Regular Article

Comparison of the Adsorption of Original and Biosimilar Preparations of Filgrastim on Infusion Sets and the Inhibition of Adsorption by Polysorbate 80

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The purpose of the study was to evaluate the adsorption of filgrastim on infusion sets (comprising infusion bag, line and filter) and to compare the adsorption of the original filgrastim preparation with biosimilar preparations using HPLC. The inhibitory effect of polysorbate 80 on this adsorption was also evaluated. Filgrastim was mixed with isotonic sodium chloride solution or 5% (w/v) glucose solution in the infusion fluid. Filgrastim adsorption on infusion sets was observed with all preparations and with both types of infusion solution. The adsorption ratio was about 30% in all circumstances. Filgrastim adsorption on all parts of the infusion set (bag, line and filter) was dramatically decreased by the addition of polysorbate 80 solution at concentrations at or over its critical micelle concentration (CMC). The filgrastim adsorption ratio was highest at a solution pH of 5.65, which is the isoelectric point (pI) of filgrastim. This study showed that the degree of filgrastim adsorption on infusion sets is similar for original and biosimilar preparations, but that the addition of polysorbate 80 to the infusion solution at concentrations at or above its CMC is effective in preventing filgrastim adsorption. The addition of a total-vitamin preparation with a polysorbate 80 concentration over its CMC may be an effective way of preventing filgrastim adsorption on infusion sets.

Key words filgrastim; adsorption; infusion set; polysorbate 80

The Japanese government now actively promotes the use of generic drugs in order to reduce national medical expenditure.1–3 As part of the drive to reduce health costs, biosimilar preparations are also beginning to be used in clinical practice in place of original biologicals. However, there are growing concerns about switching from the original preparation to biosimilar preparations in clinical practice.4 In contrast to generic preparations, it is widely considered to be impossible to make biosimilar preparations with identical biological properties to the original preparations. The uptake of biosimilar preparations has not been as rapid as the change to generic preparations as switching to a biosimilar preparation does not result in as much cost savings as switching to a generic preparation, bio-pharmaceuticals also being covered by high-cost illness insurance.

Filgrastim is a granulocyte colony-stimulating factor used to treat neutropenia. Generally, filgrastim is administered subcutaneously but in patients with a known bleeding disorder, intravenous infusions may be required. In previous reports, original and biosimilar preparations of filgrastim have been shown to have similar clinical effects.3–5

In previous reports, it has been demonstrated that protein preparations, such as insulin, adsorb to infusion sets,6–8 and filgrastim adsorption on infusion sets has also been reported.9–13 Thus, it is possible that the actual dose of filgrastim infused will be smaller than the theoretical dose predicted. Yagi and Kawa have also found that differences in flow rate affect the adsorption ratio.12 The quantitative evaluation of commercial preparations, including biosimilar preparations, from the standpoint of in vitro adsorption on infusion sets has not yet been clarified.

It has previously been reported that the adsorption of filgrastim on infusion filters varies according to the type of membrane used.14 Filgrastim adsorption on filter made from cellulose admixture ester membrane was found to be significantly higher than on other filters (including a sulfone membrane filter of the type used in the present study). Thus, the type of filter material used in the administration of filgrastim may also affect the clinical response due to a decrease in the administered dose.

Polyvinyl chloride (PVC) free infusion sets is useful for inhibiting of drug adsorption in drug of adsorption on infusion sets.15 However, it was reported that extent of filgrastim adsorption was not different between infusion sets materials which is polyvinyl chloride, ethylene–vinyl acetate copolymer and polybutadiene. This result suggested that infusion sets materials were not affected in the adsorption of filgrastim.9 Therefore, we did not compare the adsorption of the filgrastim preparations on infusion sets between infusion sets materials.

Previous papers have reported that the addition of the surfactant polysorbate 80 is an effective way to prevent adsorption on infusion sets.6–13 Polysorbate 80 is present in many total-vitamin preparations, which have also been shown to inhibit filgrastim adsorption on infusion sets.13 However, the concentration of polysorbate 80 necessary for inhibition of adsorption has not yet been reported.

In this study, therefore, we compared the adsorption of the original filgrastim preparation on infusion sets (comprising infusion bag, line and filter) with that of three biosimilar preparations using HPLC. Secondly, we determined the minimum polysorbate 80 concentration capable of preventing filgrastim adsorption on infusion sets. The effect of the pH of solution on adsorption was also examined.

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Experimental

Materials  The original preparation (A) and three biosimilar preparations (B to D) used in the evaluation of filgrastim adsorption on infusion sets are described in Table 1. Isotonic sodium chloride solution, 5% (w/v) Glucose Injection, terufusion® administration set TI-U350P and terufusion® final filter PS TF-SS430H (all Terumo Co., Ltd., Tokyo, Japan) were used in this study. Infusion set materials are summarized in Table 2. Screw-top bottle used as sample collection was made from glass. Filgrastim (Wako Pure Chemical Industries, Ltd., Osaka, Japan) as a standard was used in the adsorption inhibition study and influence pH study. Tween® 80 (polysorbate 80; Nacalai Tesque, Inc., Kyoto, Japan) was used in the adsorption inhibition study.

