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(54) **IMAGING INFECTION WITH COMPOUNDS THAT BIND TO THYMIDINE KINASE**

BILDGEBUNG VON INFEKTIONEN MIT AN THYMIDINKINASE BINDENDEN VERBINDUNGEN

VISUALISATION D'UNE INFECTION AVEC DES COMPOSES SE LIANT A LA THYMIDINE KINASE

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Description

Background of the Invention

[0001] The ability to diagnose and localize an infection in a subject are critical to the practicing clinician. Current methods to visualize bacterial infection *in vivo* use positron emission tomography with radiolabeled white blood cells or, more recently, radiolabeled antibiotics. These methods tend to be nonspecific and cannot distinguish infection from inflammation or cancer. For example, Sarda et al. tested 99mTc-labeled ciprofloxacin for imaging S. aureus infection in the knee joints of rabbits. Their results indicated that this compound lacks the specificity necessary for clinical applications ((2002) J. Nucl. Med 43:239-45). In another study, Fishman et al. determined that 18F-labeled fluconazole lacked the specificity to effectively visualize Candida in.a rabbit model of infection ((1991) J. Pharmacol. Exp. Ther. 259:1351-9)

[0002] Accordingly, the need exists for an organism specific noninvasive imaging method to detect infection, e.g., bacterial, viral or fungal infection in a subject.

[0003] US 2002/128553 discloses radiolabelled epidermal growth factor tyrosine kinase (EGFR-TK) irreversible inhibitors and their use as biomarkers for medicinal radioimaging such as Positron Emission Tomography (PET) and Single Photon Emission Computed Tomography (SPECT) and as radiopharmaceuticals for radiotherapy.

[0004] US 5,879,661 and US 6,331,287 disclose that the nucleoside analog 2'-fluoro-5-methyl-1-β-D-arabinofuranosyluracil (FMAU) has an especially desirable combination of properties for use as an imaging agent, including in particular limited in vivo catabolism.

[0005] Deng W.-P. et al., "Non-invasive in vivo imaging with radiolabelled FIAU for monitoring cancer gene therapy using herpes simplex virus type 1 k thymidine kinase and ganciclovir", EUR. J. NUCL. MED. MOL. IMAGING, vol. 31, no. 1, January 2004 (2004-01), pages 99-109, XP001119790 discloses a non-invasive imaging procedure using regulated 2'-fluoro-2'-deoxy-5-iodi-1-β-D-arabinofuranosyluracil (FIAU) as an enzyme substrate for monitoring retroviral vector-mediated herpes simplex virus type 1 thymidine kinase gene (HSV1-tk) transgene expression.

[0006] Golankiewicz B. et al., "Fluorescent Tricyclic Analogues of Acyclovir and Ganciclovir. A Structure-Antiviral Activity Study", Journal of Medicinal Chemistry, American Chemical Society, Washington, U.S., vol. 44, 2001, pages 4284-4287, XP001119790 provides an evaluation of a series of new guanine base modified tricyclic analogues of acyclovir and ganciclovir derivatives of the 3,9-dihydro-9-oxo-5H-imidiazo[1m2-a]purine system for activity against herpex simplex virus type 1 and 2. Several fluorescent analogues were obtained that showed similar potency and selectivity as the parent compounds.

[0007] Goslinski T. et al., "Synthesis and Biological Activity of Strongly Fluorescent Tricyclic Analogues of Acyclovir and Ganciclovir", J. Med. Chem., vol. 45, no. 1, 2002, pages 5052-5057 discloses strong fluorescent tricyclic analogues of acyclovir and gancyclovir and an evaluation for their activity against herpes simplex virus type 1 and type 2 in cell cultures.

[0008] Saito Y. et al., "Diagnostic Imaging of Herpes Simplex Virus Encephalitis Using a Radiolabelled Antiviral Drug: Autoradiographic Assessment in an Animal Model", Ann. Neurol. 15:548-558, 1984 discloses a new approach to the diagnosis of herpes simplex encephalitis by using a radiolabelled antiviral drug, 2'-fluoro-5-methyl-1-β-D-arabinosyluracil labelled with carbon 14 ([^{14}C]FMAU) as a probe for selectively imaging brain infection in a rat model by quantitative autoradiography.

Summary of the invention

[0009] In one aspect, the instant invention provides, for use in a method of diagnosing a bacterial infection in a subject.

[0010] In an other aspect the invention provides, for use in a method of diagnosis imaging bacteriolytic therapy.

Summary of the Invention

[0011] The compound FIAU is a nucleoside analog. In a specific embodiment, the nucleoside analog is radiolabeled, e.g., with fluorine or iodine. In certain embodiments, the nucleoside analog emits gamma particles. Exemplary compounds used in the methods include, for example, 2'-fluoro-2'deoxy-1-beta-D-arabinofuranosyl-5-iodo-uracil ([1251]-FIAU),

[0012] In a related embodiment, the nucleoside analog is fluorescent.

