Methods 127 (2017) 62–68

Contents lists available at ScienceDirect

Methods

journal homepage: www.elsevier.com/locate/ymeth

Non-invasive three-dimensional imaging of *Escherichia coli* K1 infection using diffuse light imaging tomography combined with micro-computed tomography

Luci A. Witcomb [a], Julie Czupryna [b], Kevin P. Francis [b], Gad Frankel [c], Peter W. Taylor [a,*]

[a] University College London School of Pharmacy, 29-39 Brunswick Square, London WC1N 1AX, UK
[b] PerkinElmer, 68 Elm Street, Hopkinton, MA 01748, USA
[c] MRC Centre for Molecular Bacteriology and Infection, Imperial College London, South Kensington Campus, London SW7 2AZ, UK

ARTICLE INFO

Article history:
Received 26 January 2017
Received in revised form 11 April 2017
Accepted 10 May 2017
Available online 15 May 2017

Keywords:
Bioluminescence imaging
Escherichia coli K1
Neonatal systemic infection
Gastrointestinal colonisation
Neonatal meningitis
Age-dependent susceptibility to infection

ABSTRACT

In contrast to two-dimensional bioluminescence imaging, three dimensional diffuse light imaging tomography with integrated micro-computed tomography (DLIT-μCT) has the potential to realise spatial variations in infection patterns when imaging experimental animals dosed with derivatives of virulent bacteria carrying bioluminescent reporter genes such as the *lux* operon from the bacterium *Photorhabdus luminescens*. The method provides an opportunity to precisely localise the bacterial infection sites within the animal and enables the generation of four-dimensional movies of the infection cycle. Here, we describe the use of the PerkinElmer IVIS SpectrumCT *in vivo* imaging system to investigate progression of lethal systemic infection in neonatal rats following colonisation of the gastrointestinal tract with the neonatal pathogen *Escherichia coli* K1. We confirm previous observations that these bacteria stably colonize the colon and small intestine following feeding of the infectious dose from a micropipette; invading bacteria migrate across the gut epithelium into the blood circulation and establish foci of infection in major organs, including the brain. DLIT-μCT revealed novel multiple sites of colonisation within the alimentary canal, including the tongue, oesophagus and stomach, with penetration of the non-keratinised oesophageal epithelial surface, providing strong evidence of a further major site for bacterial dissemination. We highlight technical issues associated with imaging of infections in new born rat pups and show that the whole-body and organ bioburden correlates with disease severity.

Contents

1. Introduction . . . 63
2. DLIT-μCT imaging: theory and practice . . . 63
   2.1. DLIT theory . . . 63
   2.2. DLIT-μCT in practice . . . 63
      2.2.1. Limitations and way forward . . . 64
3. Rodent model of neonatal *E. coli* K1 infection . . . 64
   3.1. Preparation and use of neonatal rat pups for DLIT-μCT imaging of infection . . . 67
   Funding . . . 67
   Appendix A . . . 67
   References . . . 67

* Corresponding author.
E-mail addresses: luci_law@hotmail.com (L.A. Witcomb), Julie.Czupryna@PERKINELMER.COM (J. Czupryna), Kevin.Francis@PERKINELMER.COM (K.P. Francis), g.frankel@imperial.ac.uk (G. Frankel), peter.taylor@ucl.ac.uk (P.W. Taylor).

http://dx.doi.org/10.1016/j.ymeth.2017.05.005
1046-2023/

1. Introduction

Escherichia coli and *Streptococcus agalactiae* (the Group B streptococcus) are the leading causes of systemic life-threatening bacterial infections in the new born infant, especially among premature, low-birth-weight neonates [1,2]. These opportunistic pathogens colonize mucosal surfaces after acquisition at or soon after birth and in a minority of cases breach the mucosal barrier to engender a rapidly progressive infection which the vulnerable infant is ill-equipped to counter. The large majority of *E. coli* strains causing neonatal systemic infections express the K1 capsule, a homopolymer of α-2,8-linked *N*-acetylneuraminic acid that constitutes the outermost layer of the cell and protects the bacteria from the host's immune clearance mechanisms [3]. *E. coli* K1 is an adept colonizer of the neonatal gastrointestinal (GI) tract from where it may translocate into the systemic circulation (sometimes causing an unwanted immune response, termed sepsis), enter the central nervous system and gain access to the meninges, producing meningitis [4,5]. *E. coli* K1 systemic infections display a strong age-dependency: the bacteria are benign, common constituents of the adult GI microbiota [6] but are potentially lethal during the first weeks of life [1,7].

