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Caco-2 monolayers in experimental and theoretical predictions of drug transport ☆

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ABSTRACT

This review examines the use of Caco-2 monolayers in the prediction of intestinal drug absorption. First, the different routes of drug transport in Caco-2 monolayers are compared with those seen in vivo. Second, the prediction of drug absorption in vivo from transport experiments in cell monolayers is discussed for different classes of drugs. Finally, the use of Caco-2 monolayers as a reference model in physico-chemical and theoretical predictions of drug absorption is discussed. We conclude that Caco-2 monolayers can be used to identify drugs with potential absorption problems, and possibly also to select drugs with optimal passive absorption characteristics from series of pharmacologically active molecules generated in drug discovery programs.

Contents

1. Introduction

During the last few years, the use of intestinal epithelial cell lines such as Caco-2 and HT29 has increased dramatically in many research fields including the pharmaceutical sciences (Fig. 1). The cell lines are now routinely cultivated as monolayers on permeable filters for studies of the transepithelial transport of drugs (for reviews, see [1, 2]; Fig. 2). Most studies of drug transport in cell monolayers have been performed using Caco-2 cells and are of a mechanistic nature. In general, the aim has been to investigate whether a drug is actively or passively transported across the intestinal epithelium and, if the transport is active, to identify the relevant carrier. Using such studies, new and sometimes unexpected drug transport routes have been identified [3–5]. Most studies on active drug transport in Caco-2 monolayers have investigated two transport systems, the dipeptide carrier [6, 7] and P-glycoprotein [8, 9]. Fewer studies have been published on the passive transport of drugs. These studies have shown that cell monolayers can be used to identify drugs with potential absorption problems and possibly also to predict drug absorption in vivo [10–12]. Since drug transport studies in cell monolayers are easy to perform and require only small quantities of drugs, they have been suggested for screening of drug absorption at an early stage in the drug development process (see the review by Bailey in this volume). Recently, Caco-2 monolayers were used to screen permeability of a synthetic peptide library containing 375 000 discrete tripeptides, divided into 150 pools [13]. Automated procedures for screening of drug transport in Caco-2 monolayers using robotics have been reported [14].

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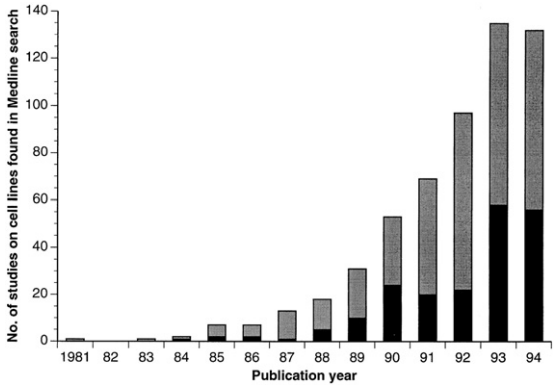

Fig. 1. Increase in the number of papers per year dealing with Caco-2 cells. The dark parts of the staples show papers dealing with absorption, transport and/or permeability. Source: Medline.

In this review, results of studies investigating the use of Caco-2 monolayers in the prediction of intestinal drug absorption are summarised. Data from a recently introduced theoretical model for prediction of passive transcellular drug absorption are also presented. For reasons of simplicity, the review deals exclusively with epithelial permeability and, therefore, other factors that may influence the extent of drug absorption and bioavailability such as solubility; formulation factors (e.g. absorption enhancers) and presystemic and systemic drug metabolism will generally not be considered. The basic characteristics of intestinal epithelial cell lines are discussed in detail in the review by Quaroni and Hochman elsewhere in this volume.

2. Transport of drugs in Caco-2 monolayers and intestinal tissues

The transport of drugs across the intestinal epithelium may occur by one or more of four different routes: the passive transcellular and paracellular routes, the carrier mediated route and by transcytosis (Fig. 2). Caco-2 monolayers have been used to study drug transport by all four routes. In this section, we will first consider how different classes of drugs are transported by these routes in the intestinal epithelium. We will then compare drug transport in Caco-2 monolayers with that in vivo.

2.1. Transport pathways across the intestinal epithelium

Rapidly and completely absorbed drugs are generally lipophilic and distribute readily into the cell membranes of the intestinal epithelium. Since the surface area of the brush border membranes is >1000-fold larger than the paracellular surface area [15], it can be assumed that these drugs are transported exclusively by the passive transcellular route. Most approved drug products which are rapidly and completely absorbed following oral administration are transported by the passive transcellular route (Fig. 2).

