

Effects of Disulfide and Thioether Linkages on Stability and Cytotoxicity of Anti-CALLA Fab-Ricin A Immunotoxins

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Anti-CALLA Fab-RTA immunotoxins were constructed using two crosslinking agent, SMPT and SMCC, to generate a disulfide and a thioether bridge between Fab fragment of K269-65 MoAb and RTA toxin moieties, respectively. These immunotoxins were selectively immunoreactive with CALLA⁺ B-lineage Daudi cells. SMPT and SMCC mediated RTA immunotoxins were prepared with 49% and 53% of the RTA conjugation yields, respectively. SDS-PAGE analysis show that immunotoxins were constructed with major Fab-1RTA (76kda), minor Fab-2RTA (106kda) and Fab-3RTA (136kda) compositions. The breakdown rates of immunotoxins were determined in the presence of glutathione by measuring the amount of reduced immunotoxins using size-exclusion HPLC. The SMCC immunotoxins were more resistant to the glutathione than SMPT immunotoxins. But, our data showed that the SMPT mediated disulfide bonded immunotoxins were much more active than the SMCC mediated thioether bonded immunotoxins to kill the target cells *in vitro*.