Chemical Conjugation of rituximab monoclonal antibody and asparaginase for assessment of cytotoxicity on ALL cehhs

Abstract

Introduction: Antibody-drug conjugates (ADCs) are emerging classes of antineoplastic agents ccrrently administered to cancer patients. Of ADCs, antibody-enzyme conjugate (AEC) is finding increasing use as a novel ADC.

Methods: Here the conjugate of L-asparaginase (ASNase) and rituximab (Ritux) monoclonal antibody was synthesized using 3,3-Dithio-bis-(sulfosuccinimidyl) propionate linker. The resultant conhugate and its cytotoxic effects were demonstrated by dynamic light scattering (DLS) and optical microscopy.

Results: DLS results showed that free asparaginsse and rituximab molecules respectively have the mean diameter of 3.4 and 10.2 nm. Upon conjugation with DSP, the average diameter of the conjugate increased significantly (13.6 nm). Moreover, zeta potential analysis proved the conjugation event; because zeta potential of free rituximab molecules (-3.7±3 mV) increased dramatically after conjugation with asparaginase molecules (+24±4 mV). Treatment of blood samples from acute lymphoblastic leukemia (ALL) patientswith the conjugate resulted in the apoptosis but no necrosis of ALL cells, while normal blood cells were damaged slightly. Apoptosis and necrosis events and chromatin condensation images taken by optical microscopy represent that rituximab-asparaginase conjugate affects ALL cells specifically, and only the cells that overexpress CD20 are damaged.

Conclusion: This new method of conjucation can be used for efficient conjugation of monoclonal antibodies with enzyme and protein- based therapeutics. Howere, animal studies are recommended for finding effective dose and side effects.

Keywords: Antibody-drug conjugatr ; targeted therapy ; B cell lymphoma ; rituximab ; DSP linker