Drugs that are slowly and incompletely passively absorbed, such as hydrophilic drugs and peptides, distribute poorly into cell membranes. It is therefore generally assumed that these drugs are transported

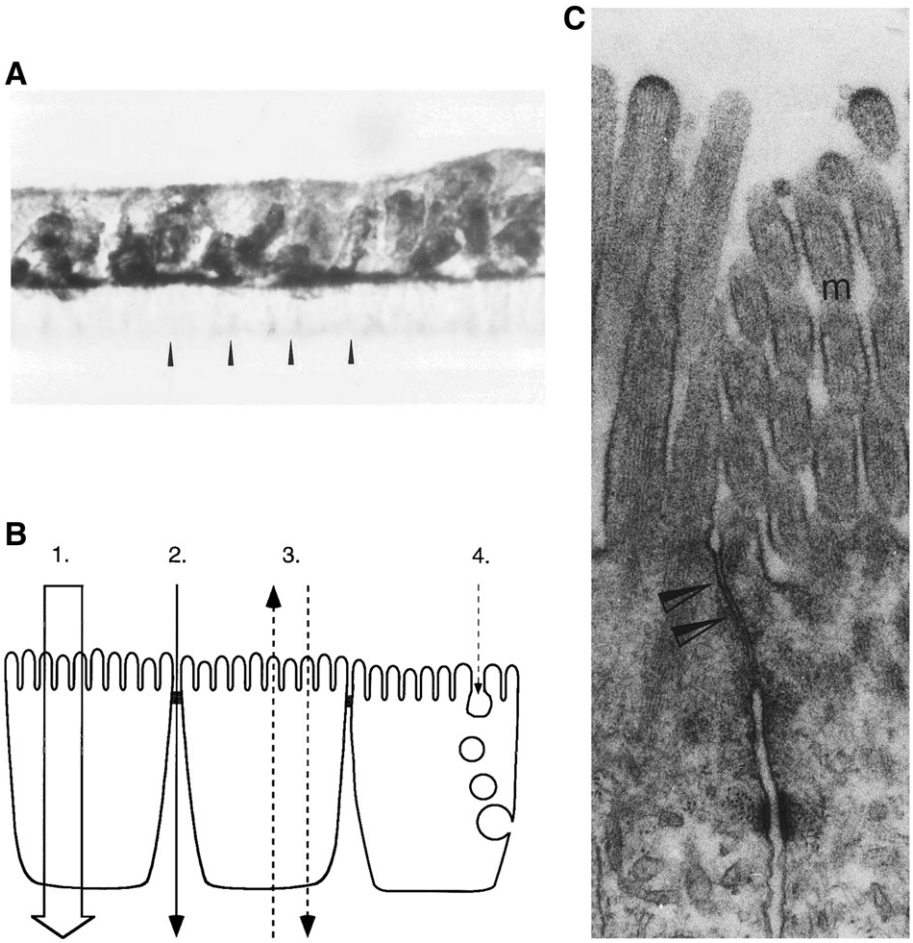

Fig. 2. A. Cryosection (4 μm) of an intestinal epithelial cell monolayer grown on a polycarbonate filter. The cells where stained with hematoxylin/eosin and fixed with formalin before sectioning with a Leica Jung CM3000 cryostat (courtesy by Dr Göran Ocklind). The arrowheads indicate the border of the premeable support. B. Schematic drawing of an intestinal epithelium. The arrows indicate the four different drug transport routes: 1, the passive transcellular; 2, the passive paracellular; 3, the active carrier-mediated transcellular and 4, the transcytosis routes. C. Transmission electron micrograph of the apical part of two Caco-2 cells with microvilli (m) and a tight junction (arrowheads).

through the water-filled pores of the paracellular pathway across the intestinal epithelium (Fig. 2). It is, however, not finally established that these drugs are transported exclusively by the paracellular route. It is possible that even very hydrophilic drugs may be transported partly by the transcellular route [16]: Consider a hydrophilic drug with a partition coefficient between the cell membrane and the extracellular fluid (P_{membr}) of 1×10^{-3}, i.e. a log P_{membr} of −3. For comparison, the log octanol/water partition coefficient (log P_{oct}; see Section 3) for molecules assumed to be transported by the paracellular route (e.g. mannitol) is also in the order of −3 [10]. Then assume that the surface area of the luminal cell membrane of the intestinal epithelium is 1000-fold larger than that of the paracellular space [15]. The larger surface area of the cell membrane will compensate for the difference in partitioning between the cell membrane and the extracellular fluid. As a result, the hydrophilic drug could be transported in equal amounts by the paracellular and transcellular routes. However, in reality, the tight junctions which gate the entrance to the paracellular pathway restrict the paracellular transport of drugs even further [17]. The low efficiency of the paracellular pathway has stimulated investigations into ways to enhance the permeability by this route (reviewed in [18]). Many of these studies have been performed in monolayers of intestinal epithelial cells and have provided new insight into the regulation of tight junctions — the rate limiting barrier of the paracellular pathway (reviewed in [19]).

