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Stability of infliximab in polyvinyl chloride bags

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Abstract

Purpose—The stability of prepared infusions of the tumor necrosis factor (TNF)-α agent infliximab after storage for up to two weeks was investigated.

Methods—To determine the feasibility of liberalized expiration dating of infliximab (current recommendations call for the infusion of prepared doses within three hours), the stability of diluted infliximab stored in polyvinyl chloride (PVC) bags at 4 °C for up to 14 days was evaluated. A known quantity of TNF-α was combined with infliximab test samples in PVC bags for one hour; immediately after the reaction period and after 7 and 14 days of storage, the residual

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amount of TNF-α (an indirect measure of the drug's biological activity) was analyzed via a validated enzyme-linked immunosorbent assay (ELISA).

Results—The mean ± S.D. amount of TNF-α consumed by infliximab was calculated to be 24.5 ± 5.6 pg/mL at baseline, 29.0 ± 4.4 pg/mL at 7 days, and 24.8 ± 17.3 pg/mL at 14 days. At all evaluated time points, ELISA results indicated that 19–24% of the original TNF-α had been consumed by infliximab (mean ± S.D. consumption: 19.6% ± 4.5% at baseline, 23.2% ± 3.5% at 7 days, and 19.8% ± 13.8% at 14 days).

Conclusion—Infliximab, when prepared at a concentration of 400 μg/mL in 0.9% sodium chloride injection, incurred no loss of biological activity when stored for up to 14 days at 4 °C in PVC bags. Changing infliximab preparation practices may improve clinic efficiency by reducing patient dissatisfaction with long wait times for infusions and avoiding costly waste.

Infliximab is a chimeric monoclonal antibody that is composed of constant human and variable murine regions. Infliximab binds with high affinity and specificity to human tumor necrosis factor (TNF)-α, an important inflammation regulator, and thereby inhibits the binding of TNF-α to its receptors.

Currently, infliximab is approved by the Food and Drug Administration (FDA) for the treatment of multiple autoimmune diseases, including rheumatoid arthritis, ankylosing spondylitis, psoriatic arthritis, plaque psoriasis, Crohn's disease, and ulcerative colitis.[1,2] It is also being investigated as an anticancer agent.[3] The use of infliximab is expected to increase due to recent studies in Crohn's disease and ulcerative colitis treatment that suggest benefits with “top-down” therapy (i.e., the early use of aggressive treatments, including biological agents, in the hope of preventing disease progression) versus “step-up” therapy (i.e., initiating newly diagnosed patients on the least aggressive forms of therapy and using more aggressive treatments with disease progression).[4]

According to the manufacturer, infliximab infusion should begin within three hours of dose preparation, and any unused portion of the single-dose vial or diluted infusion solution should be discarded.[1] To prevent the waste of infliximab, many clinics postpone preparation of doses until patients arrive for treatment, resulting in substantial infusion delays and patient dissatisfaction. If it were demonstrated that prepared infliximab doses retain their stability and efficacy for periods longer than three hours, expiration dating could be liberalized and doses could be prepared in advance, reducing waste and increasing patient convenience. The aim of the study described here was to analyze the stability of infliximab in polyvinyl chloride (PVC) bags stored at 4 °C for up to 14 days.

Methods

Need for measurement of activity

As with all monoclonal antibodies, the stability of infliximab cannot be defined on the basis of measured concentration. Stability must be measured on the basis of the activity of the monoclonal antibody— measured as a function of its ability to elicit an effect after in vitro binding to the appropriate receptor. As proteins, monoclonal antibodies may lose function (i.e., the ability to bind to relevant receptors in vivo and thus elicit a pharmacologic effect) if

the structure of the protein is altered by long-term storage. Conventional stability measurement techniques (such as high-performance liquid chromatography) quantify the presence of molecules in samples but do not detect structural changes in those molecules. The use of such standard techniques may inappropriately suggest that storage has not altered the function of the monoclonal antibody even if degradation has occurred.