[0013] In certain embodiments, the bacterial infection is caused by bacteria from a genus selected from the group consisting of *Escherichia, Bacillus Chromobacterium, Clostridium, Enteroccus, Haemophilus, Listeria, Mycoplasma, Pasteruella, Salmonella, SJaphylococcus, Streptococcus, Streptomyces, Vibrio, and Yersinia.*

[0014] In another aspect, the invention provides 2'-Fluoro-2'-deoxy-1-beta-D-arabinofuranosyl-5-iodouracil (FJAu) suitable for changing wherein the method is selected from the group consisting of planar gamma imaging and single photon emission computed tomography (SPECT) for use in imaging bacteriolytic therapy.

[0015] The compound FIAU is a nucleoside analog. In a specific embodiment, the nucleoside analog is radiolabeled,

e.g., with fluorine or iodine. In certain embodiments, the nucleoside analog emits gamma particles. An Exemplary compound used in the methods include, for example, 2'-fluoro-2'deoxy-1-beta-D-arabinofuranosyl-5-iodo-uracil ([1251]-FIAU),

[0016] In a related embodiment, the nucleoside analog is fluorescent.

[0017] In another related embodiment, the invention allows for the differentiation of bacterial infection and inflammation or cancer.

Detailed Description of the Invention

[0018] The present invention is based, at least in part, on the discovery using suitably functionatized compounds that binds to a polypeptide, *e.g.*, a thymidine kinase, expressed by an infectious organism allows for imaging of the infection. This invention is directed, at least in part, to the diagnosis and localization of infection, e.g., infections caused by bacteria, virus, or fungi. This present invention allows, for example, for the visualization of infectious foci, localization of tumors harboring anaerobic bacteria, diagnosis of infection, monitoring antibacterial therapy, studying of bacterial trafficking for emerging bacterial-based therapies of cancer and for treatment of infection by noninvasive means.

[0019] The term "treated," "treating" or "treatment" includes the diminishment or alleviation of at least one symptom associated or caused by the state, disorder or disease being treated. In certain embodiments, the treatment comprises the induction of a Pin1 inhibited state, followed by the activation of the Pin1 modulating compound, which would in turn diminish or alleviate at least one symptom associated or caused by the Pin1 associated state, disorder or disease being treated. For example, treatment can be diminishment of one or several symptoms of a disorder or complete eradication of a disorder.

[0020] The term "subject" is intended to include mammals, e.g., humans, dogs, cows, horses, pigs, sheep, goats, cats, mice, rabbits, rats, and transgenic non-human animals. In certain embodiments, the subject is a human, e.g., a human suffering from, at risk of suffering from, or potentially capable of suffering from an infection or cancer.

[0021] The term "cancer" includes malisnancies characterized by deregulated or uncontrolled cell growth, for instance carcinomas, sarcomas, leukemias, and lymphomas. The term "cancer" includes primary malignant tumors, e.g., those whose cells have not migrated to sites in the subject's body other than the site of the original tumor, and secondary malignant tumors, e.g., those arising from metastasis, the migration of tumor cells to secondary sites that are different from the site of the original tumor.

[0022] The language "effective amount" of a compound is the amount necessary or sufficient to provide a readable signal when imaged using the techniques described herein, e.g., planar gamma imaging and single photon emission computed tomography (SPECT). The effective amount can vary depending on such factors as the size and weight of the subject, the type of illness, or the particular compound. For example, the choice of the compound can affect what constitutes an "effective amount". One of ordinary skill in the art would be able to study the factors contained herein and make the determination regarding the effective amount of the compound without undue experimentation.

[0023] The phrase "pharmaceutically acceptable carrier" is art recognized and includes a pharmaceutically acceptable material, composition or vehicle, suitable for administering compounds used in the methods described herein to subjects, e.g., mammals. The carriers include liquid or solid filler, diluent, excipient, solvent or encapsulating material, involved in carrying or transporting the subject agent from one organ, or portion of the body, to another organ, or portion of the body. Each carrier must be "acceptable" in the sense of being compatible with the other ingredients of the formulation and not injurious to the patient. Some examples of materials which can serve as pharmaceutically acceptable carriers include: sugars, such as lactose, glucose and sucrose; starches, such as corn starch and potato starch; cellulose, and its derivatives, such as sodium carboxymethyl cellulose, ethyl cellulose and cellulose acetate; powdered tragacanth; malt; gelatin; talc; excipients, such as cocoa butter and suppository waxes; oils, such as peanut oil, cottonseed oil, safflower oil, sesame oil, olive oil, corn oil and soybean oil; glycols, such as propylene glycol; polyols, such as glycerin, sorbitol, mannitol and polyethylene glycol; esters, such as ethyl oleate and ethyl laurate; agar; buffering agents, such as magnesium hydroxide and aluminium hydroxide; alginic acid; pyrogen-free water; isotonic saline; Ringer's solution; ethyl alcohol; phosphate buffer solutions; and other non-toxic compatibly substances employed in pharmaceutical formulations.

