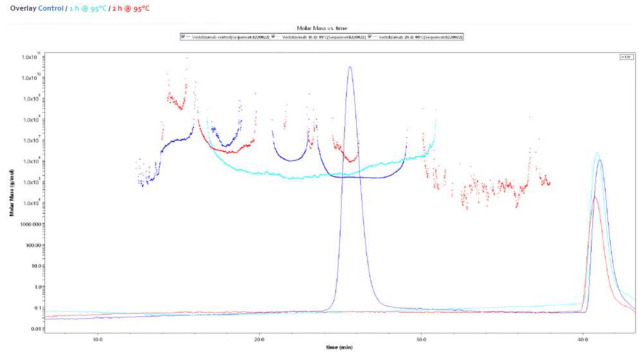

Figure 4: After 1h and 2h of incubation at 95°C, all vedolizumab signal is lost, compared to the control

DISCUSSION

According to the European guidelines for the stability of anti-tumor drugs, there is a great lack of practical information concerning the stability of anti-tumor drugs after handling in daily practice[1, 3]. Due to the inherent sensitivity of proteins to mechanical denaturation caused by shearing and shaking and subsequent foaming that might occur during transportation, many hospitals do not use their pneumatic tube transport (PTT) system for the transportation of Mabs. A worldwide study showed that only 7% of Mabs infusions are transported via PTT[13]. This could have an impact on the uniformity and the efficiency of the distribution process both at the pharmacy department and on the ward.

This study included twelve different Mabs either used to treat various cancer types or used for the treatment of inflammatory bowel disease and rheumatoid arthritis. We focused mainly on SEC-MALLS analysis and found that the antibodies after PPT were still monodisperse, in the traditional tetrameric Mab format and free of protein aggregates, the latter being the most detrimental aspect of mechanical protein destabilization[3]. Strikingly, whereas heat denaturation caused protein aggregation, even extreme vortexing of one of the samples still did not lead to protein aggregates or antibody decay, suggesting that the formulation of the Mabs used was resistant to mechanical effects of transportation[6]. One possible explanation is that these antibodies were selected during the development process to be stable upon transport, and that additives and surfactant excipients, such as polysorbate 80 or polysorbate 20 might protect the Mabs from mechanical degradation caused by stirring and shaking[6, 11, 14, 15].

One drawback of our study is that we only measured the biophysical stability of the Mabs after transport using SEC-MALLS and did not address the biological activity of the antibodies or their binding to the natural target, as suggested by the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use, and reiterated by a European Consensus guideline[3, 12]. However, it would be unlikely that in the absence of protein denaturation as shown in our study, a major loss of binding properties or biological activity would occur. We cannot however exclude chemical modifications of the Mabs. We are currently performing an experiment where we have addressed the long term storage of vedolizumab, and where there is an absolute correlation between monodispersity index and maintenance of target binding of the antibody to integrin a4b7 (data not shown). Given the multitude of antibodies tested here, we could not perform such binding studies for every single Mab before and after PTT. It is also suggested that stability measurements should be performed with at least three different preparations of each Mab. This was done for 8 out of 12 of the Mabs tested here, yet only once on paired samples of trastuzumab emtansine, rituximab, panitumumab and infliximab biosimilar.

CONCLUSION

Based on the SEC-MALLS results for multiple Mabs preparations taken from a real-life clinical situation using a pneumatic tube system for transportation from the pharmacy department to the ward, no significant effect of PTT transport on the physicochemical stability of Mabs could be demonstrated. We therefore suggest it is possible to transport infusion bags of these drugs using pneumatic tube transport systems.

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