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Radioiodination of mouse anti-III β-tubulin antibodies and their evaluation with respect to their use as diagnostic agents for peripheral neuropathies

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Abstract

The monoclonal antibody TU-20 and its scFv fragment were radiolabeled with ^{125}I in order to develop new imaging agents against the specific neuronal marker III β-tubulin. The reaction via chloramine-T using thiosulfate as a stopping reductant was determined as the most convenient way for radioiodination. The preserved immunological properties of radioiodinated species were estimated by ELISA, electrophoresis, and immunohistochemistry with autoradiography. Biodistribution studies revealed a different behavior of radioiodinated TU-20 and its scFv.

Keywords: III β-tubulin; Radioiodination; 125-Iodine; MAb; scFv; Peripheral neuropathies

1. Introduction

Peripheral neuropathies (PNs) are caused by many factors, which range from congenital defects to viral or bacterial infection, or exogenous factors such as the intake of neurotoxic agents. PNs occur along with numerous diseases, e.g. the immuno-mediated Guillain–Barre syndrome, painful axonal idiopathic neuropathy (PAIN), hereditary motor and sensory neuropathy (HMSN), or Charcot Marie Tooth disease (CMT). Furthermore, many patients with diabetes mellitus suffer from PNs. However, it is still rather problematic not only to diagnose the cause of PNs, but also to assess the degree of the degenerative process.

The principal aim of our investigation was to examine the possibility of developing a radiotracer that would be specific for cytoskeleton of degraded axons and neuronal bodies. The first step is the right choice of neuronal marker, which should be characteristic for neuronal tissue. A convenient candidate for such a marker seems to be III β-tubulin (bTcIII) that is a component of neuronal cytoskeleton and, according to Dráberová et al. (1998), it was detected only in neuronal cells and in tumors of neuronal origin. Monoclonal antibody (MAb) TU-20 raised against III β-tubulin was proposed as a promising imaging substance for bTcIII. Afterwards, a smaller scFv fragment of TU-20 was synthesized for these purposes.

Both the radiolabeled MAb and its scFv were suggested for *in vivo* applications. They are supposed to detect degraded nerves in the periphery with either standard single proton emission computed tomography (SPECT) using ^{123}I-labeled antibody or positron emission tomography (PET) by ^{124}I-labeled antibody. This work deals with ^{125}I labeling of TU-20 and its scFv and with evaluation of their properties *in vitro* and *in vivo*.

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2. Materials and methods

2.1. The monoclonal antibody TU-20 and its scFv fragment

The MAb TU-20 was purchased from Exbio, CZ. TU-20 is an IgG_1 mouse antibody with relative molecular weight of about 150 kDa. The antibody recognizes the peptide sequence ESESQGPK (aa 441–448) of human class III β-tubulin specific for neurons. The fragment scFv TU-20 was also purchased from Exbio, CZ. It is a recombinant protein expressed in *Escherichia coli* and its relative molecular weight is 27.7 kDa.

2.2. Radiolabeling with ^{125}I

Carrier-free ^{125}I as NaI was purchased from Amersham Bioscience, UK. Chloramine-T was obtained from the Department of Organic Chemistry, CU, CZ. Iodogen and polyacrylamide desalting columns with an exclusion limit 6 kDa were purchased from Pierce, USA.

TU-20 was radioiodinated using either chloramine-T or iodogen as an oxidizing agent. Iodination via chloramine-T was provided in two alternative ways: either with or without stopping the reaction by a reducing agent (Hunter and Greenwood, 1962; Robles et al., 2001). The reaction was performed under following conditions: 10 μl of TU-20 (1 mg/ml) was transferred to 10 μl phosphate buffer (PBS, 0.01 M, pH 7.4) in a reaction vessel and ^{125}I radioactivity (approximately 5.36 MBq) was added. Finally, the solution of chloramine-T in PBS (0.1 mg/ml) was added to the reaction vessel. The amount of chloramine-T ranged from 0.5 to 6 μg per 10 μg of the antibody.