Neonatal bacterial infections are medical emergencies and it is consequently difficult to obtain insights into the underlying pathogenic mechanisms of the invading bacteria from human studies alone. Importantly, key features of *E. coli* K1 systemic infections, including the age dependency, can be replicated in the neonatal rat by oral administration of bacteria, a procedure that frequently initiates stable colonisation of the GI tract [8,9]. Rat pups colonized in the first few days *postpartum* develop lethal systemic infection as the colonizing *E. coli* K1 translocate from the lumen of the GI tract to the blood circulation to cause a persistent bacteraemia [9,10]; they then elicit infection in multiple organs, including the brain [11,12]. Bacteria appear to enter the cerebrospinal compartment predominantly at the choroid plexus and penetrate superficial brain tissue [11], where they give rise to localized inflammation through cytokine-induced pathways [13]. A week after birth, the rats become refractory to systemic infection, even though *E. coli* K1 GI colonisation may persist beyond this time point [9] due to maturation of the protective mucus layer lining the GI tract [14].

Most studies with the rodent model have relied upon culling of infected animals at various stages after initiation of colonisation with *E. coli* K1 and the data provide only a "snapshot" of disease progression with time. The presence of the pathogen in selected tissues is generally revealed by viable counting, or by histological or histochemical staining of tissue sections. Real-time non-invasive imaging in the whole organism has the capacity to reveal complex patterns of dissemination from colonisation sites and the dynamic process of infection over time in individual animals. The optical imaging of bioluminescent signals from recombinant pathogenic bacteria expressing luciferase such as that provided by the *lux*CDABE operon from the terrestrial bacterium *Photorhabdus luminescens*, in combination with *ex vivo* organ analysis, has been widely adopted to furnish fresh insights into processes of infectious disease [15–18]. With conventional 2D bioluminescence imaging, the location of the signal is inferred from its location at the animal surface; a quantitative 3D reconstruction of the source of the signal may be determined by diffuse light imaging tomography (DLIT) [19]. Unambiguous non-invasive localization of bioluminescent signals from small animals without the need for organ recovery and *ex vivo* quantification can at present only be achieved using multi-modality imaging. Here, we review our recent use of a combination of DLIT co-registered with integrated micro-computed tomography (μCT) imaging to probe the infection dynamics of infection-susceptible neonatal rat pups colonized with a bioluminescent derivative of *E. coli* K1 strain A192PP. We discuss the technical challenges posed by these procedures and highlight the advantages and limitations of the technique.

2. DLIT-μCT imaging: theory and practice

Diffuse light imaging tomography utilizes a charge-coupled device (CCD) camera and band-pass emission filters to obtain a series of multi-spectral 2D images of bioluminescent sources within a living subject. The DLIT algorithms also require a measurement of the subject surface topography. Here the μCT scan serves a dual purpose: the air-tissue boundary aids in surface topography generation and provides integrated anatomical co-registration with the optical source(s).

2.1. DLIT theory

Diffuse tomographic analysis can be a rigorous approach to provide source depth and intensity information, sometimes involving complicated multi-view instrumentation [20–22] or *a priori* knowledge of allowable source distribution [23,24]. To reduce both imaging and computational time, PerkinElmer's IVIS instrumentation uses multispectral 2D image acquisitions and diffuse luminescence tomographic algorithms [19,25]. Tissue absorption of light across the 500–750 nm spectrum, mainly by haemoglobin, is significantly decreased at the red end of the spectrum compared to the blue/green end of the spectrum [26]. Therefore, the light output of an *in vivo* bioluminescent source depends on the depth of tissue it must pass through, as well as its wavelength. The DLIT algorithms exploit this property; in 2D, shallow sources appear brighter than deep sources at the blue/green end of the spectrum, while deep sources appear brighter than shallow sources at the yellow/red end of the spectrum. The DLIT algorithms produce a 3D reconstruction of the bioluminescent source(s) using a non-negative least squares optimization.

DLIT also requires a measurement of the surface topography of the imaging subject in order to convert exhibited light emission into a photon density map. In the instance of DLIT-μCT, the surface topography is derived from the μCT scan itself by delineation of the air-tissue boundary. The surface topography, when combined with the series of filtered 2D bioluminescence images, results in an improved resolution of source parameters by providing uniqueness to the algorithms.

2.2. DLIT-μCT in practice

PerkinElmer's IVIS SpectrumCT includes all hardware and software required for DLIT-μCT. The system consists of a cooled (−90'C), integrating CCD camera that is placed above a light-sealed imaging chamber (Fig. 1). The heated imaging stage is mobile along the optical axis to adjust field of view and the emission filter wheel contains 18 filters with 20 nm bandwidths. In order to keep a manageable system footprint, the X-ray source and complementary metal oxide semiconductor detector are fixed perpendicular to the optical axis, while the μCT projections are captured by rotating the subject on a unique turntable arrangement (Fig. 1). DLIT-μCT acquisition consists of a μCT scan followed by a sequence of multispectral 2D bioluminescence images. Image acquisition is performed using PerkinElmer's Living Image® software. A standard single animal μCT scan was employed to provide a quality volume for co-registration while also keeping the X-ray dose low (53 mGy).

Bioluminescence images are acquired with various emission filters in place and typically 4–6 emission filters are chosen that