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LABELLING OF ANTI-CD20 MONOCLONAL ANTIBODY CIMABior WITH 90Y.

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Radioimmunotherapy of non-Hodgkin lymphomas with anti-CD20 monoclonal antibodies (Mabs) labelled with b-emitter radionuclides has resulted promising. The aim of this work was to establish a method for the labelling Cuban chimeric anti-CD20 MAb CIMABior with 90Y.

Materials and methods: The influence of 3 BCA (NHS-DOTA, p-SCN-CHX-A"-DTPA and p-SCN-Bn-DOTA) was studied. Conjugation reaction was performed in bicarbonate buffer 0.1mol/L pH=8.5-9.0 at room temperature, varying molar ratio Mab:BCA and incubation times. After conjugation, the antibody was purified by gel filtration and by ultrafiltration through Amicon 30 kDa, and changing to ammonium acetate buffer 0.1mol/L pH=6.0-6.5. Labelling reactions were performed at room temperature and 42 ºC, when DTPA and DOTA derivatives were employed, respectively. A challenging assay against 300-fold molar excess of EDTA was used to assess the stability of radioconjugates. Immunoreactivity was assayed by flow cytometry. To assess the in vivo behaviour of 90Y- CIMABior, 9 male healthy rats received 50 µg of the Mab (37 MBq, 0.2 mL) through marginal vein of the tail. Blood samples were drawn and organ collected up to 72 h.

Results: Depending on the BCA and the molar ratio Mab:BCA, the amount of chelating groups bound to the antibody varied in the range from 2 to 13. The same way, the labelling yield also depended on the employed BCA and the number of the chelating groups in the IgG molecule. The conjugates with p-SCN-CHX-A" -DTPA and p-SCN-Bn-DOTA showed the best results of labelling efficiency (>95%). Radioimmunoconjugate CIMABior-Bn-DOTA-90Y showed the highest stability (>90% after 96 h). The affinity of Mab CIMABior for the antigen CD20 was affected by the increasing of the molar excess of BCA in the conjugation reaction. Besides, the affinity for the antigen was significantly lower in case of NHS-DOTA, with regard to the other two immunoconjugates. Immunoreactive fraction of CIMABior-Bn-DOTA-90Y was (95.27 ± 6.21) %. Labelled Mab showed a satisfactory in vivo stability. The main target organ was the spleen (1.2-2.0 %ID/g). Product had an elimination pathway through kidneys and liver. PK study showed a monoexponential with a T1/2 = 7.0 ± 3.1 h.

Conclusions: According with the outcomes of the present work, a methodology for the satisfactory labelling of anti-CD20 monoclonal antibody was established. Radioimmunoconjugate CIMABior-Bn-DOTA-90Y showed the most adequate initial characteristics to be used in the future for the radioimmunotherapy of NHL.

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