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Quantification of NC100692, a new tracer for ^{99m}Tc-imaging of angiogenesis, in human plasma using reversed-phase liquid chromatography coupled with electrospray ionization ion-trap mass spectrometry

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Abstract

NC100692 is under development as a diagnostic radiopharmaceutical for targeting angiogenesis associated with diseases, such as cancer and endometriosis. NC100692 consists of a cyclic RGD-containing peptide with an ethylene glycol chain linked to the C-terminal amino acid and a ^{99m}Tc-binding chelator linked to the N-terminal amino acid. The present report describes a method for quantification of NC100692 in human citrated plasma. The method is based on solid-phase extraction followed by reversed-phase liquid chromatography using a gradient of water and acetonitrile with 0.1% formic acid. The chromatographic system was coupled on-line with an electrospray mass spectrometer. The analyses were performed by selective ion monitoring of the $[M+2H]^{2+}$ and the $[M+3H]^{3+}$ ions of NC100692 and the internal standard, which was identical to NC100692 except for containing twice the length of the ethyleneglycol chain. The limit of quantification of the method was 0.5 ng NC100692/ml plasma. The calibration curve ranged from 0.5 to 250 ng NC100692/ml plasma and was fitted to a quadratic equation with a weighing factor of $1/y$ and found to be highly reproducible. The total precision of the method, expressed as the relative standard error of the mean, was 11.1, 10.8 and 9.7% for the low, medium and high control samples, respectively. The accuracy of the method was 103.4, 111.1 and 107.5% for the low, medium and high control samples, respectively. NC100692 was stable in human plasma during at least 3 freeze/thaw cycles, during 48 h on dry ice and at least 8 weeks when stored in a −20 °C freezer.

Keywords: NC100692; Human plasma; LC–MS; Ion-trap; Imaging; Angiogenesis

1. Introduction

The ^{99m}Tc complex of NC100692 is being evaluated as a diagnostic imaging agent for detection of angiogenesis, and was recently used for detection of malignant lesions in patients with breast cancer [1]. NC100692 consists of a peptide based pharmacophore containing a cyclic RGD sequence coupled to an ethylene glycol biomodifier in the C-terminal end of the peptide and a ^{99m}Tc-binding chelator linked to the N-terminal end. The structure of this substance is shown in Fig. 1; the synthesis was recently described by Indrevoll et al. [2].

^{99m}Tc is one of the principal radionuclides used in nuclear medicine due to its short half-life (6.02 h) and favourable γ-emitting energy resulting in low radiation exposure to the patients [3]. ^{99m}Tc-based radiopharmaceuticals are distributed to the pharmacy as lyophilised kits in a Tc-free form. Prior to use, ^{99m}Tc (in the form of the pertechnetate anion, i.e. TcO_4^-, eluted from a ^{99}Mo/^{99m}Tc generator with saline) [3] is added to the freeze-dried ligand (NC100692 in the present study) and the added ^{99m}Tc binds to the chelator part of the ligand. There is a vast excess of the ligand compared with the added ^{99m}Tc. Less than 1% of the ligand in the injected solution is in the form of a ^{99m}Tc-complex. Hence, the unlabelled ligand makes up almost the entire amount of the injected drug, despite the fact that it is the very small amount of the ^{99m}Tc-labelled agent that is responsible for the diagnostic images obtained. Accordingly, it is the

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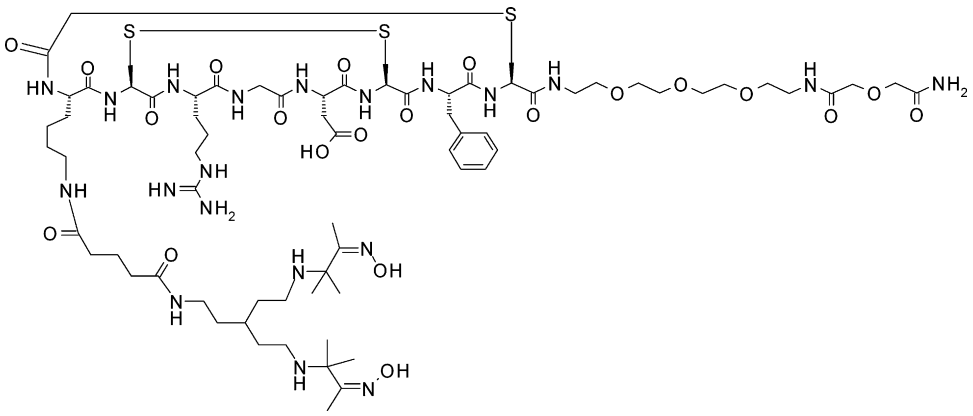

Fig. 1. The structure of NC100692.

unlabelled ligand that is used to describe the kinetic parameters for such products.

