Pharmacological Research 117 (2017) 94–102

Contents lists available at ScienceDirect

Pharmacological Research

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

Review

Passive drug permeation through membranes and cellular distribution

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ARTICLE INFO

Article history:
Received 9 June 2016
Accepted 22 November 2016
Available online 24 November 2016

Keywords:
Subcellular distribution
Membrane potential
pH Partitioning
Fick's
Nernst-Planck
Tissue targeting

ABSTRACT

Although often overlooked, passive mechanisms can lead to significant accumulation or restriction of drugs to intracellular sites of drug action. These mechanisms include lipoidal diffusion of ionized species and pH partitioning according to the electrochemical potential and to pH gradients that exist across subcellular compartments, respectively. These mechanisms are increasingly being exploited in the design of safe and effective drugs for the treatment of a wide variety of diseases. In this work, the authors review these efforts and the associated passive mechanisms of cellular drug permeation. A generic mathematical model of the cell is provided and used to illustrate concepts relevant to steady-state intracellular distribution. Finally, the authors review methods for estimating determinant parameters and measuring the net effect at the level of unbound intracellular drug concentrations.

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1. Introduction

The pharmacological and toxicological actions of many drugs occur at the intracellular level. As such, it is important to understand how drugs enter and accumulate into these sites of action. Of course, the term "intracellular" belies the heterogeneity of potential sites of action within the cell. For example, each organelle represents a physiologically unique space (e.g. surface area, volume, pH, membrane potential and membrane composition) which can differ across various types of cells. Together with the physiochemical properties of drug molecules, these physiological properties determine the rate and extent to which drugs distribute to these intracellular sites of action. Though often overlooked, this interplay can have profound implications for the achievement of drug design objectives (e.g. potency, selectivity, drug-resistance).

Drug molecules enter and accumulate into intracellular locations by both lipoidal diffusion and protein mediated transport [1–3]. It is important to understand both processes in drug design since substantial concentration gradients can be achieved through both routes of distribution. It is generally well-appreciated that active uptake or efflux of drugs via proteins embedded in the cell membrane can lead to concentration gradients across the cell. Energy for unidirectional active transport against a concentration gradient can be provided directly from ATP hydrolysis (e.g. Pgp, BCRP, MRPs1-6, BSEP) or secondary to co-transport of ions down electrochemical gradients maintained by other ATP-

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http://dx.doi.org/10.1016/j.phrs.2016.11.028
1043-6618/

dependent processes (e.g. OAT1, OAT2, OAT3, MATE1, MATE2K, OCTN1, PEPT1, NTCP) [4]. In addition, concentrative uptake can occur via other transporters which utilize mechanisms and energy sources that are less well-understood (e.g. OATP1B1, OATP2B1, OATP1B3, OCT1, OCT2, OCT3). While a holistic view of cellular drug distribution must consider the potential effect of such transporters, the scope of this review is intentionally focused upon concepts related to distribution via lipoidal diffusion. Although the relative contribution is subject to some debate, lipoidal diffusion is generally regarded to be relevant and operational in the permeation of drugs across cell membranes [2,3,5]. Most often, this is assumed to follow the concentration gradient at a rate commensurate with the molecule's lipophilicity. As such, this mechanism is not normally considered to be a means of achieving asymmetric exposure across intracellular spaces. In fact, a lack of asymmetry in intracellular-to-extracellular unbound concentrations is often interpreted as indirect evidence of passive lipoidal permeation [6,7]. However, cellular distribution via lipoidal diffusion can result in intracellular accumulation in accordance with physiological pH gradients via partitioning of ionized species that have negligible lipoidal permeability. For example, dramatic (>100-fold) accumulation of cationic amphiphilic drugs in acidic endosomal/lysosomal compartments has been well established [8]. As many hydrolytic enzymes exist and exert their physiological function within the acidic pH of the endosome/lysosome, this accumulation can have profound implications for the design of safe and effective drugs [9]. Many of these enzymes represent potential molecular targets for correcting the pathophysiologies of protein clearance that have been associated with disorders such as Alzheimer's, frontotemporal dementia, Parkinson's, Huntington's disease and osteoporosis [10,11]. For example, Black and Percival have demonstrated that lysosomal pH partitioning affected both the potency and selectivity of novel inhibitors of Cathepsin K under development for the treatment of osteoporosis [11]. Lysosomal accumulation of several antipsychotic and antidepressant drugs has also been shown to contribute to antidepressive effects through intralysosomal acid sphingomyelinase inhibition [12]. pH-dependent lysosomal sequestration of weak bases has also been implicated in enhancing the efficacy of anti-infective agents (e.g. chloroquine and primaquine) [13,14] and the propensity to produce potentially toxic accumulation of phospholipids [15]. Finally, perturbations in the local pH environment as occurs in disease states may have pharmacological and pharmacokinetic implications that extend beyond lysosomal sequestration. For example, the acidic extracellular and slightly alkaline intracellular pH of tumors has been associated with physiological resistance to weakly-basic chemotherapeutics like doxorubicin [16].

