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What would be the observable consequences if phospholipid bilayer diffusion of drugs into cells is negligible?

Douglas B. Kell

School of Chemistry and Manchester Institute of Biotechnology, The University of Manchester, 131 Princess Street, Manchester M1 7DN, UK

For drug transport across (i.e., through) an intact biological membrane, two main routes are possible: drugs may cross (i) through the phospholipid bilayer portion of the membrane, and/or (ii) via proteinaceous pores or transporters. Perhaps surprisingly, there is in fact no direct scientific evidence that the first of these takes place at any significant rate because, in the experiments performed to date, it has neither been varied as an independent variable nor measured directly as a dependent variable. Using a standard hypothetico-deductive framework, I assess the intellectual and observable consequences of assuming that, for drugs, phospholipid bilayer diffusion is negligible – 'PBIN' – (i.e., may be neglected, relative to transporter-mediated transmembrane fluxes). Predictions and postdictions of the PBIN hypothesis are not refuted by available experimental evidence.

Introduction

It is easy in science (and other fields) to take the 'textbook' or 'standard' view of a system or a process as a given, without necessarily bothering to look into the actual experimental evidence (if any) on which it might have been based. However, the history of science is full of examples in which a theory or scientific viewpoint, once widely believed, was supplanted by one that had better explanatory and predictive power (i.e., of both existing and novel data) [1,2], albeit often in the face of considerable rearguard action [3]. Sometimes this change in thinking was driven by the acquisition of new evidence, but in some cases the main driver was simply the reinterpretation of existing evidence – which newer ideas must also necessarily explain.

This widespread acceptance of a particular view seems to have come to pass in the field of cellular drug uptake, where it is commonly believed that (leaving aside endocytosis) most drugs can and do enter intact cells by passing fully through whatever unhindered phospholipid bilayer portions might exist, and – because biomembranes have lipophilic interiors – at a rate that correlates (in some fashion) with log D (see Glossary) or log P. I note, of course, that correlations of two dependent variables show nothing, except that they exist in the systems stated (while one may be causal of the other, both might instead be effects of a separate cause, or indeed entirely unrelated to each other mechanistically). However, I know of no paper in which phospholipid bilayer transport in intact biological cells has ever been varied as an independent variable (and without changing any relevant transporter-mediated uptake), nor of any in which the actual passage of a drug diffusing through the bilayer has ever been measured directly. It therefore follows that the concrete, data-driven evidence that we have that this takes place *in vivo* is, in fact, precisely zero.

What has of course been done many times is that the transfer of drugs across biological membranes has been measured, and it has been assumed or stated that this occurred via the bilayer. Obviously, assuming or stating something as a mechanism when it has not actually been measured directly can be rather hazardous, and indeed does not count as experimental evidence for it at all. I contrast the situation with experiments in which the activity of genetically encoded protein transporters has been varied independently (Figure 1).

Glossary

BDDCS: the Biopharmaceutics Drug Disposition Classification System, a 2×2 matrix that can be used to classify drugs into four main classes depending on whether their solubility and/or extent of metabolism is 'high' or 'low'; see [54].

KNIME: the Konstanz Information Miner (http://www.knime.org), a freely available workflow environment for creating and running cheminformatics and related workflows.

log D: the logarithm of the distribution coefficient, D. D is the ratio of the sum of the concentrations of all forms of a compound (ionised plus non-ionised) in each of two phases, typically 1-octanol and an aqueous buffer, whose pH must be specified.

log P: the logarithm of the partition coefficient, P. P is a measure of the hydrophobicity of a molecule; log P is the logarithm (base 10) of the ratio of the concentration of a solute molecule in an organic solvent, usually 1-octanol, to that of the non-ionised form of the same molecule in water.

Corresponding author: Kell, D.B. (dbk@manchester.ac.uk).

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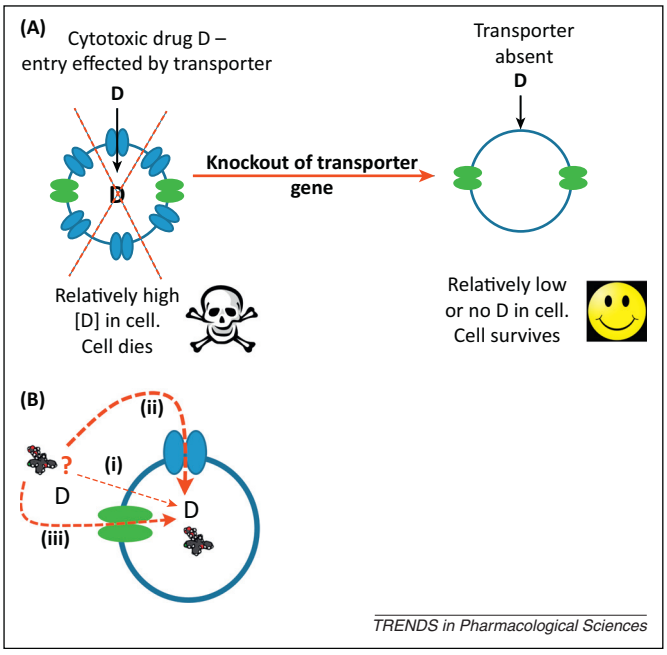

