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HOW DRUGS PASS THROUGH BIOLOGICAL CELL MEMBRANES – A PARADIGM SHIFT IN OUR UNDERSTANDING?

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ABSTRACT

It was once widely believed, and text books (e.g. [1]) still portray it thus, that much (or even most) of the flux of pharmaceutical drugs into biological cells occurs by diffusion through whatever lipid bilayers may be present. A number of logical fallacies underpin this belief. Instead, an increasing amount of evidence indicates, perhaps surprisingly, that such phospholipid bilayer-mediated diffusion is in fact negligible in

intact cells. Drugs that pass through cell membranes (and I ignore endocytosis) do so by hitchhiking on transporters whose normal roles involve endogenous cellular metabolism. This recognition has some important and beneficial implications for the design of safe and efficacious drugs, in particular that they may be targeted to specific cells and tissues that express the relevant transporters [2].

INTRODUCTION - A FIELD OF LOGICAL FALLACIES

Membranes surround biological cells and determine what can enter into and exit from them. The general assumption is that such membranes are normally rather impermeable, else cells would be full of anything, including cytotoxic molecules, to which they happen to be exposed. (Indeed, mammalian cells had four billion years of evolution to learn how not to let any old rubbish enter them freely, which is also why they are osmotically active). Nevertheless, such molecules would therefore be more concentrated outside and have a tendency to fall down their concentration gradients to seek to enter cells, and indeed pumps exist to pump them out. However, these general considerations present something of a quandary for oral pharmaceutical drugs, since they must at least cross the intestinal epithelia, and for that majority whose targets are intracellular they must also cross other cell membranes. The question is how they do so.

We first need to mention the grotesque misuse of the word 'passive' in this field. This word has a well-established thermodynamic meaning (where concentrative transport is 'active', and equilibrative transport is 'passive'). However, many workers also ascribe a mechanistic meaning to it ('passive' is then considered to mean 'transport through the bilayer', i.e. bilayer diffusion, as opposed to transporter-mediated uptake). In the worst cases (intellectually) the equilibrative nature of a particular drug's transport is demonstrated but the mechanistic meaning is then inferred (yet another logical fallacy). All of this is best solved [3] either by avoiding completely the use of the word 'passive' in these discussions or by clearly defining the intended meaning of "passive transport" immediately beforehand.

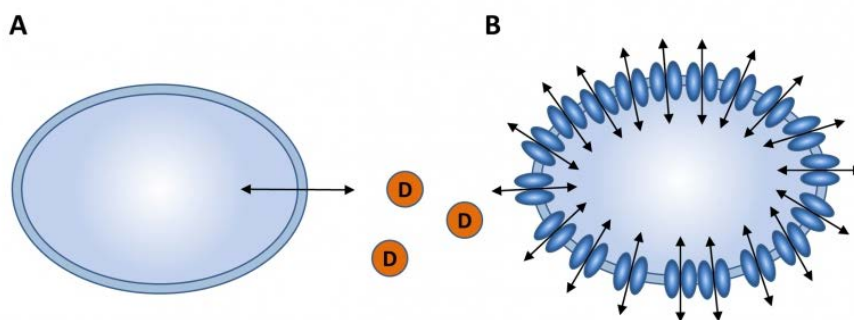


Figure 1. Two main means by which drugs might pass through biological cell membranes. © D.B.Kell

The nub of the problem that I am discussing is shown in Figure 1. It concerns how pharmaceutical drugs (or for that matter any other small molecule) can pass through a typical cell membrane. In Figure 1 the drug is shown (in cartoon form) as having a 'choice' of passing through the phospholipid bilayer (Figure 1A), or through the many protein transporters (SLCs) that are known to exist. This is illustrated in 1B, that also serves to remind us that most membranes are approximately 3:1 protein:lipid by mass, and probably 1:1 by area, so that very little free bilayer probably exists. These transporters are present, of course, not for the benefit of pharmaceutical companies but for the normal purposes of intermediary metabolism; indeed fully one third of the reactions identified in a recent reconstruction [4] of the human metabolic network

involve transporters.