Instrument Preparation  All instruments except infusion sets were coated according to a common procedure, with 1% bovine serum albumin in phosphate-buffered saline (PBS) to prevent adsorption of filgrastim.

Methods

Comparison of Filgrastim Adsorption on Infusion Sets from Original and Biosimilar Preparations

The scheme of sample collection is shown in Fig. 1(a). Firstly, the filgrastim preparation (150 µg) was added to either isotonic sodium chloride solution or 5% (w/v) glucose solution in a screw-top bottle. The solutions were gently agitated and a 1 mL sample collected (baseline solution, Sample A). The bottled solutions were then put into the infusion bag and gently agitated before a second 1 mL sample was collected (Sample B). The infusion line was then connected to the bag and the fluid allowed to flow from the bag into a screw-top bottle (flow rate: 100 mL/60 min). A 1 mL sample was collected from a screw-top bottle (Sample C). Finally, the collected solutions were returned to the infusion bag, and the infusion line and filter were connected. Solutions were allowed to flow from the bag through the line and filter (flow rate: 100 mL/60 min), into a screw-top bottle from where the final 1 mL sample was collected (Sample D). Filgrastim concentrations of samples A to D were measured using HPLC. Adsorption ratios were calculated from the following equations:

\[
\text{Adsorption ratio on infusion bag (\%)} = \frac{(A - B)}{A} \times 100 \quad (1)
\]

\[
\text{Adsorption ratio on infusion line (\%)} = \frac{(B - C)}{B} \times 100 \quad (2)
\]

\[
\text{Adsorption ratio on total infusion set (\%)} = \frac{(A - D)}{A} \times 100 \quad (4)
\]

The concentrations of filgrastim in each sample solution were determined using HPLC. For HPLC, 100 µL was injected onto a chromatograph (LC-10AT VP, Shimadzu Corporation, Kyoto, Japan) equipped with a UV detector (SPD-10A VP, Shimadzu Corporation, Kyoto, Japan), an integrator (LC solution, Shimadzu Corporation, Kyoto, Japan) and reverse-phase column (CAPCELL PAK C18 UG120 S5: 4.6 mm, i.d. 250 mm; Shiseido Co., Ltd., Tokyo, Japan). The column oven and auto sampler temperature were kept at 60 and 4°C, respectively. The mobile phase composition was: (A) acetonitrile–water–trifluoroacetic acid (30:70:0.1, v/v/v) and (B) acetonitrile–water–trifluoroacetic acid (90:10:0.1, v/v/v), respectively, and the flow rate was 0.65 mL/min. Run time was 40 min with linear gradient elution: 0–5 min (25–51% B), 5–15 min (51–85% B), 15–20 min (85–25% B), 20–40 min (25% B). The wavelength was set at 215 nm.

Determination of Polysorbate 80 Concentration Required to Inhibit Filgrastim Adsorption on Infusion Sets

The scheme of sample collection is shown in Fig. 1(b). Filgrastim (150 µg) and various volumes of polysorbate 80 (0.020, 0.033, 1.4, 40, 80 mg) were added to the isotonic sodium chloride solution or 5% (w/v) glucose solution in the screw bottle. The solutions were gently agitated. The sample collection and concentration measurements were conducted as described above. The polysorbate 80 concentrations selected were the minimum and maximum content (by volume) of the levels present in the filgrastim preparations used (0.020, 0.033 mg, respectively, see Table 1), the critical micelle concentration (CMC) of polysorbate 80 (1.4 mg/ml) and the minimum and maximum content by volume of the total-vitamin preparations currently available on the Japanese market (40 and 80 mg, respectively).
Influence of pH on Filgrastim Adsorption on Infusion Sets

To evaluate the influence of pH on filgrastim adsorption, filgrastim (30 µg) was added to 20 mL of PBS at various pHs (4.5, 5.0, 5.5, 5.65, 6.0, 6.5) in a screw-top bottle. The solutions were gently agitated and a 1 mL sample collected (baseline solution, Sample E). The bottled solutions were transferred to an injection syringe and an infusion filter was connected directly to the injection syringe. A further 1 mL sample was collected after the solution had passed through this system (Sample F).

\[
\text{Adsorption ratio } \% = \frac{(E - F)}{E} \times 100
\]  

Statistical Analysis  The relationships between filgrastim adsorption, polysorbate 80 concentration, and the pH of solution were analysed using Dunnett’s test; statistical significance was accepted at the \( p < 0.01 \) level.

Results and Discussion

The adsorption of the original and three biosimilar preparations of filgrastim on infusion sets (bag, line and filter) is shown in Fig. 2. Filgrastim adsorption was observed with all preparations and total adsorption ratio were about 30%. There were no differences in the adsorption ratios when filgrastim was delivered in isotonic sodium chloride solution or 5% (w/v) glucose solution. These results suggest that filgrastim adsorption on infusion sets is similar for all filgrastim preparations tested.

