Available online at www.sciencedirect.com

SCIENCE DIRECT®

Advanced Drug Delivery Reviews 57 (2005) 43–61

Advanced DRUG DELIVERY Reviews

www.elsevier.com/locate/addr

Intracellular trafficking pathways and drug delivery: fluorescence imaging of living and fixed cells

Peter Watson[a], Arwyn T. Jones[b], David J. Stephens[a,*]

[a]*Department of Biochemistry, University of Bristol, School of Medical Sciences, University Walk, Bristol, BS8 1TD, UK*
[b]*Welsh School of Pharmacy, Cardiff University, Redwood Building, Cardiff CF10 3XF, UK*

Received 8 March 2004; accepted 5 August 2004
Available online 28 September 2004

Abstract

Cellular processes depend on the fidelity of intracellular membrane traffic. Lipids, proteins, receptor ligands and solute molecules are trafficked to distinct compartments within the cell through both the biosynthetic and endocytic pathways. An appreciation of these pathways is vital for a complete understanding of intracellular drug delivery. Recent advances in fluorescence imaging have facilitated the analysis of these pathways in great detail. It is now possible to gain insight into the real-time dynamics of cellular components and macromolecular pharmacological agents as they are delivered into and traffic within single cells. Here, we discuss the analysis of intracellular drug delivery from the perspective of fluorescence imaging of both living and fixed cells. This review aims to cover trafficking pathways, markers for subcellular compartments, fluorescent labels for intracellular structures and pharmacological agents and relevant recent developments in imaging technology. In particular, we shall focus on the application of live cell imaging to the study of endocytic drug delivery.

Keywords: Endocytosis; Membrane traffic; Protein sorting; Live cell imaging; GFP

Contents

* Corresponding author. Tel.: +44 117 928 9955; fax: +44 117 928 8274.
E-mail address: david.stephens@bristol.ac.uk (D.J. Stephens).

0169-409X/$ - see front matter © 2004 Elsevier B.V. All rights reserved.
doi:10.1016/j.addr.2004.05.003

1. Introduction

The organization of mammalian cells is highly complex. Compartmentalization is maintained through the organization of the cytoplasm into multiple intracellular structures that have their own distinct identity and function. Proteins, lipids and solutes are transported between these compartments in an ordered manner by membrane traffic pathways [1]. The biosynthetic pathway delivers newly synthesised proteins through the cell to their final destination; this might be lysosomes, the Golgi apparatus or, for secretory proteins, the extracellular space. The endocytic pathway controls the transport of molecules, such as receptor ligands and solutes entering the cells [2]. These pathways are obviously interdependent and many of the compartments and protein machines are common to both. An understanding of the trafficking itinerary of pharmacological agents delivered to cells via endocytic pathways requires knowledge of intracellular membrane traffic, the molecular dynamics of the organelles concerned and an appreciation of the capabilities and limitations of state-of-the-art technology that is utilised to increase knowledge in this field [2–6].

Individual compartments within the endocytic pathway are continually in flux [2]. The steady-state distribution of compartment "markers" is maintained through a balance of targeting, retention and retrieval mechanisms. A critically important concept is that there are no truly "resident" components of compartments. Proteins and lipids are continually moving between compartments and the balance of traffic between them defines the steady-state localization. Trafficking is not 100% efficient leading to a broad distribution across compartments. In the context of drug delivery, the endocytic pathway is most relevant and it is on this that we shall focus this review. Modern drug design requires the delivery of drugs to a specific location in the cell. Their site of action may be exclusively within the inside of a range of organelles or the agent may require escape from endosomes (endosomolytic) or lysosomes (lysosomolytic) prior to reaching its target. Clearly, different systems are required to promote early escape from endosomes compared with a requirement for delivery to and utilisation of a lysosomal protease for release of drug into the cytosol. In most cases, it is also important that the drug is not exported from the cell following its entry into recycling or secretion pathways. The plasma membrane and downstream organelles, therefore, pose major barriers to effective drug delivery but increasing knowledge of endocytosis and agents that perturb or manipulate endocytic pathways are opening new avenues for more efficient intracellular delivery.

Endocytosis is a term used to describe multiple methods of internalisation. These include clathrin-dependent endocytosis, clathrin-independent endocytosis, macropinocytosis and internalisation via caveolae [7,8]. Many agents that are delivered to cells will also be delivered to compartments outside of the