While lipoidal diffusion is commonly assumed to occur exclusively via the neutral species, ionized species can also permeate via lipoidal diffusion, though at rates that are often 3–4 orders of magnitude lower [17,18]. An important consideration for permeation of ionized species is that it follows the electrochemical gradient according to Nernst-Planck [19,20]. In cases where the ionized species are present in such high relative abundance that the permeability penalty is negated, significant asymmetry in intracellular-to-extracellular unbound drug may be observed due to the inward negative membrane potential that is maintained across many membranes in mammalian cells (e.g. accumulation of cations and exclusion of anions). For example, this phenomenon has been implicated in the dramatic accumulation (>100 fold) of cationic molecules in mitochondria in accordance with the relatively large membrane potential difference (i.e. approximately −150 mV) in this organelle [21,22]. This propensity for mitochondrial accumulation has been exploited in the design of cationic drugs for the treatment of cancer and a wide range of degenerative diseases involving the mitochondria [23–25]. Conversely, the general intracellular exclusion of anionic drugs by the same mechanism coupled with substrate affinity for OATP-mediated uptake has been exploited in the design of hepatoselective glucokinase activators [26].

Clearly, many opportunities exist to better understand and optimize the cellular distribution of molecules. In support of these efforts, mathematical models have been constructed which account for the intracellular distribution of both the ionized and neutral species of ionizable molecules according to all of the aforementioned mechanisms of passive distribution [9,26–29]. In the next section, we will review the theoretical aspects of passive lipoidal diffusion culminating with the description of a generic cell model of passive lipoidal drug permeation. The cell model will be used to generate simulations illustrative of key concepts related to the steady-state accumulation of ionizable drugs within cells. The authors will also provide an overview of experimental methods for generating estimates of key model parameters and for measuring drug accumulation in cells. While much work remains to develop a quantitatively-validated experimental and mathematical framework, the concepts and methods discussed in this work provide a useful starting place and are conceptually applicable in the rational design of safe and effective drugs.

2. Kinetics of passive cellular permeability

A detailed mathematical treatment for cellular distribution of ionizable molecules (i.e. both the neutral and ionized forms) in accordance with the physiochemical properties of a drug (permeability, pKa), the physiological properties of the cell (pH, membrane potential, surface area, volume), Fick's law (diffusion of neutral species), the Nernst-Planck relationship (diffusion of charged species) and the Henderson-Hasselbalch relationship (ionization state) is described in the supplementary material. In this framework, the surface-area normalized, net flux of monoprotic acids and bases can be described by Eqs. (1) and (2), respectively. In these equations, P_n and P_i represent the intrinsic permeability of the neutral and ionized forms of the drug. Further, fu_d, C_d, fu_r and C_r represent the unbound fraction and total drug concentration in the donor (d) and receiver (r) sides. For acids, H_d and H_r represent the fraction ionized in the donor and receiver side. K_d and K_r represent the neutral fraction in the donor and receiver side.

$$J = P_n\left(fu_d K_d C_d - fu_r K_r C_r\right) - \frac{\nu P_i\left(fu_r H_r C_r - e^{-\nu} fu_d H_d C_d\right)}{1 - e^{-\nu}} \quad (1)$$

For bases, H_d and H_r represent the neutral fraction in the donor and receiver side. K_d and K_r represent the fraction ionized in the donor and receiver side.

$$J = P_n\left(fu_d H_d C_d - fu_r H_r C_r\right) - \frac{\nu P_i\left(fu_r K_r C_r - e^{-\nu} fu_d K_d C_d\right)}{1 - e^{-\nu}} \quad (2)$$

In both Eqs. (1) and (2), ν represents the following function (Eq. (3)). Where z = −1 and 1 for acids and bases, respectively.

$$\nu = \frac{zF\Delta\phi}{RT} \quad (3)$$

In Eq. (3), F,$\Delta\phi$, R and T represent the Faraday constant, membrane potential, gas constant and temperature, respectively.

Accounting for the volume (V_r) contained within a given surface area of cell membrane (SA_d), one can obtain the following general pharmacokinetic equations for distribution of an acidic (Eq. (4)) or basic (Eq. (5)) drug into a cellular subcompartment.

$$V_r \frac{dC_r}{dt} = SA_{dr} P_n\left(fu_d K_d C_d - fu_r K_r C_r\right) - \frac{\nu P_i SA_{dr}\left(fu_r H_r C_r - e^{-\nu} fu_d H_d C_d\right)}{1 - e^{-\nu}} \quad (4)$$

$$V_r \frac{dC_r}{dt} = P_n SA_{dr}\left(fu_d H_d C_d - fu_r H_r C_r\right) - \frac{\nu P_i SA_{dr}\left(fu_r K_r C_r - e^{-\nu} fu_d K_d C_d\right)}{1 - e^{-\nu}} \quad (5)$$