The inhibitory effects of polysorbate 80 on filgrastim adsorption on infusion sets is shown in Fig. 3. Filgrastim adsorption on infusion bag, line and filter was not decreased by the addition of 0.02 or 0.033 mg polysorbate 80, the minimum and maximum contents, respectively, of the filgrastim
preparations used. Thus, current levels of polysorbate 80 in filgrastim preparations have no influence on filgrastim adsorption on infusion sets. Filgrastim adsorption on infusion sets was significantly decreased by the addition of polysorbate 80 at concentrations at or above its CMC. However, significantly higher concentrations of polysorbate 80 were no more effective in preventing drug adsorption on the infusion set than the CMC concentration. This result is supported by the findings of Mollmann et al., who reported that polysorbate 80, at concentrations above its CMC, can be used to protect insulin against surface adsorption.20) When polysorbate 80 concentrations were above the CMC, polysorbate 80 was adsorbed over the whole hydrophobic solid.20) It is suggested that the adsorption sites of filgrastim decrease because polysorbate 80 is preferentially adsorbed over the whole infusion set when its concentration is above the CMC. These results suggest that the addition of polysorbate 80 at a concentration at or above its CMC is an effective way to prevent filgrastim adsorption on infusion sets. The total adsorbed on the infusion sets was the same whether isotonic sodium chloride solution or 5% (w/v) glucose solution were used as medium. The polysorbate 80 concentration in commercial total-vitamin preparations ranged from 40–80 mg/preparation.21–23) When we assume the condition of the commercial total-vitamin preparations (polysorbate 80 concentration of 40–80 mg/preparation) were mixed with filgrastim preparation in isotonic sodium chloride solution or 5% (w/v) glucose solution (100 mL), the concentration of diluted polysorbate 80 could be expected to maintain concentration over 1.4 mg/100 mL. Therefore our experimental conditions on polysorbate 80 concentrations seem cover clinical condition such as mixing of filgrastim preparation and commercial total-vitamin preparation.

The filgrastim adsorption ratio was highest at a solution pH of 5.65, which is the isoelectric point (pI) of filgrastim (Fig. 4). Hydrophobic and electrostatic interactions are known to be involved in the mechanism of protein adsorption on interfaces.24,25) It is therefore probable that hydrophobic interactions are involved in filgrastim adsorption on infusion sets.

Figure 5 is a representation of the assumed filgrastim adsorption process on infusion sets.26) Unlike sorption, drug adsorption to the infusion set surface is monomolecular adsorption.27) Therefore, the adsorption no longer produces it if the infusion set surface is saturated with an adsorbed drug. In the absence of polysorbate 80 (Fig. 5(a)), the preferential adsorption of filgrastim is probably the result of interactions between hydrophobic moieties of the filgrastim molecule and hydrophobic portions of the infusion set. At low concentrations of polysorbate 80 (Fig. 5(b)), it is suggested that the polysorbate 80 is mostly in solution and its effect on adsorption of filgrastim is therefore limited. Increasing concentrations of polysorbate 80 have an increasing inhibitory effect on filgrastim adsorption (Figs. 5(c), (d)), up to the point when the CMC is reached, when the maximum effect is seen (Fig. 5(e)) and filgrastim is displaced from all the adsorption sites of the infusion sets. It is suggested that there is a competitive interaction between hydrophobic moieties on the filgrastim molecule and hydrophobic portions of polysorbate 80. Thus, the addition of polysorbate 80 to the infusion solutions at a concentration at or above the CMC significantly decreases filgrastim adsorption on infusion sets, due to the greater affinity of polysorbate 80 for the infusion set interface than filgrastim.

![Fig. 3. Inhibitory Effect of Polysorbate 80 on Filgrastim Adsorption Ratios on Infusion Sets (Bag, Line and Filter)](image-url)

(a) In isotonic sodium chloride solution, (b) in 5% (w/v) glucose solution. Each value is the mean±S.D. of three. Dunnett test, **p<0.01 vs. polysorbate 80 concentration of 0 mg.

![Fig. 4. Influence of Solution pH on Filgrastim Adsorption Ratio on Infusion Filters](image-url)

Filgrastim adsorption ratio on infusion filters at various solution pHs. Each value is the mean±S.D. of three. pH 5.65 is the pI of filgrastim. Dunnett test, **p<0.01 vs. pH 5.65.
Conclusion

Filgrastim adsorption on infusion sets was confirmed for the original preparation as well as three biosimilar preparations, and there were no significant differences between them. These results show that the problem of filgrastim adsorption on infusion sets cannot be solved by switching from the original to a biosimilar preparation. There is description on the filgrastim package insert under precautions, which is that filgrastim and other preparations should not be administered simultaneously. However, sometimes mixed administration of filgrastim and total-vitamin preparation was occurred in hyperalimentation and previous paper have reported that total-vitamin preparations, which have also been shown to inhibit filgrastim adsorption on infusion sets. It was clear that the addition of polysorbate 80 to the infusion solution at a concentration at or above its CMC significantly decreases filgrastim adsorption on infusion sets in our report. Thus, the co-administration of a total-vitamin preparation containing polysorbate 80 at concentrations above its CMC might be an effective way to prevent filgrastim adsorption on infusion sets.

Conflict of Interest The authors declare no conflict of interest.

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