Some hydrophilic drugs whose chemical structures mimic those of various nutrients can be transported across the intestinal epithelium by active, carrier-mediated transport (Fig. 2). Often, transport is mediated partly by the carrier and partly by passive routes. Since carrier-mediated transport is saturable, the contribution of the passive route will increase with increasing dose. If the drug has a low passive permeability, saturation of the carrier will result in a decreased absorbed fraction. This may occur either when the carrier is saturated by nutrients or at high dose levels of the drug [20]. There are also active transporters such as P-glycoprotein, which mediate drug transport in the serosal to mucosal direction [4, 7–9, 21, 22]. In this case, saturation of the carrier could result in an increase in the absorbed fraction of drug [8].

The low capacity of the transcytosis route from the mucosal to the serosal side of the intestinal epithelium makes this route less attractive for the transport of drugs (Fig. 2). It has therefore mainly been considered as a route for highly potent drugs (such as peptide antigens) which are excluded from the other transport pathways due to their size [23]. Another disadvantage is that transport generally occurs in membrane vesicles which contain large amounts of proteolytic enzymes. As a result most exogenous proteins are extensively degraded during transcytosis both in situ and in cell monolayers [24–30]. The transport of vitamin B_{12} is perhaps the best example of naturally occurring receptor-mediated endocytosis/transcytosis across enterocytes in the mucosal to serosal direction [31]. The B_{12}-transport system has a low capacity and requires specific binding to an intrinsic factor, suggesting that this pathway is of limited value as a general drug transport route. However, transcytosis of macromolecules and even small microparticles is more effective in M-cells, specialised epithelial cells overlying the lymphoid tissue of the intestinal epithelium [32]. This cell type, which may have lower proteolytic activity in its transport vesicles, is the main target for antigen-containing microparticulate delivery systems intended for oral vaccination [33]. Unfortunately, the low number of M-cells in the intestinal epithelium reduces the possibility of using these cells as a general pathway for epithelial drug transport. Therefore, this transport route will not be further considered in this review.

2.2. Comparison of drug transport in cell monolayers and intestinal tissues

The first study attempting to correlate passive drug permeability in Caco-2 monolayers with drug absorption in humans after oral administration suggested that the cell monolayers might be used to identify drugs with potential absorption problems [10]. Completely absorbed drugs were found to have high permeability coefficients ($P_{app} > 1\times10^{-6}$ cm/s) whereas incompletely absorbed drugs had low permeability coefficients ($P_{app} < 1\times10^{-7}$ cm/s) in the Caco-2 monolayers. Other more recent studies suggested that the cell monolayers ranked the permeabilities of drugs in the same order as more complex absorption models such as in situ perfusion models [11, 12, 34, 35]. These correlation studies were mainly performed with passively transported drugs. Other recent studies indicate how well passive transcellular and paracellular drug transport in Caco-2 monolayers can model the drug transport in vivo: the effective permeabilities for three different classes of drugs were investigated in Caco-2 monolayers and in human jejunum in situ [36] using a double balloon technique and single pass perfusion [37, 38]. Drugs which are rapidly and completely absorbed by a passive (transcellular) route and those which are slowly and incompletely absorbed by a passive (paracellular) route were investigated. Drugs and nutrients transported by active carrier-mediated routes were also studied. The comparison was unbiased by extracellular barriers such as the 'unstirred water layer' [39, 40]. Surprisingly, the effective permeabilities of the rapidly and completely absorbed compounds (transported by the passive transcellular route) differed only 2- to 4-fold between the models [36] (Fig. 3). These results indicate that Caco-2 monolayers are an excellent model of the passive transcellular pathway, the most common drug permeation route in the intestine. Since Caco-2 monolayers are flat compared to the extensively folded human jejunum, the results also support the hypothesis that only a fraction of the anatomical surface area of the intestine — the villi tips — participates in the absorption of this class of drugs [41].