Compounds of the Invention

[0024] The methods described herein make use ofcompounds that bind to thymidine kinase polypeptides, in an organism and produce a detectable signal that can used to obtain an image of a subject and, thereby, determine the presence and location of the organism. The compounds used in the methods of the invention bind to a thymidine kinase, with greater affinity than they bind to a kinase, e.g., a thymidine kinase, in the subject to which they are administered. Thymidine kinases are particularly well suited for the methods of the invention. The bacterial thymidine kinases have a consensus sequence in the kinase catalytic domain that is not present in the kinase catalytic domain of mammalian

thymidine kinases (see the Examples). Accordingly, compounds with high affinity for bacteria thymidine kinases exhibit greatly reduced affinity for mammalian thymidine kinases.

[0025] The invention utilizes compounds that are easily synthesized and are detectable to an imaging apparatus, e.g., a PET or SPECT instrument. The compounds are nucleoside analogs that bind to a thymidine kinase. Bioinformatic analysis of the 53 pathogenic bacteria whose genomes have been sequenced reveled that every species has a thymidine kinase (Bettegowda et al. (2005) PNAS 102:1145-50).

[0026] In specific embodiments the nucleoside analogs are labeled with a radioisotope, e.g., a radioisotope of iodine or fluorine. In another embodiment, the nucleoside analogs may be fluorescent.

[0027] Preferred radiolabeled compounds of the invention are nucleoside analogs that are easily synthesized and limited *in vivo* catabolism. Compounds such as those described in USPNs:5,879,661 and 6,331,287 can be used with the methods of the invention. Other exemplary compound useful in the methods of the invention include, for example, 2'-fluoro-2'deoxy-1-beta-D-arabinofuranosyl-5-iodo-uracil ([1251]-FIAU),

[0028] Exemplary fluorescent compounds that may be used in the methods of the invention have recently been described by Golankiewicz et al. ((200)) J. Med. Chem. 44:4284-7) and Goslinski et al. ((2002) J. Med. Chem. 45:5052-7). The fluorescent tricyclic acyclovir and ganciclovir analogs described by Goslinski *et al*.,particularly GCV3, cam be used in the aforementioned methods

Imaging

[0029] Generally, imaging techniques involve administering a compound to a subject that can be detected externally to the subject. Images are generated by virtue of differences in the spatial distribution of the imaging agents which accumulate in various locations in a subject. The methods of the present invention, the imaging techniques rely on the compounds being preferentially bound by the organism, e.g., the infectious organism. The spatial distribution of the imaging agent accumulated in a subject, e.g.; in an infected region, may be measured using any suitable means, for example, planar gamma imaging and single photon emission computed tomography (SPECT). Alternatively, imaging techniques that detect fluorescence may be used in the methods of the invention.

[0030] Isotopes that decay by electron capture and/or y emission are used in SPECT, and include, for example, ^{123}I and ^{124}I.

[0031] Specifically, imaging is carried out by scanning the entire patient, or a particular region of the patient using the detection system, and detecting the signal, e.g., the radioisotope signal. The detected signal is then converted into an image. The resultant images should be read by an experienced observer, such as, for example, a physician. The foregoing process is referred to herein as "imaging" the patient. Generally, imaging is carried out about 1 minute to about 48 hours following administration of the compound used in the methods of the invention. The precise timing of the imaging will be dependant upon such factors as the clearance rate of the compound administered, as will be readily apparent to those skilled in the art. Preferably, imaging is carried out between about 1 minute and about 4 hours following administration.

[0032] Once an image has been obtained, one of skill in the art will be able to determine the location of the compound. Using this information, the artisan can determine, for example, if an infection is present, the extent of the infection, or the efficacy of treatment which the subject is undergoing. Images obtained at different time points, e.g., 12, 24, 36, 48 or more, hours apart are particularly useful in determining the efficacy of treatment, e.g., antiviral, antibacterial or antifungal treatment.

[0033] Unlike methods currently used, the imaging methods described herein allow the clinician to distinguish infection from inflammation and cancer.

Dosage and Formulation

[0034] The compounds for use in the methods of the present invention can be administered orally using any pharmaceutically acceptable dosage form known in the art for such administration. The compound can be supplied in solid dosage forms such as dry powders, granules, tablets or capsules, or in liquid dosage forms, such as syrups or aqueous suspensions. The compound can be administered alone, but is generally administered with a pharmaceutical carrier. A valuable treatise with respect to dosage forms is Remington's Pharmaceutical Sciences, Mack Publishing.

