OTR.18 - Establishing a lyophilized presentation for mAb 4G2

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Introduction:

Monoclonal antibodies (mAbs) have highly efficient immune response for many diagnostic and therapeutic researches. Detection of flavivirus by using mAbs in diagnostic kits, for instance, can be lower cost and faster alternative, creating important autonomy in the production of this input to our country. Lyophilized presentations for mAbs should be more stable and suited to milder conditions of storage and distribution, 2 to 8°C instead of -70°C, as they are currently costly, provided to assure their quality.

Objective

This work intends to establish a lyophilized presentation for the mAb 4G2 assuring its physico-chemical and biological stability.

Methodology:

D1-4G2-4-15 murine hybridoma was grown in suspension in high glucose DMEM medium supplemented with fetal bovine serum and L-Glutamine, and supernatant harvested. Subsequently, downstream techniques were used to get mAb formulations in ranges from 0.54 to 3.00%. Differential scanning calorimetry and electric resistivity analysis were used to specify thermophysical properties. Preliminary lyophilization runs were carried out, followed by an evaluation over the final product for a set of quality parameters, including also a characterization by SEC on Superdex 200 Column, SDS-PAGE (8-25%) and IEF-PAGE (3-9). Tryptophan fluorescence spectra and circular dichroism were also evaluated to verify conformational modifications.

Results:

Results from electric resistivity and thermal analysis identified phase transitions, enthalpies and critical temperatures of interest for the lyophilization cycle design, which was based specially on the minimum temperatures that mAb formulation should reach during freezing and the maximum temperature that mAb formulation should not reach during sublimation. Evaluation over final products showed a formation of homogeneous and pharmaceutically elegant cake, low residual moisture and rapid reconstitution in water for injection. The mAb 4G2 presented by SEC a major peak with average homogeneity corresponding to 98.2% peak area and a fronting peak with MW similar to a dimeric form (1.8% peak area), that was related to a reversible aggregation of 4G2. Samples of 4G2 lyophilized, stored for 6 and 12 months, showed an increase around 2.28% and 6.55% in the dimeric form and also presented a high MW aggregate (> 600kDa). Results from SDS-PAGE profiles suggest no degradation of the polypeptide chains and IEF-PAGE results indicate that the polysaccharide content was maintained with the lyophilization process. Circular dichroism and fluorescence spectra were identical, which suggest there were no conformational changes in the lyophilized monoclonal with the storage.

Conclusion:

After lyophilization process, the mAb 4G2 did not lose their physico-chemical and conformational characteristics, although the aggregation content was altered. Formulation studies by adding stabilizers, modifying the pH or even the ionic strength of the medium followed by an optimization of lyophilization cycle may decrease the aggregation content. Studies of the biological activity of 4G2 should be conducted to corroborate the stability of this monoclonal desirable in lyophilized presentation.

Keywords: Monoclonal antibody; Lyophilization; Dengue