After the reaction time, during which the reaction mixture was gently agitated, the reaction alternatively might be stopped or not with 100 μl of the solution $Na_2S_2O_3 \cdot 5H_2O$ in water (4 mg/ml). The reaction time was same (60 s) in both cases—iodination via chloramine-T with or without stopping reaction with reducing agent.

Radioiodination of TU-20 via iodogen was also proceeded in two alternative ways: by a direct (Fraker and Speck, 1978) or an indirect method (Chizzonite et al., 1991). Iodination tubes for both methods were prepared in the same way. One hundred microliters of iodogen dissolved in chloroform (10–500 μg/ml) were given in a glass tube and chloroform was evaporated under a slow stream of nitrogen. The prepared iodination tubes were used immediately. The procedure for the direct method consisted in adding 10 μl of TU-20 (1 mg/ml) into the reaction tube with 50 μl of phosphate buffer (PBS, 0.05 M, pH 8.5) and an equal amount of $Na^{125}I$ around 5.4 MBq. Reaction time was 15 min. The indirect method was performed in two steps. First, radioactivity in PB was added into the tube coated with iodogen. After 15 min the activated iodide was withdrawn, transferred into the vessel containing 10 μl of the antibody, and the mixture was agitated for 20 min.

Radioiodination of scFv TU-20 was performed via chloramine-T without stopping reaction with thiosulfate as described previously for TU-20. The fragment to radioactivity ratio was 1 μg to 1.5 MBq. The reaction time was altered from 1 to 30 min.

After each radiolabeling procedure mentioned above, the reaction mixture was loaded on the top of a BSA-blocked polyacrylamide desalting column with an exclusion limit 6 kDa. The fractions were eluted with 0.1% BSA in PBS (PBS/BSA) and measured for radioactivity. The radiochemical yield Y of reaction was calculated as a ratio of high molecular weight fractions activity (A_{HMW}) to all fractions activity (A_s) after purification. Moreover, standardized yield Ψ (μg/MBq) was calculated as Ym_a/A_0, where m_a is the initial amount of antibody in μg and A_0 is the activity of ^{125}I in MBq entering to the reaction. A molar ratio of ^{125}I to antibody is represented by N_{125-I}/N_a and indicates the degree of antibody labeling. $N_{125-I}/N_a = (2.4 \times 10^{-24} A_{HMW} A_0 T_{1/2} M_r)/A_s m_s$, where $T_{1/2}$ is the half-life of ^{125}I, M_r the relative molecular weight of either TU-20 or scFv TU-20, and m_s is the initial amount of antibody.

2.3. Quality control

The immunoreactivity of the radiolabeled MAb TU-20 was determined immediately after the reaction by an enzyme-linked immunosorbent assay (ELISA) using a commercial set for detection of mouse anti-III β-tubulin antibodies from VIDIA, CZ. I_p was assayed as a percentage of preserved immunoreactivity of the [^{125}I]TU-20 to immunoreactivity of unlabeled antibody in the ELISA test.

Radiochemical stability in time was investigated in the same way as purification after labeling was done on desalting columns and activity of high and low molecular weight peek was measured for each time interval after iodination.

Furthermore, electrophoresis on 4–12% bis–tris gel was performed in order to find radiolabeled impurities such as antibody fragments or adjuvans in the samples of [^{125}I]TU-20 and [^{125}I]scFv TU-20. Electrophoresis was done in the same day as radioiodination. Protein bands were visualized by staining the gels with Silver Stain Plus (Biorad, USA). ^{125}I-labeled immunoconjugates were visualized using autoradiography exposing plate BAS-SR 2025, and developed by BAS-1800II (both from FUJIFILM, Japan). The autoradiographs were evaluated by AIDA 2.0 software (Raytest, Germany). Parameter S was estimated as a percentage of the radioactivity bound in the band belonged to the entire antibody to the total radioactivity of a lane. In fact, S is a rate of radiochemical purity.

2.4. In vitro evaluation of binding specificity

Immunohistochemistry and autoradiography of the mice brain tissue slices were performed in order to evaluate