[0035] The compounds for use in the methods of the present invention can be administered in such oral dosage forms as tablets, capsules (each of which includes sustained release or timed release formulations), pills, powders, granules, elixirs, tinctures, suspensions, syrups, and emulsions- Likewise, they may also be administered in intravenous (bolus or infusion), intraperitoneal, subcutaneous, or intramuscular form, all using dosage forms well known to those of ordinary skill in the art.

[0036] The compounds for use in the methods of the invention can be administered by any means that produces contact of the compound with the compound's site of action in the body of a host, such as a human or a mammal. They

can be administered alone or with pharmaceutical carrier selected on the basis of the chosen route of administration and standard pharmaceutical practice.

[0037] The dosage regimen for the compounds determined from the present invention will, of course, vary depending upon known factors, such as the pharmacodynamic characteristics of the particular agent and its mode and route of administration; the species, age, sex, health, medical condition, and weight of the recipient; the nature and extent of the symptoms; the kind of concurrent treatment; the frequency of treatment; the route of administration, the renal and hepatic function of the patient, and the effect desired. An ordinarily skilled physician or veterinarian can readily determine an effective amount of the compound to administer to a subject.

[0038] The compounds for use in the methods of the present invention can be administered in intranasal form via topical use of suitable intranasal vehicles, or via transdermal routes, using those forms of transdermal skin patches wall known to those of ordinary skill in that art.

[0039] In the methods for use of the present invention, the compounds described herein can be administered in admixture with suitable pharmaceutical diluents, excipients, or carriers (collectively referred to herein as carrier materials) suitably selected with respect to the intended form of administration, that is, oral tablets, capsules, elixirs, syrups and the like, and consistent with conventional pharmaceutical practices.

[0040] For instance, for oral administration in the form of a tablet or capsule, the active drug component can be combined with an oral, non-toxic, pharmaceutically acceptable, inert carrier such as lactose, starch, sucrose, glucose, methyl cellulose, magnesium stearate, dicalcium phosphate, calcium sulfate, mannitol, sorbitol and the like; for oral administration in liquid form, the oral drug components can be combined with any oral, non-toxic, pharmaceutically acceptable inert carrier such as ethanol, glycerol, water, and the like. Moreover, when desired or necessary, suitable binders, lubricants, disintegrating agents, and coloring agents can also be incorporated into the mixture. Suitable binders include starch, gelatin, natural sugars such as glucose or beta-lactose, corn sweeteners, natural and synthetic gums such as acacia, tragacanth, or sodium alginate, carboxymethylcellulose, polyethylene glycol, waxes, and the like. Lubricants used in these dosage forms include sodium oleate, sodium stearate, magnesium stearate, sodium benzoate, sodium acetate, sodium chloride, and the like. Disintegrators include, without limitation, starch, methyl cellulose, agar, bentonite, xanthan gum, and the like.

[0041] The compounds for use in the methods of the present invention can also be administered in the form of liposome delivery systems, such as small unilamellar vesicles, large unilamallar vesicles, and multilamellar vesicles. Liposomes can be formed from a variety of phospholipids, such as cholesterol, stearylamine, or phosphatidylcholines.

[0042] Compounds for use in the methods of the present invention may also be coupled with soluble polymers as targetable drug carriers. Such polymers can include polyvinylpyrrolidone, pyran copolymer, polyhydroxypropylmethacrylamide-phenol, polyhydroxyethylaspartamidephenol, or polyethyleneoxidepolylysine substituted with palmitoyl residues. Furthermore, the compounds determined from the present invention may be coupled to a class of biodegradable polymers useful in achieving controlled release of a drug, for example, polylactic acid, polyglycolic acid, copolymers of polylactic and polyglycolic acid, polyepsilon caprolactone, polyhydroxy butyric acid, polyorthoesters, polyacetals, polydihydropyrans, polycyanoacylates, and crosslinked or amphipathic block copolymers of hydrogels.

[0043] Gelatin capsules may contain the active ingredient and powdered carriers, such as lactose, starch, cellulose derivatives, magnesium stearate, stearic acid, and the like. Similar diluents can be used to make compressed tablets. Both tablets and capsules can be manufactured as sustained release products to provide for continuous release of medication over a period of hours. Compressed tablets can be sugar coated or film coated to mask any unpleasant taste and protect the tablet from the atmosphere, or enteric coated for selective disintegration in the gastrointestinal tract. Liquid dosage forms for oral administration can contain coloring and flavoring to increase patient acceptance. In general, water, a suitable oil, saline, aq ueous dextrose (glucose), and related sugar solutions and glycols such as propylene glycol or polyethylene glycols are suitable carriers for parenteral solutions. Solutions for parenteral administration preferably contain a water soluble salt of the active ingredient, suitable stabilizing agents, and if necessary, buffer substances. Antioxidizing agents such as sodium bisulfite, sodium sulfite, or ascorbic acid, either alone or combined, are suitable stabilizing agents. Also used are citric acid and its salts and sodium EDTA. In addition, parenteral solutions can contain preservatives, such as benzalkonium chloride, methyl- or propyl-paraben, and chlorobutanol.

