Published in final edited form as:
Am J Health Syst Pharm. 2013 March 1; 70(5): 436–438. doi:10.2146/ajhp120035.

Stability of Stock and Diluted Rituximab

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Abstract

Purpose—The stability of rituximab was studied in the manufacturer-supplied concentration and diluted with 0.9% sodium chloride injection in polyvinyl chloride (PVC) bags at 4 °C for up to 14 days.

Methods—Ten milliliters of manufacturer-supplied rituximab (10 mg/mL) was directly drawn into each of three empty and sterile glass vials. Three milliliters of the rituximab 10 mg/mL and 17 mL of 0.9% sodium chloride injection were mixed and injected into each of three empty and sterile PVC bags. Samples were analyzed immediately after preparation and again after storage at 4 °C for 3, 7, and 14 days. Rituximab activity was measured using a previously described enzyme-linked immuno sorbent assay (ELISA) method. Stability was defined as no more than 15% loss from the initial activity. Physical stability was examined by observing color changes or precipitations under normal laboratory lighting.

Results—The percentage of initial activity of rituximab in both conditions studied remained over 85% stored at 4 °C after 14 days. No changes in color or turbidity were observed in any of the preparations.

Conclusion—Rituximab is stable at 4 °C for up to 14 days, either at the manufacturer-supplied 10 mg/mL concentration or diluted to 1.5 mg/mL with 0.9% sodium chloride injection in PVC bags. The extended beyond-use dating has significant waste-reduction and cost-saving implications.

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Introduction

Rituximab (Rituxan®, Genentech, Inc.) is a chimeric monoclonal antibody directed against CD20, an antigenic marker on mature B-cells but absent on stem cells, normal plasma cells or tissues.[1-3] It is infused intravenously to deplete circulating B-cells via antibody-dependent cellular cytotoxicity, complement fixation, and apoptosis induction, indicated as a treatment for non-Hodgkins lymphoma (NHL), chronic lymphocytic leukemia (CLL) and moderate to severe rheumatoid arthritis (RA). The typical initial dose for NHL and CLL is 375 mg/m^2, while two-1000 mg infusions separated by 2 weeks are usually given to RA patients in combination with methotrexate.[1] Additionally, clinical efficacy has been demonstrated in several off-label uses in primary immune thrombocytopenia, solid organ transplants, endocrine disorders, and dermatology.[4-6]

Rituximab is available at the concentration of 10 mg/mL in either 100-mg (10 mL) or 500-mg (50 mL) single-use vials without preservatives. Prior to administration, rituximab is diluted to 1-4 mg/mL directly into an infusion bag containing either 0.9% sodium chloride injection or 5% dextrose in water, and the drug remaining in the vial is discarded.[7] The package insert dictates that diluted solutions are stable at 2-8 °C for 24 hours, and at room temperature for an additional 24 hours. Consequently, the unused drug must frequently be discarded, as patients are usually dosed weekly or biweekly during treatment courses.[4] Rituximab is extremely expensive - $3,693 for each 500 mg vial.[8] Therefore the ability to reduce waste by extending the expiration dating of prepared doses and residual vials would produce significant cost-saving. This study sought to determine the stability of stock or diluted rituximab stored at 4 °C for up to 14 days, utilizing a sandwich ELISA based on a methodology validated by Hampson *et al*.[9]

Materials and Methods

Description of the ELISA Technique

As with all monoclonal antibodies, stability can not 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 binding *in vitro* to the appropriate receptor. As proteins, monoclonal antibodies may lose function (their ability to bind to relevant receptors in vivo, eliciting a pharmacological effect) if the structure of the protein is altered by long-term storage. Conventional stability measurement techniques, such as (such as high performance liquid chromatography, HPLC) only quantify the presence of molecules in samples, but do not detect structural changes in those molecules. 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 immuno sorbent assay (ELISA). ELISA is a specific and highly sensitive method for quantitative measurements of monoclonal antibodies or other analytes in solutions. In ELISA, a receptor specific to the monoclonal antibody being tested is coated on a microtiter plate. This receptor is analogous to the receptor in the body that the monoclonal antibody is intended to interact with 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 color reaction that is measured providing a quantifiable indication that the complex has formed. If the monoclonal antibody being tested is degraded after 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.

The detection limits for ELISA are commonly in the lower picogram/ml range. Since samples 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. For more information about ELISA, please refer to the FDA guidance on the testing of monoclonal antibodies.[10]

Sample Preparation

To evaluate the stock solution stability for rituximab, 10 mL of the 500-mg manufacturer vial (10 mg/mL) of rituximab[a] was directly injected into each of three sterile, empty glass vials. To evaluate the stability of diluted rituximab, 3 mL withdrawn from the 100-mg manufacturer vial (10 mg/mL)[b] was mixed with 17 mL of 0.9% sodium chloride injection in each of three sterile, empty polyvinyl chloride (PVC) bags to yield a concentration of 1.5 mg/mL. Immediately after removing aliquots for time zero (time-0) measurements, the samples were stored at 4 °C, where they remained for the duration of the study. Calibration standards were prepared freshly at time-0, day 3, 7 and 14 from a rituximab stock vial.[a]

Enzyme-linked immunosorbent assay (ELISA)