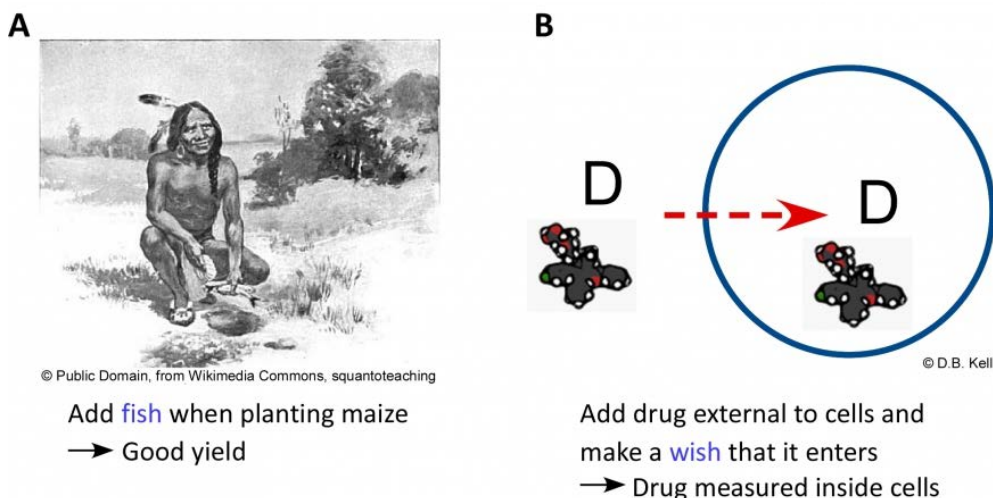


Figure 2. (A) A logical fallacy (inferencing mechanisms solely from initial and later stages) illustrated using a maize God (as commonly invoked in meso-American agriculture) as an example. (B) A similar (il)logical structure is frequently applied to infer, incorrectly, mechanisms of drug uptake. (Fig. A Squantoteaching © Public Domain, taken from Wikipedia, via Wikimedia Commons, Fig. B and compilation © D.B.Kell)

The surprising thing is that, despite the non-existence of any actual evidence (which in itself is also surprising), many workers believe that mechanism A is prevalent, if not even dominant [5]. The absence of evidence stems from a series of logical fallacies by which mechanisms are inferred when they are not actually measured. I illustrate this in Figure 2. This shows a logical fallacy (known variously as “affirming the consequent” or “post hoc ergo propter hoc”) by which associating inputs (the fish, along with the maize seed) and outputs (a better yield) is attributed to a mechanism (placating the maize God) that is not measured Fig. 2A) but simply assumed or inferred. Obviously one alternative mechanism is that maize is a nitrogen-hungry crop and that the fish provides the nitrogen. This hypothesis is better, in part because it is testable (e.g. by using a nitrogen source different from a fish), and the intermediates (nitrogen derivatives) are measurable. However, inferring mechanisms of drug transport simply by observing that it occurs has an (il)logical structure that is formally identical (Fig. 2B) to that of the maize God example.

In a similar vein, but as a separate logical fallacy, the fact that molecules can pass ‘through’ synthetic experimental configurations such as artificial ‘black’ lipid membranes or liposomes tells us precisely that, but it is not logically possible to infer that therefore they also do so in biological membranes, just because they too may contain phospholipid bilayers. This is because (inter alia) biomembranes are stuffed with proteins (Fig 1) that affect the lipid organisation strongly. This particular logical fallacy (inferences made from studies of varying X do not apply to Y if Y and X differ) is apparently sufficiently extreme, or indeed frankly obvious, that it does not even seem to have a codified name in formal logic.

The essential problem [3], in comparing the relative fluxes of substances (i) through bilayers or (ii) via transporters in real membranes, is that experimenters neither vary the former as an independent variable, nor measure it as a dependent variable. By contrast, those who believe in the overwhelming predominance of the transporter route, as I do (e.g. [2; 3; 6-9]), can easily vary the expression of transporters genetically [10]. An especially nice example of this type [11] shows that approximately 99.5% of the transcellular uptake of sepantronium bromide is mediated by a solute carrier called SLC35F2, leaving at most 0.5% for bilayer transport (or more likely transport via SLCs such as the ‘organic cation transporters’ OCT1 and OCTN that were previously believed to be involved [11]). As we tend to put it [3; 8], this means that the best analysis indicates that the transport of drugs across the membranes of biological cells through the Phospholipid Bilayer Is normally Negligible (PBIN) [3; 8].