As part of the development of NC100692 it was necessary to establish a method to describe the kinetics of this agent in humans receiving doses close to the clinical imaging dose (pharmacokinetics). As one clinical imaging dose consists of not more than 75 μg NC100692 [1], a method with low limit of quantification was needed for this purpose. We here report validation data obtained following normal guidelines for such work [4,5], with a method based on solid phase extraction (SPE) of human citrated plasma followed by reversed-phase liquid chromatography coupled with electrospray ion-trap mass spectrometry (LC–ESI-MS). This method was found suitable for the intended use and has been used to describe the pharmacokinetics of NC100692 in humans (to be published elsewhere).

2. Experimental

2.1. Materials

Acetonitrile and methanol were LiChrosolve grade and acetic acid was pro-analysis grade, all from Merck (Darmstadt, Germany). Formic acid was pro-analysis grade from Rathburn Chemicals LTD (Walkerburn, Scotland) and ammoniumhydroxid was from Sigma (Sigma–Aldrich Co., St.Louis, MO, USA). NC100692 and the internal standard (NC100682) were from GE Healthcare (Oslo, Norway); the internal standard was identical to NC100692 except for having twice the length of the ethylene glycol chain. Water was purified by reversed osmosis; ion exchanged and filtrated through a 0.45 μm filter using a Milli-Q system (Millipore, Bedford, MA, USA). Other chemicals were of analytical grade. Citrated human blood for standard and control samples and specificity and selectivity experiments were obtained from 8 healthy volunteers.

2.2. Standard and control samples

The calibration standards and the control samples were made by dilutions in human citrated plasma (Blood Bank, Ullevål University Hospital, Oslo, Norway) and stored at −20 °C. The calibration standards contained 0.5 to 250 ng NC100692/ml, and the control samples were made at 3 concentration levels, i.e. 1.5 ng NC100692/ml (low), 75 ng NC100692/ml (medium) and 200 ng NC100692/ml (high).

2.3. Sample preparation

Plasma was prepared by centrifugation at 3000 × *g* for 10 min at room temperature. The internal standard (50 μl) was added to the plasma samples (250 μl) and extracted by a SPE procedure using Gilson ASPEC XL4 from Gilson Inc. (Middleton, WI, USA). The SPE columns (30 mg Waters Oasis HLB) obtained from Waters (Milford, MA, USA) were preconditioned with 1 ml of methanol and 1 ml of water. The cartridges were washed with 2 ml of water and 2 ml of methanol + ammoniumhydroxid + water (35 + 2 + 63, v/v/v). Finally, NC100692 and the internal standard were eluted with 0.75 ml of methanol + acetic acid + water (80 + 2 + 18, v/v/v). The samples were evaporated under a stream of nitrogen at room temperature and the residues were dissolved in 125 μl of 20% (v/v) acetonitrile in water; 30 μl were injected onto the LC-column.

2.4. Chromatographic conditions

A TSP Surveyor LC-system (ThermoFinnigan, San Jose, FL, USA) was used with a Supelco Discovery HS C18, 100 × 2.1 mm i.d. (3 μm particle diameter) column (Supelco, Bellafonte, PA, USA) fitted with a Phenomenex C8 4 × 2.0 mm i.d. guard column (Phenomenex, Torrance, CA, USA). Mobile phase A was 0.1% (v/v) formic acid in water and mobile phase B 0.1% (v/v) formic acid in acetonitrile. To separate NC100692 from endogenous peptide and protein material, a gradient was run starting at 95% mobile phase A, decreasing to 50% A in 8 min, holding for 1 min, and then returning to 95% A in 1 min. Including 9 min equilibration time, the total chromatographic run time was 19 min. The flow rate was 0.3 ml/min and the analysis was performed at ambient temperature (approximately 22 °C). The samples were kept in the autosampler at 4 °C during the entire analytical sequence.