Rituximab activity was measured using a previously validated ELISA.[8] Rat anti-rituximab antibodies (MB2A4)[c] were diluted to 1 μg/mL in a coating buffer containing 0.015 M sodium carbonate and 0.0285 M sodium bicarbonate at pH 9.6. Immunoplate Maxisorp Certified Plates[d] were coated with 100 μL of this antibody solution, and left to incubate overnight at 4 °C. To reduce nonspecific binding of rituximab to the surface of the plastic ELISA plate, the plate wells were then blocked with 200 μL of 1% (w/v) BSA in PBS for two hours at room temperature. The plates were washed three times with a wash buffer[e] containing 0.138 M NaCl, 0.0027 M KCl, and 0.05% Tween-20 at pH 7.4. A dilution buffer prepared with 1% (w/v) BSA and 0.05% Tween-20 in PBS was used to dilute samples to 50 ng/mL, and prepare calibration standards at 5, 10, 25, 50, 100, 500 and 1,000 ng/mL. Following dilution, 100 μL of each sample or standard was added to triplicate wells in the plate, which was then incubated for 60 minutes at room temperature and washed 5 times with the wash buffer. The goat anti-human IgG HRP-conjugated secondary antibody[f] was

[a]Rituxan 500mg, Genentech, Inc., South San Francisco, CA (Lot number 857556)
[b]Rituxan 100mg, Genentech, Inc., South San Francisco, CA (Lot number 862198)
[c]MCA2260, AbD Serotec, Raleigh, NC (Lot number 250510)
[d]96-Well Flat Bottom MICROLON 600 Plate, High-Binding, ISC Bio Express, Kaysville, UT (Lot number 1022209)
[e]ELISA Wash Buffer Powder, eBioscience, San Diego, CA (Lot number E00003-1273)
[f]AHI1304, Invitrogen Corporation, Camarillo, CA (Lot number 747038)

then diluted 1:2500 with the blocking solution, and 100 mL was added to wells. This solution was allowed to incubate for 60 minutes at room temperature before washing 5 times with the wash buffer. A 0.4 mg/ml substrate solution of o-phenylenediamine[g] (o-PD) was prepared with hydrogen peroxide according to the manufacturer's specifications, and 100 μL was added to the wells. The substrate solution was allowed to incubate in the dark at room temperature for 15 minutes before adding 50 μL of 3 M sulfuric acid to terminate the reaction. Absorbance was read at 490 nm on a plate spectrophotometer.[h]

Rituximab activity was measured in three replicates for each glass vial containing 10 mg/mL rituximab or PVC bag containing 1.5 mg/mL diluted rituximab. Stability was assessed by measuring the percentage of the initial activity remaining at the end of each time interval. Stability was defined as the retention of at least 85% of the initial activity. Physical stability was determined by visual inspection for evidence of precipitation under normal laboratory fluorescent light.

Results

The stability data of rituximab is summarized in Table 1. The percentage remaining of the initial activity of rituximab stored at 4 °C for up to 14 days exceeded 85% in both tested conditions. No evidence of precipitation or color change was observed in any of the solutions within the study period.

Discussion

Rituximab is an expensive and widely used medication with only two available single-use vial sizes. The manufacturer only recommends 24-hour stability for diluted rituximab stored at 2-8 °C. The weight-based dosing for the treatment of NHL and CLL commonly leads to residual drug that is discarded in the absence of longer storage stability.

This study has extended the rituximab stability at 4 °C for up to 14 days either in the undiluted manufacturer vials or diluted in 0.9% sodium chloride injection in infusion bags. As the average retail price of each 500 mg vial is $3,693, reduction in drug waste may result in cost-savings.[8] At our cancer center, unused portions of rituximab vials were regularly discarded every Friday afternoon, as the residual would not be expected to be used until the following Monday (beyond the manufacturer-recommended expiration dating). Assuming residuals of approximately 25% of each vial each week, extending the expiration dating of vials would be expected to result in annual savings of over $40,000. Additional savings are associated with the use of prepared doses that are not administered to patients as planned.

Conclusion

Rituximab 10 mg/mL undiluted in glass vials and 1.5 mg/mL diluted in 0.9% sodium chloride injection in PVC bags are stable at 4 °C for up to 14 days. Substantial cost savings

[g]Sigma-Aldrich Inc., St. Lous, MO (Lot number 029K-8217)
[h]SpectraMax 190, Molecular Devices Inc., Sunnyvale, CA

can be realized if the expiration dating is extended on prepared but unused doses and on residual drug in vials.

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Table 1

Stability of Rituximab in Stock Vials and in Diluted Infusion Bags*

Sample Format	% Initial Activity Remaining (Mean ± SD)		
	Day 3	Day 7	Day 14
Glass vial (n = 3), 10 mg/mL†	103.7 ± 6.6	111.1 ± 6.6	114.8 ± 3.3
PVC bag (n = 3), 1.5 mg/mL†	93.9 ± 4.3	94.1 ± 4.1	100.4 ± 4.3

*For each vial or PVC bag, triplicate ELISA assays were performed and averaged, and the three averages were used to compute standard deviation (S.D.).

†Samples were serially diluted to 50 ng/mL before added to the ELISA plate. Absorbance was read at 490 nm.