Figure 1. Varying the mechanistic basis for drug uptake as an independent variable causes predictable and measurable consequences. **(A)** This is straightforwardly done for proteinaceous transporters, for instance by knocking out the relevant genes, as illustrated for the case of a cytotoxic drug whose entry to the cell is via a transporter coloured in blue. Other types of transporters (in green) exist but are not used by the drug. Precisely these types of experiments have been carried out for antimetabolites (e.g., [15]) and candidate anticancer drugs [22], leading to straightforward and accurate inferences about the role of such transporters in the uptake of the target drug(s). The uptake (and effectiveness) of many other drugs has also been found to covary with the expression levels of particular transporters (e.g., gemcitabine and ENT1 [46]). **(B)** By simply observing the extracellular disappearance or intracellular uptake of a drug, one may seek to infer that the transport of the externally added drug entering a cell occurs (i) via its transport through the bilayer. Obviously, other interpretations of the mechanisms of this drug transport are possible, however, such as transport through the blue (ii) or green (iii) transporters (or both). Thus, no logically correct inference is possible if neither bilayer diffusion nor the presence or activity of relevant transporters are varied in known ways, nor measured directly. We are aware of no experiments in which phospholipid bilayer transport has been varied independently in this way, and under circumstances where any changes in competing transporter-mediated uptake have also been monitored (figures not drawn to scale).

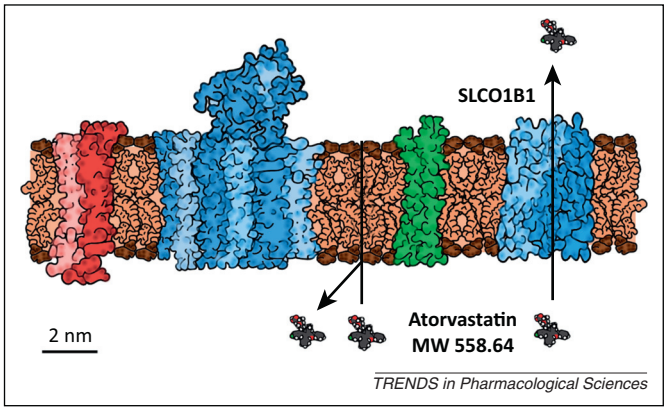

Figure 2. A typical biomembrane, drawn approximately to scale. The illustration uses common protein:phospholipid mass ratios, and shows a typical drug (atorvastatin), also drawn to scale. Phospholipids are in brown, proteins in other colours. The question arises as to whether any of the drug passes through those phospholipid bilayer regions that remain unaffected by the presence of the proteins. According to this Opinion, it is much easier to account for many types of observations if one recognises that in fact the overwhelming bulk of transmembrane drug transport occurs via proteinaceous pores and transporters (that are involved in intermediary metabolism) and not through phospholipid bilayers. Abbreviation: SLCO1B1, solute carrier organic anion transporter family, member 1B1.

In a separate strand of activity, researchers have studied the transport of drugs across protein-free lipid (or other hydrophobic) bilayers or membrane structures. On the assumption that cells also contain similar phospholipid bilayers, and that these are not modified materially by the presence of (what is, by mass, usually considerably more) protein (Figure 2), they have been tempted to extrapolate such *in vitro* findings to biological membranes *in vivo*. This again lacks real logic because the properties of biological membranes do differ in many ways from those of these artificial ones (not least by the presence of aquaporins [4]).

Few studies have systematically sought to understand how drug uptake and cellular lipid composition may covary; however, if one believes (as I do not) that most (or a significant part) of the uptake flux of drugs in intact cell membranes occurs via phospholipid bilayers, such lipid variation is not to be invoked as a major mechanism of variable uptake anyway because, for a significant number of drugs, the majority of the difference in ('background') rates between, for example, MDCK and Caco-2 cells does not vary more than twofold [5].

Thus, starting in 2008 [6], my colleagues and I have been developing the idea that the phospholipid bilayer portion of intact biological membranes is not in fact naturally significantly permeable to drugs and common xenobiotics because evolution simply selected against that (and note of course that biomembranes are osmotically active). Arguably it is precisely the 'laminated' hydrophilic–hydrophobic–hydrophilic structure of phospholipid bilayers that stops real, undamaged biomembranes from being leaky to small molecules (in either direction). Leaving aside endocytosis, paracellular transport, and so on, then, and concentrating on cases where a drug genuinely traverses a cellular (or indeed intracellular) membrane, how drugs do cross membranes is to be seen as being via the many proteinaceous transporters (e.g., [7,8]) (Figure 2) encoded by the relevant genome. Many of those for small molecules are recorded in the metabolic networks reconstructed and curated, for example for yeast [9] and humans [10,11]. Of course, these transporters are taken to be there not specifically for the benefits of pharmaceuticals companies but for the purposes of intermediary metabolism. To this end we have summarised the evidence for the dominance of transporter-mediated uptake in several review and experimental articles [5,6,12–19].

However, such reviews are necessarily retrospective. By contrast, if one starts (as a hypothesis [20,21]) by accepting that the phospholipid bilayer diffusion of drugs across intact biological membranes is negligible (PBIN), a great many interesting and thus prospective consequences follow, that I explore below. I shall point at some of the relevant evidence, but readers will wish to judge for themselves the extent to which the observations (including their own) have yet been made or not, and whether the PBIN view thus has useful predictive (or postdictive) power. I group the consequences into three classes; some consequences really relate to more than one class.