[0044] Suitable pharmaceutical carriers are described in Remington's Pharmaceutical Sciences, Mack Publishing Company, a standard reference text in this field.

EXAMPLES

Materials and Methods

[0045] In Vitro Bacterial Susceptibility Assays. 1-(2'-deoxy-2'-fluoro-β-D-arabinofuranosyl)-5-iodouracil (FIAU) (Moravek catalog no. M251) and penciclovir (Moravek catalog no. M972) were purchased from Moravek Biochemicals (Brea, CA). Zidovudine was purchased from Glaxo Wellcome. Bacterial susceptibility tests were performed in 96-well

microtiter plates (VWR Scientific), with serial dilutions of drug placed in each well. Each well was inoculated with *Escherichia coli* (Yale University E. coli Genetic Stock Center, New Haven, CT) and grown in Luria broth (Invitrogen) at 37°C. E. *coli* TK mutants were generated by selecting for spontaneously resistant colonies on plates containing 1 mg/ml Zidovudine. Ten resistant clones were selected and screened for deletions in the TK gene by using the PCR primers SZ46-eTKKO20F and (5'-TGATGAAAAGTAGAACAGTCG-3') SZ49-eTK-KO789R (5'-ATCAAGACGCAGCACCATG-3')- One resistant clone was found to contain a deletion in the TK gene and was used for subsequent experiments. As a control for integrity of the DNA ofthis clone, its 16S rRNA-gene was amplified by using the primers SZ-16S-Ecoli993F (5'-ACATCCACGGAAGTTT-TCAG-3') SZ 16S-Ecoli454R (5'-CCGAAGGTTAAGC-TACCTAC-3').

[0046] Tumor Inoculation and Spore Administration. All animal experiments were overseen and approved by the Animal Welfare Committee of The John Hopkins University and were in compliance with university standards. Six- to 8-wk-old athymic *nu/nu* or BALB/c mice, purchased from Harlan Bioproducts for Science (Indianapolis), were used for tumor implantation studies. Five million cells were injected s-c, into the right flank of each mouse. Tumor volume was calculated as length X width^2 X 0.5, and mice were treated with *Clostridium novyi-NT* spores when tumors occupied ~250 mm^3. C. *novyi*-NT spores were prepared as described (15), and mice were i.v. injected with 300 million spores suspended in 250 μl of PBS.

Table 1. Bacterial strains imaged after i.m. injection.

Organism	Clinical significance
E. coli	Adult and infantile diarrhea, urinary tract infection, pneumonia, meningitis, and abscess
E. faecalis 49532	Nosocomial infection including vancomycin-resistant enterococci, urinary tract infection, endocarditis, abscess, and meningitis
S. pneumoniae 49619	Pneumonia, meningitis, sinusitis, osteomyelitis, and sepsis
S. aureus 29213 and 25293	Cellulitis, indwelling medical device infection, diabetic ulcer, postsurgical wounds, osteomyelitis, endocarditis, meningitis, mastitis, phlebitis, pneumonia, boils, furuncles, and impetigo
S. epidermidis F362	Endocarditis, cellulitis, urinary tract infection, and indwelling medical device infection

[0047] [^{125}I]FIAU Preparation. Briefly, 1-(2'-deoxy-2'-fluoro- β-D-arabinofuranoside)-uracil (300 μg, 1.22 mmol, Moravek) was dissolved in 170 μl of 2 M HNO_3. To this solution, 1.5 mCi (1 Ci = 37 GBq) of [I-^{125}] Nal-(ICN) was added and the contents heated at 130°C for 45 min. The reaction was quenched with 150 μl of HPLC mobile phase (20:79.9: 0.1% MeCN:H_2O:triethylamine). The resulting [^{125}I]FIAU was purified by reverse-phase HPLC by using two passages over a Phenomenex Luna C_{18} semiprep column (10 μm, 4.6 x 250 mm, Phenomenex, Torrance, CA) by using the above-mentioned isocratic mobile phase at a flow rate of 2 ml/min. The product was concentrated under reduced pressure and formulated in 0.9% physiological saline before sterile filtration through a 0.22-μm syringe filter. Formulations were kept at I mCi/ml to minimize the injection volume. The final radiochemical yield was = 50%, the radiochemical purity was >99%, and the specific radioactivity was>2,000 Ci/mmol.

