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Bioimaging predictors of rilpivirine biodistribution and antiretroviral activities

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HIGHLIGHTS

- Theranostic antiretroviral nanoparticles form intracellular macrophage drug depots.
- Coordinate SPECT/CT or MRI signal intensities and UPLC MS/MS measured ARV concentrations.
- IICP-MS for theranostic particle metal detection can validate ARV tissue concentrations.
- Bioimaging can provide a rational platform to predict ARV nanoparticle tissue biodistribution.
- Complex physicochemical biodistribution characteristics not predicted by available mathematical modeling can be affirmed through theranostic platforms.

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ABSTRACT

Antiretroviral therapy (ART) has changed the outcome of human immunodeficiency virus type one (HIV-1) infection from certain death to a life free of disease co-morbidities. However, infected people must remain on life-long daily ART. ART reduces but fails to eliminate the viral reservoir. In order to improve upon current treatment regimens, our laboratory created long acting slow effective release (LASER) ART nanoformulated prodrugs from native medicines. LASER ART enables antiretroviral drugs (ARVs) to better reach target sites of HIV-1 infection while, at the same time, improve ART's half-life and potency. However, novel ARV design has been slowed by prolonged pharmacokinetic testing requirements. To such ends, tri-modal theranostic nanoparticles were created with single-photon emission computed tomography (SPECT/CT), magnetic resonance imaging (MRI) and fluorescence capabilities to predict LASER ART biodistribution. The created theranostic ARV probes were then employed to monitor drug tissue distribution and potency. An intrinsically radiolabeled "^{111}Indium (^{111}In)", europium doped cobalt-ferrite particles and rilpivirine were encased in a polycaprolactone core surrounded by a lipid shell (^{111}InEuCF-RPV). Particle cell tissue distribution, and antiretroviral activities were sustained in macrophage tissue depots. ^{111}InEuCF-PCL/RPV particles injected into mice demonstrated co-registration of MRI and SPECT/CT tissue signals with RPV and cobalt. Cell and animal particle biodistribution paralleled ARV activities. We posit that particle selection can predict RPV distribution and potency facilitated by multifunctional theranostic nanoparticles.

1. Introduction

The development of effective antiretroviral therapy (ART) for human immunodeficiency virus type one (HIV-1) infection has dramatically reduced disease morbidities and mortality [1,2]. ART's success, over the past several decades, is substantive as evidenced by the

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Abbreviations:

^{111}In	^{111}indium
^{177}Lu	^{177}lutetium
AFM	atomic force microscopy
ART	antiretroviral therapy
ARV	antiretroviral
BD	biodistribution
Bq	Becquerel
BSA	bovine serum albumin
Ci	Curie
DCM	dichloromethane
DMSO	dimethyl sulfoxide
DSPE-PEG$_{2000}$	1,2-distearoyl-*sn*-glycero-3-phosphoethanolamine-*N*-[amino(polyethylene glycol)-2000]
EuCF	europium cobalt ferrite
FACS	fluorescence-activated cell sorting
FOV	Field of view
GALT	gut-associated lymphoid tissue
HIV-1	human immunodeficiency virus type 1
ICP-MS	inductively coupled plasma mass spectrometry
IV	intravenous
kVp	kilovoltage peak
LASER ART	long acting slow effective release antiretroviral therapy
MBq	mega becquerel
MDM	human monocyte-derived macrophage
MOI	multiplicity of infection
MRI	magnetic resonance imaging
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
NanoART	nanoformulated antiretroviral therapy
NRPV	nanoformulated rilpivirine
PBS	phosphate-buffered saline
PC	phosphatidylcholine
PCL	polycaprolactone
PDI	polydispersity index
PFA	paraformaldehyde
PK	pharmacokinetic
ROI	region of interest
RPV	rilpivirine
RT	reverse transcriptase
SPECT/CT	single photon emission computed tomography and computed tomography
TEM	transmission electron microscopy
μCi	Microcurie
UPLC-MS/MS	ultraperformance liquid chromatography tandem mass spectrometry
UPLC	ultra-performance liquid chromatography
XRD	X-ray diffraction

