DESIGN OF RADIOPHARMACEUTICALS FOR MONITORING GENE TRANSFER THERAPY

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Abstract

The development of radiopharmaceuticals for monitoring gene transfer therapy with emission tomography is expected to lead to improved management of cancer by the year 2010. There are now only a few examples and approaches to the design of radiopharmaceuticals for gene transfer therapy. This paper introduces a novel concept for the monitoring of gene therapy. We present the optimisation of the labelling of recombinant human β-NGF ligands for *in vitro* studies prior to using ^{123}I for SPET and ^{124}I for PET studies.

INTRODUCTION

The first [1] clinical experience employing gene therapy was gained in 1989. Peripheral blood leukocytes were transduced with heterologous DNA to examine the biological *in vivo* properties of tumour-infiltrating lymphocytes, and thereby optimising antitumour immunotherapy strategies. Since then numerous approaches have been undertaken to apply powerful strategy of therapeutic gene transfer to almost all kinds of human diseases. However, despite substantial progress there are still a number of key technical issues to be resolved before gene therapy can be safety and effectively applied in the clinic [2]. Major limitations still include the lack of efficient vector application procedures, efficient restrictions of the vector tropism to the tissues to be targeted as well as an insufficient long-term expression of transferred therapeutic genes.

In this context, it is of major importance to efficiently monitor the short term as well as the long term effects of technical improvements in gene therapy techniques [3,4]. Information documenting the respective vector profiles and application techniques is supposed to exert a vigorous effect on the design of subsequent, improved vector generations and application procedures. Therefore, *in vivo* molecular imaging using emission tomography may be one of the best methods to assess the expression of a gene transduced in a target tissue and to monitor *in vivo* gene transfer as a therapeutic approach to cancer, chronic viral infections and mono-genetic inherited diseases. The concepts of gene therapy have been reviewed [5].

There are now only a few examples and approaches to the design of radiopharmaceuticals for suicide gene transfer therapy. See TABLE I.

This paper summarises the concepts, current status, and an example using ligands of nerve growth factor, and prospects for this new direction of radiopharmaceutical sciences and oncology. Conventional procedures for *in vivo* monitoring of therapeutic gene transfer are based on the molecular characterisation of repetitively collected serum and tissue probes for detection of therapeutic RNA's, DNA's and proteins. These highly sensitive and specific techniques are

TABLE I. RADIOTRACERS AIMED TO GENE TRANSFER THERAPY

TRACER	GENE / VECTOR TYPE	REFERENCE
^{131}I-FIVAU, FIVRU	HSV-tk / STK retrovirus	Wiebe, et al, 1995 [6]
^{131}I-/^{124}I-FIAU	HSV-tk / gpSTK-A2 retrovirus	Tjuvajev, et al., 1995 [10]
^{3}H-5FC	HSK-tk	Haberkorn, et al., 1996 [7]
^{18}F-Acyclovir	HSK-tk / Adenovirus	Srinivasan et al., 1996 [8]
^{18}F-FHPG	HSK-tk	Goldman, et al., 1996 [13]
^{125}I-/^{124}I-β-NGF	Adenovirus / PLDNSN retrovirus	This Work, 1997 [15]
^{125}I-/^{124}I- ----MAb	Adenovirus / PLDNSN retrovirus	This Work, 1997 [24]
^{18}F-Purines	HSK-tk	Barrio, et al., 1997 [28]
^{125}I-Bombesin	Adenovirus / AdCMGRPr	Rogers et al., 1997 [29]

FIVAU = (E)-5-2-(iodovinyl)-2-deoxyuridine, R = ribofuranosyl

FIAU = Fluoro-1 b D-arabinofuranosyl-5-iodouracil

5FC = 5-Fluorocytosine

FHPG = 9-1-(1-Fluoro-3-hydroxy-2propoxyl)-methylguanidine

β-NGF = Recombinant β-Nerve Growth Factor

Bombesin = mIP-Des^{14}-Bombeson-(7-13)NH_2

laborious and require a continuous control of expression strength, kinetics and localisation following gene transduction. In order to expand gene transfer to the patient setting, it is essential to understand *in vivo* cell cycle control, proliferation, apoptosis and neovascular activity, e.g. within tumours. The goal for PET is to define a convenient way for *in vivo* monitoring of the kinetics, the definition and continuous monitoring of a distinct vector tropism and distribution particularly with the initial 72 hours following *in vivo* transduction as well as over longer time periods (i.e., months to years subsequent to the initial gene transfer procedure).

