LABELING OF AN ANTI-VEGF MONOCLONAL ANTIBODY WITH RADIOACTIVE ARSENIC ISOTOPES

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Introduction: The inhibition of tumor-induced angiogenesis is an emerging therapeutic strategy in clinical oncology aiming at halting cancer progression by suppressing tumor blood supply. One of the better-defined factors, involved in the angiogenesis process, is vascular endothelial growth factor (VEGF). Tumor-derived VEGF is a new target in the design of anticancer medicines, since blocking VEGF with the adequate monoclonal antibody may block tumor development. VG76e, an anti-VEGF monoclonal antibody, has been labeled with ^{124}I, ^{99m}Tc, ^{153}Sm and ^{177}Lu for tumor detection using SPECT/PET imaging [1], with encouraging results which warrant the need for further investigation using other radionuclides. Since the enrichment of antibodies in tumor tissue is a slow process, covering several days, radionuclides with a long physical half-life are necessary to assess their pharmacokinetics. Recently, ^{72}As and ^{74}As have been identified as positron emitting radionuclides with long physical half-lives of 26 h and 17.4 d, respectively [2].

Experimental: The labeling of proteins with radioactive arsenic isotopes is based on their high affinity to free –SH groups. As a direct method, the reduction of disulfides of the antibody was performed via TCEP*HCl (tris(2-carboxyethyl)phosphine hydrochloride). The number of created -SH groups was estimated before each labeling experiment. The modified antibody VG76e was directly incubated with an ethanolic solution of nca $[^{72/74/77}As]AsI_3$ at 37°C for 30 minutes.

The labeling of VG76e was optimized with reactor produced nca ^{77}As. The labeling yields were determined by SEC-HPLC. Purification of VG76e was performed by gel-filtration.

Results and Discussion: The direct method of endogenous disulfide reduction with TCEP*HCl was optimized. The resulting number of -SH groups was 4 per antibody for the direct method. Labeling was quantitative at 37°C and 30 min. The stability of a purified antibody fraction was monitored over 100 h in PBS buffer and BSA containing solution and showed no loss of activity. The immunoreactivity has not yet been tested.

Conclusion: A method for the labeling of VG76e with arsenic isotopes has been optimized with nca ^{77}As to give quantitative yields after 30 minutes reaction time at 37°C. The label is stable in vitro for more than 100 h. The in vivo evaluation of VG76e will be performed with ^{72}As or ^{74}As labeled antibody via small animal PET.

References:

1. Collingridge D.R., Carroll V.A., et al., Cancer Research, 62, pp 5912-5919 (2002).
2. Bouziotis, P., Fani, M., et al., Anticancer Research, 23/3A, pp 2167-2171 (2003).
3. Jennewein M., et al., Applied Radiation and Isotopes, 63(3), p 343-351 (2005).

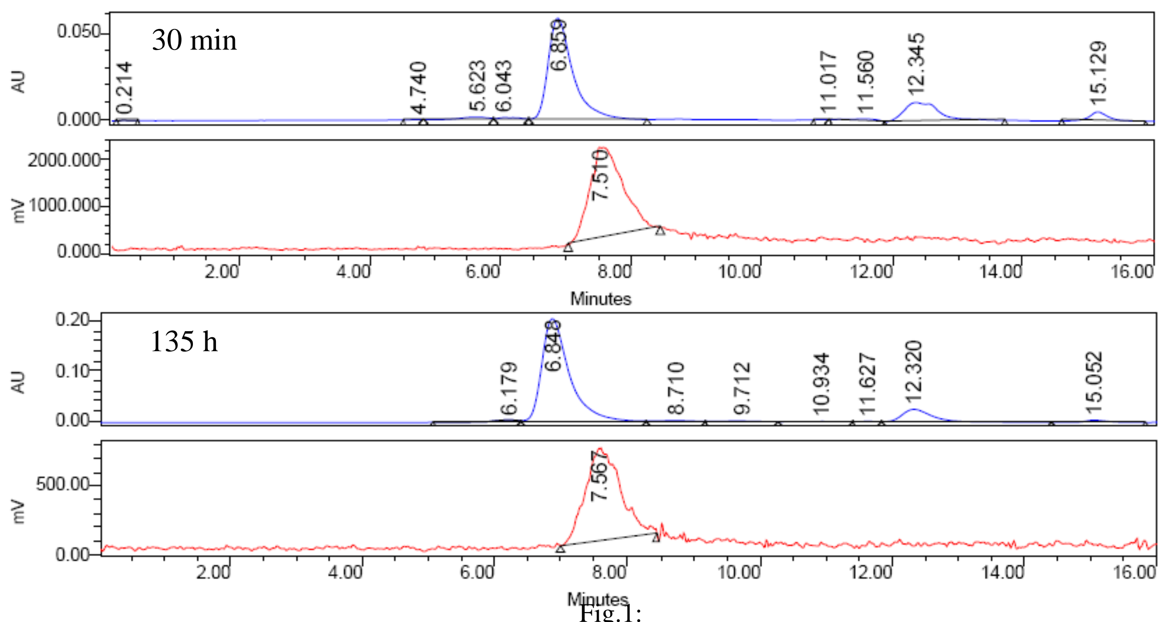

Fig.1:
SEC-HPLC (1 ml PBS / min) after 30 min and 135 h. VG76e detection at 6.8 min (UV) and 7.5 min