LONG-TERM STABILITY OF DILUTED SOLUTIONS OF THE MONOCLONAL ANTIBODY INFlixIMAB

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Objective: To assess the long term stability of the therapeutic monoclonal antibody Infliximab (Remicade®) reconstituted in water for injection and in two diluted preparations in 0.9 % NaCl and stored at 4 °C and frozen at -20 °C.

Background: Infliximab (IFX) is a chimeric human-murine monoclonal antibody (mAb) directed against tumor necrosis factor alpha (TNF-α). It is indicated in the treatment of an important number of pathologies: rheumatoid arthritis, Crohn’s disease, psoriatic arthritis, psoriatic plaque psoriasis and ulcerative colitis. It blocks the damage caused by high levels of TNF-α by forming the Infliximab-TNF-α immune complex. This complex is responsible for the reduction of severe levels of proinflammatory components as Interleukin-6.

Materials and methods: Adsorbent methods for assessing the physicochemical properties of Infliximab were developed and ICH validated: reverse phase high performance liquid chromatography with diode array detector (RP-HPLC/DAD) for quantification; weak cation exchange high performance liquid chromatography (WCX-HPLC/DAD) to track changes in the isoform profile; size exclusion chromatography high performance liquid chromatography with diode array detector (SE-HPLC/DAD) for aggregates detection; and matrix assisted laser desorption ionisation mass spectrometry (MALDI-TOF/MS) to obtain peptide mass fingerprinting (PMF) in order to detect major changes in the chemical structure. Biological activity was assessed using a specific immunoassay based on the ELISA technique using plates sensitized with the Tumor Necrosis Factor α (TNF-α).

Results: 24 hours after preparation of the solutions the loss of biological activity was close to 50 %, rising to 63 % at day 2, a value that remained constant until the last control day (7). The overall quantity of IFX was assessed for a month during which it remained unchanged. No aggregate formation was detected in two weeks of testing. Slight changes in the chromatographic isoforms profile were detected after a week. Fingerprinting indicated minimal changes in the IFX structure (three months).

Conclusion: Despite the fact that no major changes were detected in the physicochemical properties of IFX after a week of testing (changes in the isoform profile at day 7), the ELISA results indicated an important decrease in the biological activity when the medicine was reconstituted 24 hours after preparation of the solutions. We have so far been unable to corroborate this result using a different method for evaluating biological activity -such as flow cytometry- due to technical problems which we are currently investigating. Authors declare no conflict of interest.