The stability of monoclonal antibodies and other biological products used therapeutically must be tested using other techniques, such as enzyme-linked immunosorbent assay (ELISA). ELISA is a specific and highly sensitive method for quantitative measurements of monoclonal antibodies or other analytes in solutions. In this type of ELISA, a receptor specific to the monoclonal antibody under investigation is coated onto a microtiter plate; this receptor is analogous to the receptor in the body with which the monoclonal antibody is intended to interact in vivo. After the microtiter plate is dried, a sample of the monoclonal antibody is added and binds to the receptor (provided the monoclonal antibody is still functional and has the ability to bind). After a defined period of time, the unbound monoclonal antibody sample is washed away, and additional detector reagents are added. These detector reagents attach only to the receptors that have captured the monoclonal antibody being tested and undergo a measurable color reaction, providing a quantifiable indication that the complex has formed. If the monoclonal antibody being tested is degraded due to storage, it will not be bound to the receptor (just as it would not elicit the desired therapeutic effect if administered to a patient) and less light is emitted and measured.

The detection limits for ELISA are commonly in the lower picogramper-milliliter range. Since amounts of monoclonal antibodies used in clinical practice exceed that concentration, samples prepared in stability studies are serially diluted until they reach the detection range of the assay. More information about ELISA methods may be found in published FDA guidance on the testing of monoclonal antibodies.[5]

In the case of infliximab, measuring the effect of storage on the retention of the drug's function (i.e., stability) required the use of an indirect ELISA method, as described above. Infliximab from stored samples was combined with a known quantity of TNF-α, and the two agents were allowed to react for one hour (per the assay manufacturer's recommendation); the combination of infliximab and TNF-α was then placed on microtiter plates coated with a TNF-α detector antibody. Whereas free TNF-α was bound to the detectors on the plate, the TNF-α captured by infliximab (an indication of the retention of the drug's ability to provide a therapeutic effect) did not bind to the detectors and was instead washed away after the completion of the reaction period.

Materials and sample preparation

Infliximab in 100-mg vials[a] was obtained from commercial sources. Commercially available 0.9% sodium chloride injection,[b] empty PVC infusion bags,[c] and sterile water for injection[d] were also obtained. A human TNF-α/TNFSF1A immunoassay kit (DTA00C)[e] was used.

[a]Remicade 100 mg, Centocor Ortho Biotech, Inc., Horsham, PA, lot 9GZ62013P1.
[b]Sodium chloride for injection, Hospira Inc., Lake Forest, IL, lot C783621.
[c]PVC bags, Baxter Healthcare Corporation, Deerfield, IL, lot UR09E15180.
[d]Sterile water, Hospira Inc., lot 78-219-DK.

Each test sample was prepared by reconstituting the contents of a 100-mg vial of infliximab with 10 mL of sterile water for injection. Three samples were prepared by adding an 8-mL (80-mg) portion of reconstituted infliximab to 192 mL of 0.9% sodium chloride injection in an empty PVC bag to attain an infliximab concentration of 400 μg/mL.

Stability analysis

After incubation with infliximab, free TNF-α was used as a measure of infliximab activity, as previously described for other monoclonal antibodies and measured with ELISA.[6] The TNF-α assay was validated over the range of 15.6–1,000 pg/mL, with a demonstrated correlation coefficient of 1.0 over that range. To examine the biological activity of infliximab, 100-μL portions of the standard infliximab solution diluted to 0.4, 40, 4,000, and 400,000 ng/mL were mixed with 100 μL of TNF-α (250 pg/mL), resulting in final initial concentrations of in-fliximab ranging from 0.2 to 200,000 ng/mL. These varying concentrations are necessary with ELISA use, as the detection range of the assay is established experimentally. The final concentration of TNF-α was 125 pg/mL. After one-hour reaction periods (as recommended by the assay manufacturer), the concentrations of residual free TNF-α in the mixture of infliximab and TNF-α were detected.

As described above, the consumption of TNF-α was calculated by subtracting the value for the residual free concentration from the initial concentration value. The biological activity of infliximab was then expressed as the percentage of TNF-α consumed. By comparing the activity of infliximab at different time periods with its baseline activity, a relative stability value was computed. Samples were tested in triplicate at baseline and after storage for 7 and 14 days at 4 °C.

Results

Table 1 provides data on the stability of infliximab tested at an initial concentration of 20 ng/mL, a concentration within the detection range of the assay. The relative activity of infliximab was unchanged after storage at 4 °C in PVC bags for 7 and 14 days.