Iodine-124 is the positron-emitting radionuclide of choice in the design and development of therapeutic radiopharmaceuticals. Iodine-124 has a 4.16 day half-life, and 25% on the nuclear decay events are associated with emission of a positron ($E_{\beta+}$ = 2.134 MeV). The radioisotope has been produced [30] for PET applications [31] by using either the ^{124}Te(d,2n)^{124}I and the ^{124}Te(p,n)^{124}I nuclear reactions by irradiation of high isotopic enrichment (>97%) of ^{124}Te with 15 MeV deuterons or 12 MeV protons, respectively.

Approaches by Wiebe et al., [6], Haberkorn et al., [7], Srinivasan et al., [8], Monclus et al. [9], and Tjuvajev et al. [10,11,12] and Goldman et al [13] used a retroviral vector to transfer the suicide gene coding for the thymidine kinase of the herpes simplex virus (HSV-tk) in tumour cells. The indirect measurement of gene expression was attempted [14] through visualisation of enzyme activity of a tracer pro-drug (e.g., ganciclovir or 5-fluorocytosine) to the toxic product to follow the metabolism of the radioactive probe.

Our novel approach [15] focuses on the design of a suitable cell surface reporter system that incorporates a receptor protein and its specific ligand for the in vivo monitoring system of target restricted tropic vectors for selected targets of gene transfer therapy. This is exemplified by diverse asialoglycoprotein-receptor restricted, hepatotropic vector systems [16,17,18].

The cytokine nerve growth factor (NGF) comprised of 118 amino acid residues was chosen as the ligand, and a cytoplasmatically truncated mutant of the low affinity nerve growth factor receptor P75 (delta-p75) as the appropriate marker gene. The tertiary structure is comprised of three cystine disulfide residues and three Beta hairpins. The NGF is present as a dimer for biological activity [19,20]. NGF has a high affinity to at least two broad cell surface receptors [21] called p75 (Kd of 10^{-9}) and TrkA (Kd of 10^{-11}). The interaction with p75 seems to involve Lysine residues. The structure of NGF also contains two tyrosine residues that are not required for binding to p75, and therefore do not influence the biological activity when radio-iodinated [19].

There is evidence that NGF-R has a role in programmed cell death of neurons, regulation and coordination neuronal proliferation, differentiation and maintenance in the adult, the information flow between the nervous and immune system, and pain causing events.

EXPERIMENTAL

Stable LNGF-R cell lines have been generated by transduction of amphotropic PA317 packing cells with LNGF-R encoding retroviral vector pLDNSN (Boehringer Mannheim). Stable cell lines were characterised by PCR and immunochemistry. Supernatant of stable producer clone PALDNS#12 yields a titre of 7 x 10^5 cfu/ml, and was used for transduction of NIH 3T3 fibroblasts, and two hepatoma cell lines (HuH7 and Morris Hepatoma). Thereby, pairs of LNGF-R receptor positive and LNGF-R receptor negative (negative controls) cell lines were made available subsequent for binding studies. In addition, LNGF-R cDNA encoding recombinant adenoviral vectors were transfected which allow also the monitoring of the adenoviral gene transfer. Construction was achieved by homologous recombination of the respective cDNA with the adenoviral shuttle vector in E. coli [22], and subsequent plaque purification. These adenoviral vectors are currently used for LNG-R transduction of NIH 3T3 and Morris hepatoma recipient cells thereby allowing functional analysis of the adenoviral mediated gene transfer.

Initially ^{125}I was used for the optimisation of the labelling of recombinant human β-NGF ligands prior to using ^{123}I for SPET and ^{124}I for PET studies. Two methods were evaluated: lactoperoxidase, and as a superior approach using the mild oxidising agent N-bromosuccinimide (NBS). The radiochemical yields with ranged ^{125}I ranged from 85 to 95% with lactoperoxidase, and from 95 to 98% with NBS. The NBS reaction was carried-out as follows: 10 µg NGF was incubated with 0.5 mCi ^{125}I-iodide (in NaOH) and 0.3 µg NBS in a final volume of 15 µl Na-Phosphate buffer (0.13 M, pH 7.4) on ice for 5 min. The reaction was terminated by dilution with Na-phosphate buffer containing 0.5% BSA.