The correlation of the permeabilities of the slowly and incompletely absorbed drugs in the Caco-2 monolayers and human jejunum was qualitative rather than quantitative. These drugs were transported at a 30- to 80-fold slower rate in the Caco-2 monolayers than in the human jejunum [36] (Fig. 3). While this may be an advantage for the in vitro identification of drugs with potential absorption problems the results clearly indicate a large quantitative difference between the two models. The two most likely explanations for this discrepancy are related to possible differences in the permeability of the paracellular pathway and in the absorptive surface areas. Thus, electrophysiological and permeability data indicate that the permeability of the tight junctions in Caco-2 monolayers is lower than the average permeability observed in the human intestine in vivo (e.g. [42–44]). However, studies using hydrophilic markers that permeate the cell monolayers by the paracellular route indicate that the difference is of a quantitative rather than qualitative

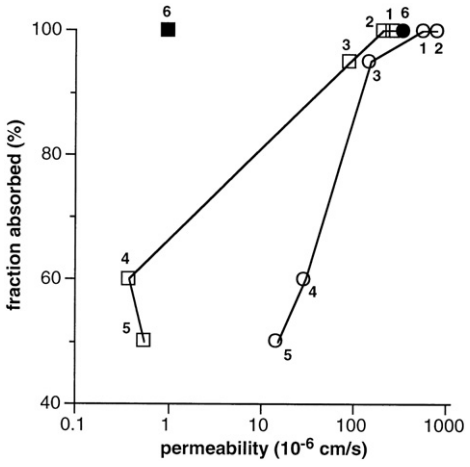

Fig. 3. Fraction absorbed in humans after oral administration as a function of permeability coefficients in the Caco-2 model (-□- passive transport; ■ L-dopa) and human jejunum (-○- passive transport; ● L-dopa). The numbers refer to: 1, antipyrine; 2, naproxen; 3, metoprolol; 4, terbutalin; 5, atenolol; 6, L-dopa. Data compiled from [36] with permission from the publisher.

nature. The paracellular permeability of polyethylene glycols decreased with molecular weight in a comparable fashion in Caco-2 monolayers and human intestine in vivo [45], although the permeabilities of the polyethylene glycols were almost 100-fold lower in the cell monolayers. These results suggest that there are fewer openings (pores) in the tight junctions in the Caco-2 monolayers but that the average pore diameters are comparable in the two models. Recently, evidence supporting this hypothesis was provided by Tanaka et al. who showed that the ratio of the permeabilities of low and high molecular weight compounds and Cl^- were comparable in Caco-2 monolayers, rat jejunum and rat colon [46].

Another explanation for the difference in paracellular permeability between human jejunum and Caco-2 monolayers involves differences in the absorptive surface area (Fig. 4). Drugs having a lower permeability will remain longer in the intestinal lumen before they are absorbed. These drugs may therefore diffuse further down the length of the villi as compared to drugs having a high permeability (which are rapidly and completely absorbed through the villus tips). This diffusion would not only increase the absorptive surface area, but also allow a fraction of the drug absorption to occur through the leakier paracellular pathway in the crypt region [47].

A third, more speculative explanation for the low paracellular permeability in Caco-2 monolayers could be related to differences in the regulation of tight junction permeability and paracellular water fluxes in the cell monolayers [48] as compared to intestinal tissues in situ [49]. For instance, conditions which change water flux induced paracellular solute transport in excised intestinal tissues [50] and in experimental animals in situ [51] seem to have little effect on the absorption of marker molecules and drugs in the perfused human jejunum [52, 53]. An explanation for this difference has been suggested by Karlsson et al. in a recent study in Caco-2 monolayers [54]. In this study an osmotically induced water flux in the apical to basolateral direction enhanced the transport of small drugs (m.w.<130) but had no or only a limited effect on drugs of conventional size (m.w.>130). Thus, it may be speculated that paracellular water flow occurs through aqueous pores that are too small to allow significant 'solvent drag' of drug molecules of conventional size in vivo [53, 54].