drug's ability to efficiently reduce circulating plasma HIV-1 loads to undetectable levels. In so doing ART protects CD4$^+$ T cells and reduces comorbid disease [3,4]. However current treatment limitations include drug pharmacokinetics (PK) and biodistribution profiles, viral mutations and drug toxicities [5,6]. All affect optimal therapeutic efficacy [7]. Additionally, short antiretroviral (ARV) drug half-lives necessitate daily dosing and strict regimen adherence [8]. Reduced ARV access to virus target tissues can also affect the maintenance of drug levels at action sites and the ability to contain CD4 + T cell infection [9]. Innate inflammatory and adaptive immune responses tied to HIV-1 infection continue despite therapeutic drug regimens, leading to diabetes, osteoporosis, cardiovascular diseases and neurocognitive disorders [10–15]. Each and all of these limitations have led to the development of longer acting nanoformulated ART [16,17] and recently to the chemical synthesis of lipophilic prodrug nanocrystals coined long acting slow effective release LASER ART. The noted scientific advances have further extended drug ARV half-lives and potencies [18,19]. LASER ART rests on four pillars; creation of hydrophobic prodrug nanocrystals, enhanced drug lipophilicity associated with improved cell membrane drug penetration, slow drug release and hydrolysis, and facilitated viral reservoir drug penetrance. These transformative technologies create ARVs that best penetrate viral reservoirs and increase the drug's apparent half-life creating medicines that maximally restrict viral growth [20]. However, to realize the potential of long acting tissue viral reservoir penetrating ARVs, the drugs' pharmacokinetic (PK) and pharmacodynamic (PD) profiles must be optimized to minimize on and off-target effects. Moreover, while mathematical descriptions of long-acting nanoformulated drug distribution have been developed, these cannot reflect the diversity of chemical alterations and physical characteristics now required for LASER ART nanoformulations [21]. To this end, multimodal theranostic ARVs were made as an effective descriptor for drug biodistribution.

In the current report, highly stable europium cobalt-ferrite (EuCF) nanocrystals, intrinsically radiolabeled with ^{111}indium were loaded with the ARV drug, rilpivirine (RPV). The newly minted drug particles facilitated real-time non-invasive detection of drug and antiretroviral drug biodistribution and screening. These findings forge a significant improvement over first generation EuCF-PCL particles [22] by extending ARV encapsulations and bioimaging capabilities to assess drug biodistribution. Indeed, these particles improve signal intensities from both single photon emission computed tomography (SPECT/CT) and magnetic resonance imaging (MRI) in order to accurately predict drug distribution. Validation of this predictive potential is provided by ultra-performance liquid chromatography tandem mass spectrometry (UPLC-MS/MS) and inductively coupled plasma mass spectrometry (ICP-MS) made possible by the metals encased in the particles. The data demonstrate, for the first time, that theranostics ARV particles can provide a platform to facilitate clinically effective LASER ART development.

2. Materials and methods

The reagents used in this report, animal sources, purchasing and handling, preparation of RPV freebase, manufacturing of nanoformulated RPV (NRPV) and NRPV-CF®633, isolation of human monocytes from human immunodeficiency virus types 1,2 (HIV-1,2) and hepatitis B seronegative donors and cultivation of monocyte-derived macrophages (MDM) are described in the supplemental data section and follow published protocols [23]. The physiochemical methods used in this report for particle characterization and stability, UPLC-MS/MS and ICP-MS parameters for drug and metal concentration determinations were performed as previously reported [24,25] and are briefly described in the supplemental information.

2.1. Preparation of radiolabeled multimodal theranostic particles

Monodisperse and high quality crystalline EuCF nanocrystals were synthesized and characterized via state of the art techniques as previously described [22]. Production of chelate free, non-leachable and highly stable radiolabeled ^{111}InEuCF nanocrystals was accomplished by intrinsic co-doping of the ^{111}In radionuclide along with europium into the cobalt ferrite lattice structure. In a typical synthesis ~555 MBq (~15 mCi) of $^{111}InCl_3$ was mixed with 750 μL of benzyl alcohol, vortexed and stirred for 30 min at room temperature. The mixture was slowly transferred into a Teflon container for hydrothermal synthesis. The formulations were prepared with cobalt acetylacetonate, iron (III)