The NGF (2.5 µg) was incubated with ^{124}I (17 mCi/ml from UKV-Essen) and 0.08 µg NBS. The radiochemical yield was determined by tlc on silica gel 60 using a mobile phase of (acetone / n-butanol / ddH_2O / ammonia) (65/20/5/10). The radiochemical yield with ^{124}I was >90%. The product was purified with a Biogel P6 Micro BioSpin Column, that was equilibrated with Na-Phosphate

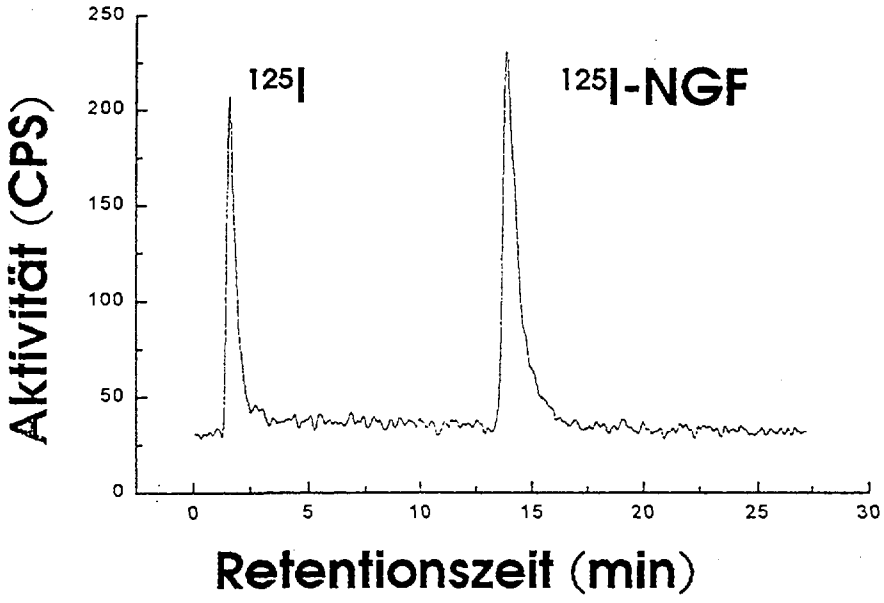

FIG. 1. RADIO-HPLC FOR PURIFICATION OF RADIO-IODINATED NGF.

buffer containing 0.5% BSA and 0.1% Protaminsulfate. Radio-HPLC was performed with a C_{18} column using a gradient of H_2O and Acetonitrile (with 0.1% mTFA). See FIG. 1

Characterisation of the retrovirally transduced cell lines has yielded NGF-R positive clones both in immunofluorescence as well as functional binding with labelled NGF. The biological NGF activity was estimated using the NGF induce neuron growth on PC12 cells to be 80% of the native NGFs. The specific binding of the labelled NGF to the PC12 cells was 70%.

A Micro-imager I (Biospace, Paris) with 30 micron resolution gave *in vitro* images confirming the radioiodinated NGF was bound to the LNGF-R surface receptor reporter protein expressed on both stable transduced NIH 3T3 as well as on Morris Hepatoma cells, but not on untransduced control cells [15]. These results provide the first experimental evidence that the chosen ligand/receptor system (LGF/LNGF-R) vector transduced target cells. This novel approach differs from applications of radiopharmaceuticals restricted for usage in suicide gene transfer therapy only (See TABLE I) in that usage of a non-functional marker gene (here: the LNGF-R gene devoid of any intrinsic receptor functions) enables an universal application of such "monitoring" vectors without interfering with the cellular functions of the vector addressed target cells.

Currently, biodistribution in rodents, and *in vivo* emission tomographic studies are planned [23,24] at DKFZ using a high resolution PC 2048-7WB PET scanner [25]. For this purpose, the Morris hepatoma animal model [26] will be used. The model closely resembles the tumour biology of human hepatocellular carcinomas in tumour growth rate, blood supply characteristics and metastases. Tumour implantation was performed by insertion of ~ 3 mm^3 of Morris hepatoma tumour 3924 tissue into the sub-capsular pocket of the left lobe of the liver [27]. After 10 - 14 days the tumours were injected with LNGF-R transducing retroviral or adenoviral vectors. The PET study [24] is planned 48 hr after the transduction procedure.

CONCLUSION

The development of radiopharmaceuticals for monitoring gene transfer therapy with emission tomography is expected to lead to improved management of cancer by the year 2010. This paper summaries the progress to date.

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