WHAT KINDS OF DATA, EVIDENCE AND PREDICTIONS ARE MORE EASILY EXPLAINED VIA TRANSPORTER-MEDIATED UPTAKE OF PHARMACEUTICAL DRUGS RATHER THAN BY ANY SUPPOSED BILAYER DIFFUSION?

Much of the evidence that we have come to consider hinges on the highly differential ability of drugs to enter cells whose phospholipids (and their biophysical properties) are barely different. Thus in the study of Winter et al. [11] mentioned above, the expression level of (the transcript for) SLC35F2 varied several hundredfold in different cell lines, broadly mirrored by the sensitivity of the same cell lines to sepantronium bromide (which is cytotoxic).

So, if drugs were able to leak across the bilayer portions of intact cell membranes at any kinds of functionally significant rates, the default assumption is that their unbound forms would be more or less uniformly distributed within and between the cells of interest (at least if significant potential differences and pH gradients are lacking). Any number of studies (cited in references such as [2; 3; 6-9]) show that the distribution of drugs is in fact highly heterogeneous between cells in an organ, between tissues of an individual organism, and even more so between organisms of different species. Specifically, the distributions were measured directly, for instance by mass spectrometric imaging (e.g. [12-14])

If transporters are important, it might be assumed either that they were essential for cell growth or that their importance had then simply selected for redundancy. A very nice recent paper [15] shows that the second of these is true, as just 12 of the >500 known SLCs were found to be essential for haploid cell growth, consistent with the earlier findings [10; 11] on the mainly nonlethal effects of individual genetic knockouts of SLCs.

The view that uptake is predominantly via transporters (and that any 'background' rates through the bilayer are correspondingly negligible) also provides a simple explanation for 'barriers', such as the blood-brain [16], blood-retina and blood-testis barriers, on the basis that phospholipid bilayer transport is anyway negligible and these 'barrier' membranes simply lack most of the necessary transporters (though those that they possess might then be usefully exploited). The (statistically) known rates and expression profiles are also ample [17; 18] to account for such transport. Finally, if these drugs are hitchhiking on transporters used in intermediary metabolism, the expectation is that drugs would be chemically similar to metabolites, an expectation that is borne out [19; 20].

Possibly the most exciting aspect is the fact [2] that the essentially negligible rates of transmembrane transport in the absence of transporters means that one can seek to target drugs to tissues that express (or can be induced, by pharmacological or other means, to express) the necessary transporters selectively. Two very nice examples by Pfefferkorn and colleagues illustrate this. Activating liver glucokinase can provide a rational strategy for a drug to control type II diabetes, but activating this enzyme in other tissues can be toxic [21]. However, by ensuring that the candidate drug was a substrate for members of the concentrative organic anion transporting polypeptide (OATP) (SLCO/SLC21 [22]) family, its activity was largely confined to the liver at the concentrations that could then be applied [23]. Some HMGCoA reductase inhibitors (colloquially 'statins') can induce myopathies (muscle disorders). A similar strategy for hepatoselective HMGCoA reductase inhibitors [24] yielded molecules with a hepatocyte:myocyte concentration ratio of 250,000:1.

A PARADIGM SHIFT TOWARDS A RECOGNITION OF 'TRANSPORTER-ONLY' TRANSMEMBRANE TRANSPORT OF DRUGS?

Thirty years ago, it was widely believed that general anaesthetics (narcotics) worked solely via phospholipid bilayers, whereas now the hydrophobic pockets on the many target proteins (e.g. GABA_A receptors, treK channels, etc.) to which they bind, are in fact reasonably well understood. The infiltration of any narcotics into phospholipid bilayers is thus seen as irrelevant to their function. I anticipate a similar paradigm shift occurring as the explanatory power of the predominance of transporter-mediated fluxes of pharmaceutical drugs becomes more established. According to a populist view (based originally on William James (see [25]), scientific understanding passes through three phases ("it's wrong; it's not important; we knew it all along"). There are still some workers who are firmly rooted in the first stage, and for whom any kind of acceptance of the ideas of predominant transporter-mediated uptake will take longer. The history of science is full of this. Fortunately, the existing literature, as well as the actual evidence, leads me to suppose that we are somewhere between the second and third stages for most informed workers who have been prepared to study the evidence properly. One day, the community (and sociologists of Science) will wonder how we could possibly have believed otherwise.

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