[0048] Experimental Infections. *E. coli* strains or clinical isolates from the Johns Hopkins Hospital Microbiology Laboratory, including *Staphylococcus aureus* 29213 and 25923, *Streptococcus pneumoniae* 49619, *Enterococcus faecalis* 49532, and *Staphylococcus epidermidis* F362, were used to create experimental infections. Bacteria were grown to log phase in Mueller Hinton Broth with Cations (Remel, Lenexa, KS) or BBL Todd Hewitt Broth (Becton Dickinson). Localized infections were generated by injecting ≈ 1 x 10^9 *E. coli* and ≈1 x 10^8 of the other bacterial strains into mouse thighs. Morphologic examinations of the infectious lesions in the thighs of mice injected with the TK-deficient (TK-) strain of *E. coli* showed that they were as intense as those resulting from WT *E. coli.* To quantify the minimal number of bacteria required to generate signals upon imaging, mice were injected in the thigh with various amounts of *S. aureus* 25923. One hour later, the mice were killed, the muscles were harvested and homogenized, and the extracts spread on blood agar plates (Becton Dickinson) for colony counting. The plating efficiency of *S. aureus* 25923 grown in liquid media was found to be >95%.

[0049] In Vivo Imaging. Mice were injected with 225 μCi of [^{125}I]FIAU via the tail vein and imaged at various time points thereafter. Before imaging, mice were anesthetized via s.c. administration of acepromazine and ketamine. Each scan took ≈10 min with a dedicated small-animal single-photon emission computed tomography (SPECT)/computed tomography (CT) camera (Gamma Medica X-SPECT, Northridge, CA) in planar acquisition mode using a low-energy high-resolution (LEHR) parallel-hole collimator. For each bacterial strain used, at least two mice were injected and image. To obtain SPECT/CT images, animals were first scanned for ≈ 40 min by using a small-animal SPECT camera in tomographic acquisition mode, using two LEHR parallel-hole collimators. The animals then underwent CT by using appropriate fiducial markers that allowed coregistration.

[0050] Biodistribution. Imaging experiments showed that high signal-to-noise ratios in infectious foci could be con-

sistently obtained 24 h after [^{125}I]FIAU administration. Accordingly, this time point was chosen for detailed analyses. Biodistribution studies were performed in mice injected with ≈ 1x10^{8} *S. aureus* 25923 into one thigh. Six hours later, the mice were injected with 2 μCi of [^{125}I]FIAU, and, after another 24 h, the mice were killed, their organs were harvested, and radioactivity was determined.

[0051] **Susceptibility of E. *coli* to Nucleoside Analogs**. To determine whether endogenous bacterial TK could provide a reporter enzyme suitable for imaging, the susceptibility of *E. coli* to a variety of common nucleoside analogs was examined *in vitro.* Growth inhibition indicated that the nucleoside analog was a substrate for the *E. coli* TK and could thereby serve as an imaging reporter when radiolabeled. *E. coli* proved resistant to genciclovir and penciclovir but quite sensitive to FIAU and Zidovudine.

[0052] To determine whether the TK gene was responsible for this sensitivity, a derivative of *E. coli* in which the TK gene was deleted was created. PCR was used to demonstrate the absence of the TK gene in this derivative. The TK strain was moderately resistant to Zidovudine and highly resistant to FIAU. Because FIAU can be radiolabeled by using commercially available reagents and has been successfully used to image tumor cells transfected with HSV1-TK, it was elected to test its potential for imaging bacterial infections.

[0053] **In Vivo Imaging of *E. coli* Infections**. [^{125}I]FIAU was synthesized by standard methods and injected i.v. into animals 6 h after intramuscular inoculations of bacteria into the thighs of mice. Whole-body planar scintigram demonstrated the uptake of [^{125}I]FIAU within the thighs of mice harboring WT *E. coli* bacteria. Signals from the infectious lesions could be seen as early as 2 h after injection of [^{125}I]FIAU and were optimal ≈ 16 h after injection. Infections of the same mice inoculated with TK- *E. coli* in the opposite thighs showed no discernable uptake of [^{125}I]FIAU.

[0054] ***In Silico* Analysis of Bacterial TK**. An *in silico* assessment of TK genes in all 53 pathogenic bacteria whose genomes have been sequenced and made publicly available was preformed. This assessment revealed that each of these bacterial species possessed TK genes. Moreover, the homology between these TK genes was striking, with a clear consensus within the kinase catalytic domain. Each of the 53 bacteria contained at least 25 residues that were identical to those of the consensus. In contrast, this consensus sequence was not found in mammalian TKs, presumably accounting for the differential capacities of the mammalian enzymes to phosphorylate substrates such as FIAU.

