Abstract

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Name of the work: In vitro saturation study of gallium-67 and zirconium-89 labelled monoclonal antibody ramucirumab on SKOV-3 cell line.

Targeted biological treatment becomes more and more important with the development of a new therapy in oncology. It stimulates immune system to eliminate cancer cells. Significant progress has been made since the introduction of monoclonal antibodies. They represent one of the newest possibility used in diagnosis and treatment of tumours. The ability of the monoclonal antibody ramucirumab is to recognize and bind specifically to tumour cell antigens such as the VEGF type 2 receptor (VEGFR-2) and thus to supress angiogenic process. Anti-angiogenic ramucirumab inhibits this receptor via blocking of VEGF binding sites, which prevents the growth of tumours. It is possible to increase antitumor effect of monoclonal antibodies by their combination with other molecules like radionuclides, toxins and cytostatics when forming the so called conjugates. Prepared immunoconjugates serve as diagnostic and therapeutic tools also in Nuclear Medicine.

The aim of the experimental work in was the preparation of radiolabelled monoclonal antibody ramucirumab with two radionuclides (^{67}Ga and ^{89}Zr) with the use of the chelating agent deferoxamine (DFO). The prepared radiopharmaceuticals were analyzed on their radiochemical purity and stability with the employment of the analytical methods high-performance liquid chromatography with radiometric detection and instant thin layer chromatography. The preserved binding affinity of radiolabelled antibody ramucirumab to VEGFR-2 receptor was verified on SKOV-3 cell culture with the use of the classical manual saturation technique. The equilibrium dissociation constant (K_D) for this interaction was determined too. The biological properties testing of zirconium-89 labelled ramucirumab also included the *in vivo* organ biodistribution and PET/CT imaging of the induced VEGFR-2 positive tumour formation in mice.

The results of the radiochemical purity determination demonstrated that the prepared radioimmunoconjugates had a purity above 95 % as required by the European Pharmacopoeia for radiopharmaceuticals administered in patients. Moreover, the prepared radiopreparations maintained the required radiochemical purity for three days. The results of *in vitro* saturation studies provided the mean value of the dissociation constant of three independent measurements for ^{67}Ga[Ga]-DFO-ramucirumab K_D = 35.94 (± 7.30) nM, and for ^{89}Zr[Zr]-DFO-ramucirumab K_D = 27.44 (± 8.14) nM. Although, the obtained K_D values were lower than for unlabelled (native) RAM (K_D = 1–2 nM), the affinity to the target receptor was still sufficient for further potential studies. *In vivo* testing with ^{89}Zr[Zr]-DFO-ramucirumab confirmed data obtained from *in vitro* measurements. An *ex vivo* biodistribution study demonstrated the accumulation of radiolabelled ramucirumab in the induced SKOV-3 tumours as early as day 1 after administration with maximal tumour activity measured on day 3. At the same time, the PET/CT imaging method supported *ex vivo* experiments with clear imaging of the tumour in the resulting images.

In conclusion, the obtained results confirmed the potential of ramucirumab for imaging of VEGFR-2 positive tumours thanks to the successful testing of the prepared ^{67}Ga- and ^{89}Zr-labelled ramucirumab. Besides, the use of ^{89}Zr for radiolabelling underscores the advantage of a prepared radiopharmaceutical for imaging of the angiogenic process in the diagnosis and monitoring of treatment due to its promising imaging properties as the PET emitter. However, the disadvantage remains in its high cost.

Key words: gallium-67, in vitro saturation study, monoclonal antibody, PET/CT, radiolabelling, ramucirumab, SKOV-3, VEGFR-2, zirconium-89