Discussion

Cytokines are synthesized by many types of cells and exert their physiological or pathological effects after binding to cell surface receptors. TNF-α, one of the key proinflammatory cytokines, has the following proposed functions: apoptosis of immune cells, stimulation of the release of other proinflammatory cytokines, stimulation of the release of matrix metalloproteases, assistance in the expression of endothelial adhesion molecules, and blockade of lipoprotein lipase.[7] Infliximab neutralizes the biological activity of TNF-α by binding with affinity and specificity to the soluble and transmembrane forms of TNF-α, an action that, in turn, inhibits the binding of TNF-α with its receptor.[8,9] Since its market launch in the United States in 1999, sales of infliximab have grown dramatically. In 2008, over $2 billion was spent on the drug in U.S. hospitals and clinics.[10]

[e]Quantikine Human TNF-α Immunoassay, R&D Systems, Minneapolis, MN.

Currently, infliximab is the only anti-TNF-α agent approved by FDA for both of the main forms of inflammatory bowel disease (IBD), Crohn's disease and ulcerative colitis. In clinical trials, infliximab has been found to be effective for both the induction and maintenance of IBD remission when given every eight weeks[11,12]; in patients with Crohn's disease, it has also been shown to promote fistula closure.[13] Although the yearly cost of infliximab treatment exceeds $16,000 when the drug is administered at eight-week intervals, multiple studies have found infliximab to be cost-effective and to improve health-related quality of life.[14-17]

Infliximab is supplied in vials containing 100 mg of lyophilized in-fliximab powder; in clinical practice, the powder is reconstituted with 10 mL of sterile water for injection and then diluted with 250 mL of 0.9% sodium chloride injection for i.v. administration. Due to manufacturer recommendations on expiration dating[1] and the high cost of infliximab, which may exceed $2000 per dose, many clinics prepare doses only after patients arrive and are prepared for treatment. Extended stability dating (i.e., out to 14 days after reconstitution) would permit the preparation of infliximab doses well in advance of scheduled treatments, which could improve patient satisfaction by reducing wait times. The demonstration of extended stability may also help reduce drug costs by enabling the use of prepared but unadministered doses in other patients and by decreasing the routine discarding of unused diluted drug.

To our knowledge, before the study reported here, little was known about the activity and stability of stored infliximab. In addition to the manufacturer recommendations on beyond-use dating,[1] Beer et al.[18] recently reported that infliximab was stable when stored undiluted in syringes over six weeks at 4 °C for use in the treatment of diabetic macular edema and neovascular age-related macular degeneration. However, the administration of infliximab in clinical practice for indications such as Crohn's disease and ulcerative colitis entails the use of diluted doses larger than those previously studied; the stability of these larger doses of the drug in dilute form has not been previously reported.

Conclusion

Infliximab, when prepared at a concentration of 400 μg/mL in 0.9% sodium chloride injection, incurred no loss of biological activity when stored for up to 14 days at 4 °C in PVC bags. Changing infliximab preparation practices may improve clinic efficiency by reducing patient dissatisfaction with long wait times for infusions and avoiding costly waste.

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Table 1

Assay Results for Infliximab Stored for Up to 14 Days[a]

Variable	Storage Time (days)		
	0	7	14
Mean ± S.D. final TNF-α concentration (pg/mL)	100.5 ± 5.6	96.0 ± 4.4	100.2 ± 17.3
Mean ± S.D. amount TNF-α consumed after reaction (pg/mL)[b]	24.5 ± 5.6	29.0 ± 4.4	24.8 ± 17.3
Mean ± S.D. % TNF-α consumed by infliximab[c]	19.6 ± 4.5	23.2 ± 3.5	19.8 ± 13.8
% Initial infliximab activity remaining[d]	100	118	101

[a] Infliximab was prepared at a concentration of 400 mg/mL and assayed, after dilution, at a concentration of 20 ng/mL. Mean ± S.D. values are based on triplicate assays. TNF-α = tumor necrosis factor-α.

[b] Calculated by subtracting the mean final TNF-α concentration from the initial TNF-α concentration of 125 pg/mL.

[c] Calculated by dividing (1) the mean amount of TNF-α consumed by (2) the initial TNF-α concentration of 125 pg/mL and multiplying the product by 100.

[d] A value of 100% was assumed at baseline (0 day). For 7 and 14 days, the value was calculated by dividing (1) the mean % TNF-α consumed by (2) the mean value for that variable at baseline (19.6%) and multiplying the product by 100.