[0055] **Imaging Infections Caused by Pathogenic Bacteria**. In light of this high sequence conservation, it was expected that [^{125}I]FIAU could be used as a tracer for pathogenic bacteria in general. Four patient-derived strains identified in the Johns Hopkins Hospital Microbiological Laboratory were selected to test this expectation. The identities and clinical properties of the selected strains are listed in Table 1. Infectious foci due to *E. faecalis, S. aureus, S. epidermidis,* and *S. pneumoniae* could all be readily imaged with [^{125}I]FIAU. Robust signals could be observed as early as 4 h after administration of [^{125}I]FIAU. Time-course studies showed that [^{125}I] remained in the infected tissues for long time periods, presumably because [^{125}I]FIAU was incorporated into the DNA of the bacteria- In contrast to the maintenance of this bacterial signal, the background signal in noninfected tissues gradually decreased, presumably because of continuing metabolism and excretion of [^{125}I]FIAU. This resulted in very high signal-to-noise ratios by 48 h after administration of the tracer.

[0056] For quantitative distribution measurements, mouse thighs were infected with *S. aureus* 25923 and [^{125}I]FIAU was administered 6 h later. Tissues were harvested after another 24 h and radioactivity measured. The infected muscle contained much higher levels of [^{125}I]FIAU than the other tissues, with the ratio of radioactivity in infected thighs to uninfected (contralateral) thighs exceeding 14:1.

[0057] To determine the minimal number of bacteria that could be imaged with this approach, various numbers of *S. aureus* 25923 were injected into mouse thighs. One hour later, the thigh tissue was excised, homogenized, and spread on blood agar plates. The 1-h time point was chosen because this was the earliest time point at which injections of [^{125}I] FIAU consistently produced discernable scintigraphic images of infectious foci. As few as 2x10^{6} colony-forming units per gram of muscle tissue produced discernable signals.

[0058] **Imaging Intratumoral Infections**. Imaging of infectious foci that were created by a process other than i.m. injection was performed. It has been shown that the spores of anaerobic bacteria, when systemically administered to mice, germinate only within tumor tissues. *C. novyi-NT* is a derivative of *C. novyi* that is devoid of its major systemic toxin gene and can therefore be safely delivered to animals. When injected i.v. into mice bearing tumors, <1% of the spores localize within tumors, the remainder being sequestered in the spleen and liver. The few spores localized within the tumor germinate rapidly, achieving a density of ≈ 10^{8} per gram of tissue by 24 h.

[0059] BA LB/c mice bearing CT-26 mouse colon tumors were treated with a single i.v. injection of *C. novyi-NT* and [^{125}I]FIAU was administered 24 h later. Serial images showed that the tumors could be visualized as early as 16 h after injection of tracer, with maximum uptake observed 24-48 h after injection of [^{125}I]FIAU. No uptake was observed in tumors that had not been treated with *C. novyi-NT.* Similar results were obtained in nude mice harboring HCT116 and HT-29 colon cancer xenografts.

[0060] Because planar y camera imaging is limited in its ability to reveal anatomical detail, SPECT/CT imaging was also performed. As observed in tumor-bearing rabbits treated with *C. novyi-NT* spores, areas of gas produced by the bacteria within CT-26 tumors could also be visualized upon CT, providing definitive evidence for infection. Coregistration

of CT images with corresponding SPECT images demonstrated that bacterial germination and tracer uptake were limited to the tumor region. Untreated mice showed no signs of gas or tracer uptake within their tumors.

Imaging of Bacteriolytic Therapy

[0061] The following example sets forth the imaging of bacteria sequestered within the hypoxic core of a tumor.

[0062] A hallmark of almost all solid malignancies is the presence of significant hypoxia and necrosis. The engineered anaerobic bacterium Clostridium novyi-NT can selectively target and destroy experimental tumors. In order to follow C. novyi-NT in vivo after injection into mice iodine-125 labeled 2'-Fluoro 2'-deoxy 5-iodouracil-β-D-arabinofuranoside (I-125 FIAU) was used.

[0063] *In vitro* susceptibility tests were performed on C. *novyi-NT* using 96 well plates containing two-fold serial dilutions of FIAU (Moravek Biochemicals) in reinforced clostridial media (Difco). Approximately, 10^5 -10^6 bacteria were inoculated into each well and incubated at 37 C overnight in an anaerobic chamber. C. *novyi*-NT growth was measured using an OD_{600}. In order to test the mechanism of action of FIAU on bacteria, the thymidine kinase (TK) gene in *E. coli* was knocked out. An *in vitro* FIAU susceptibility test comparing wild type *E. coli* with the mutant TK deficient *E. coli* was preformed. 96 well plates were used containing two-fold serial dilutions of *FIAU* in Lauria Broth. Approximately, 10^5-10^6 bacteria were inoculated into each well and incubated at 37 C overnight. Growth was measured using OD_{600}.