Even if the tight junction permeability turns out to be normal in the Caco-2 monolayers, the relationship between the permeability and the fraction absorbed is very steep for incompletely absorbed drugs. Thus, when the permeability coefficient increases from 1×10^{-7} to 1×10^{-6} cm/s, i.e. with one order of magnitude, the predicted absorbed fraction of a drug increases from 0 to 100% in vivo [10]. However, several studies using peptidomimetics have shown that it is possible to obtain reasonable monolayer-in vivo correlations for slowly and incompletely absorbed drugs as well [34, 35, 55]. However, it is clear that the predicted fraction absorbed for this group of compounds is more sensitive to variations in cell monolayer permeability than is that for the rapidly and completely absorbed drugs, which cover a larger range of permeabilities.

At least two approaches have been used to produce cell culture models with a more leaky paracellular pathway. In the first approach, monolayers of cell lines displaying a higher paracellular permeability were used. For instance, monolayers of the normal but relatively undifferentiated intestinal epithelial cell line IEC-18 had a lower electrical resistance and a higher paracellular permeability than Caco-2 cell monolayers [56]. Preliminary results indicate that the permeabilities of incompletely absorbed drugs span a wider range in IEC-18 monolayers than in Caco-2 monolayers [57]. Similar results have been obtained in our laboratory using the conditionally immortalised intestinal epithelial cell line 2/4/A1 [58, 59].

In the second approach, co-cultures of the cell lines Caco-2 and HT29-H were established to represent the two most abundant cell populations in the intestinal epithelium, absorptive cells and goblet cells [60]. HT29-H cells form monolayers of mucin secreting human intestinal goblet cells [61]. It was reasoned that since the paracellular permeability of HT29-H monolayers was 50-fold higher than that of Caco-2 monolayers [61], the co-cultures should have a paracellular permeability that was closer to the human situation. Moreover it was assumed