[0064] For *in vivo* studies, FIAU was labeled with I-125. The optimal time course for imaging mice harboring HCTI 16 colon cancer xenografts was determined empirically by varying the time of injection of FIAU in relation to C. *novyi*-NT. Biodistribution studies were performed in 12 athymic nu/nu mice harboring HCT116 xenografts ~350-400 mm^3, six of which were injected with 300 million *C. novyi-NT* spores 24 hrs prior to 2 uCi of I-125 FIAU. Eight hours after I-125 FIAU injection, all twelve mice were euthanized by cervical dislocation and the brain, lungs, heart, blood, small and large intestine, liver, kidneys, muscle and tumor were harvested and weighed. The activity in each tissue was measured using an automated gamma counter (LKB Wallace 1282 Compugamma CS Universal Gamma Counter). The percent-injected dose per gram of tissue (%ID/g) was calculated by comparison with samples of a standard dilution of the initial dose. At least three mice each harboring HCT116, HuCCT1 biliary cancer xenograft, or CT26 mouse colon tumors were imaged using a dedicated Gamma Medica X-SPECT small animal SPECT camera after being injected with *Clostridium novyi*-NT spores and 150-200 uCi of I-125 FIAU.

[0065] *Clostridium novyi*-NT had a minimum inhibitory concentration 50 (MIC_{50}) of ~20 ug/ml of FIAU. Wild type *E. coli* had a MIC_{50} of -10 ug/ml while the TK deficient *E. coli* were not inhibited at any concentration of FIAU tested. It was determined that C. *novyi*-NT germination could be optimally imaged under the following conditions: inject 300 million spores of C. *novyi*-NT and 24 hrs later inject 150-200 uCi of FIAU and image 8 hrs later.

[0066] The biodistribution studies revealed a 7:1 tumor:muscle ratio. All tumor types responsive to bacteriolytic therapy were able to be imaged using I-125 FIAU.

[0067] *In vitro* susceptibility tests suggested that *Clostridium novyi*-NT could potentially be imaged using I-125 FIAU. The putative mechanism off accumulation of FIAU within the bacteria is via phosphorylation of FIAU and its subsequent integration into bacterial DNA. Biodistribution and imaging data suggest that I-125 FIAU is a facile and robust method for imaging bacteriolytic therapy in mice.

Claims

1. 2'-Fluoro-2'-deoxy-1-beta-D-arabinofuranosyl-5-iodouracil (FIAU) suitable for imaging for use in a method of diagnosing a bacterial infection in a subject, wherein the imaging method is selected from the group consisting of planar gamma imaging and single photon emission computed tomography (SPECT),

2. FIAU for use in the method of claim 1, wherein the FIAU is ^{125}I-FIAU.

3. 2'-Fluoro-2'-deoxy-1-beta-D-arabinofuranosyl-5-iodouracil (FIAU) suitable for imaging for use in a method of diagnosis imaging bacteriolytic therapy, wherein the imaging method is selected from the group consisting of planar gamma imaging and single photon emission computed tomography (SPECT)

Patentansprüche

1. 2'-Fluor-2'-desoxy-1-beta-D-arabinofuranosyl-5-ioduracil (FIAU), das sich zur Bildgebung eignet, zur Verwendung in einem Verfahren zur Diagnose einer bakteriellen Infektion in einem Subjekt, wobei das Bildgebungsverfahren ausgewählt ist aus der Gruppe, bestehend aus planare Gamma-Bildgebung und Einzelphotonen-Emissionscom-

putertomographie (SPECT).

2. FIAU zur Verwendung in dem Verfahren gemäß Anspruch 1, wobei das FIAU ^{125}I-FIAU ist.

3. 2'-Fluor-2'-desoxy-1-beta-D-arabinofuranosyl-5-ioduracil (FIAU), das sich zur Bildgebung eignet, zur Verwendung in einem Verfahren zur diagnostischen Bildgebung bei bakteriolytischer Therapie, wobei das Bildgebungsverfahren ausgewählt ist aus der Gruppe, bestehend aus planare Gamma-Bildgebung und Einzelphotonen-Emissionscomputertomographie (SPECT).

Revendications

1. 2'-Fluoro-2'-désoxy-1-bêta-D-arabinofuranosyl-5-iodouracile (FIAU) approprié pour l'imagerie, utilisable dans un procédé de diagnostic d'une infection bactérienne chez un sujet, le procédé d'imagerie étant choisi dans le groupe constitué de l'imagerie gamma planaire et de la tomographie d'émission monophotonique (SPECT).

2. FIAU utilisable dans le procédé selon la revendication 1, dans lequel le FIAU est le ^{125}I-FIAU.

3. 2'-Fluoro-2'-désoxy-1-bêta-D-arabinofuranosyl-5-iodouracile (FIAU) approprié pour l'imagerie, utilisable dans un procédé d'imagerie diagnostique d'une thérapie bactériolytique, le procédé d'imagerie étant choisi dans le groupe constitué de l'imagerie gamma planaire et de la tomographie d'émission monophotonique (SPECT).

REFERENCES CITED IN THE DESCRIPTION

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