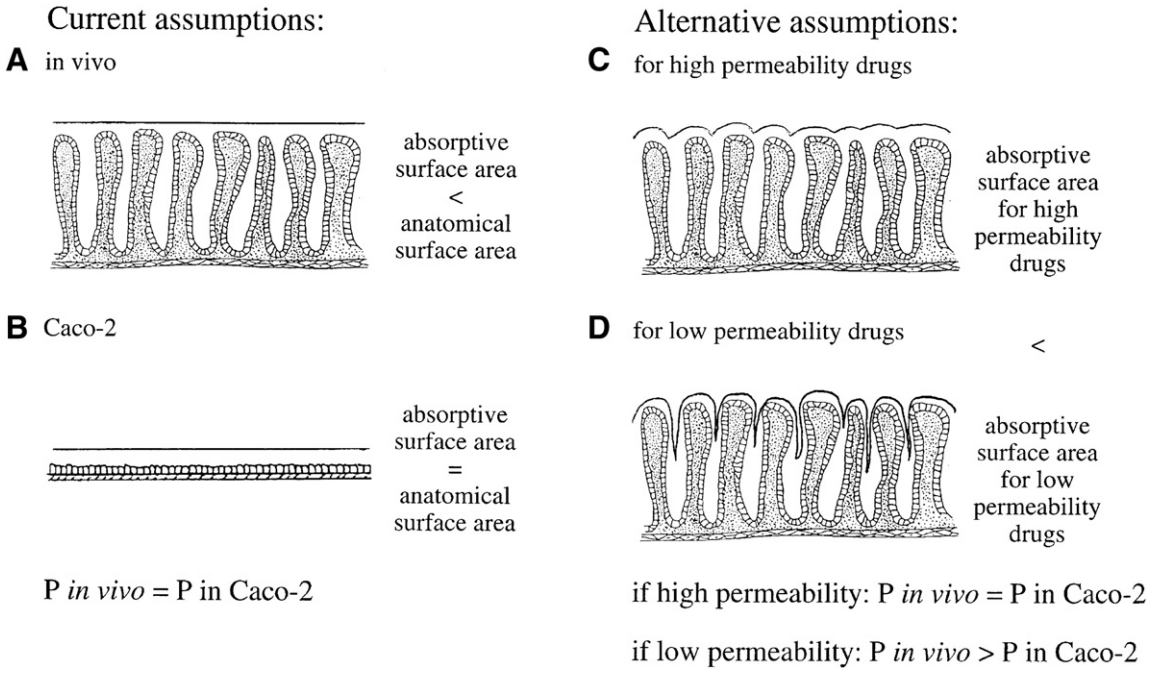

Fig. 4. Comparison of absorptive surface areas for high and low permeability drugs in the intestine in vivo and in Caco-2 monolayers. In perfusion experiments, the absorptive surface area of the small intestine is normally assumed to be equal to the inner surface area of a smooth tube (A). This surface area, which is much smaller than the anatomical surface area of the small intestine, is directly comparable to that in flat Caco-2 monolayers (B). It is therefore assumed that permeability coefficients in vivo and in cell culture are directly comparable. Alternatively, it can be hypothesised that the absorptive surface area in the small intestine varies with drug permeability (C, D). Drugs displaying a high intestinal permeability would have a smaller absorptive surface area (C) than drugs displaying a low intestinal permeability (D), see text for explanation. According to this hypothesis, the permeability coefficients of highly permeable drugs would be comparable in vivo and in Caco-2 monolayers while drugs displaying a low permeability would have a higher permeability in vivo than in the cell cultures. The thin lines above the intestinal segments in A, C and D and the cell monolayer in B represent the absorptive surface areas.

that, if the cells could be mixed in the proportions normally found in vivo, the goblet cells would produce a protective mucus layer (as they do in monocultures [61]) and the unique transport properties of the Caco-2 cell line would be maintained in the co-cultures. The approach was only partly successful since the co-cultures formed tighter monolayers than expected under the applied culture conditions and, at most, a fivefold increase in paracellular permeability was observed. Moreover, the two cell populations did not mix as well as in vivo (probably a result of differential expression of cell adhesion molecules) and the goblet cells did not produce detectable amounts of mucus. These results indicate that, in contrast to previous suggestions [62], the establishment of co-cultures mimicking in vivo conditions may be much more difficult than the establishment of monocultures. An alternative method of establishing co-cultures of intestinal epithelial cells could be to isolate cells from transgenic mice bearing an inducible growth promoting gene [63]. If such a gene could be switched on in precursor cells of the major epithelial cell populations after isolation from the intestine, co-cultures comprised of all these cell populations could theoretically be established. However, this approach may be limited by so far undefined requirements of the different cell populations for growth factors, extracellular matrix, etc.

The limitation of many of the early studies on the correlation between Caco-2 monolayer permeability and in vivo absorption was the use of drugs which are mainly transported passively by the transcellular and paracellular routes without consideration of drugs transported by a carrier-mediated mechanism. Recent attempts to include actively transported drugs in the correlation have given variable results. In the study by Lennernäs et al., the drug l-dopa (which is normally completely and rapidly absorbed, mainly via the carrier for large neutral amino acids in the human jejunum) [20] was found to have a >100-fold lower effective permeability in Caco-2 monolayers than in the human jejunum in the investigated concentration interval [36] (Fig. 3). The difference could be attributed to the lower expression of this carrier in the cell monolayers than in vivo, resulting in saturation of the carrier in the cell monolayers [64, 65]. Similar results were reported for L-leucine and D-glucose, leading to the conclusion that the expression of carrier-mediated transport processes in the cell monolayers must be fully characterised before correlations with the in vivo situation can be established. However, in another study which included actively transported compounds, the drug permeabilities in Caco-2 monolayers correlated with those in the perfused rat intestine [66]. This suggests that in some circumstances it may be possible to predict the absorption of both passively and actively transported drugs in Caco-2 monolayers. The best correlation between the two models was obtained for small organic molecules; larger and more complex peptidomimetics displayed a weaker correlation [66]. Thus, it seems possible that the strength of the Caco-2 monolayer vs. in vivo correlations may vary for different groups of drugs. Some aspects of this variability may be related to the factors discussed above, such as differences in absorptive surface area, or to variations in the luminal content or extracellular mucus layer [67–69]. Other potential sources of variability are related to experimental conditions and the cell line itself: Kim et al. found that the permeabilities of a set of model peptides in the perfused rat intestine were lower than the corresponding permeabilities in Caco-2 monolayers [34] while Stewart et al. found that the permeabilities for peptidomimetics in the perfused rat intestine were higher than those in Caco-2 monolayers [66]. This discrepancy was attributed to differences in the experimental conditions [66]. In the study by Kim et al., the permeabilities were obtained after non-steady state perfusion and the appearance of the compounds in the mesenteric circulation was measured, whereas Stewart et al. used steady-state drug input and measured the disappearance of the drug from the intestinal lumen.

Another source of variability is the cell line itself. Caco-2 cells are a heterogeneous cell population [70] which is exposed to different selection pressures in different laboratories. The properties of Caco-2 cells in one laboratory may therefore differ from those in another. This was clearly illustrated in a recent study by Walter and Kissel, which showed that only one of the two populations of Caco-2 cells originating from different laboratories displayed active transport of thyrotropin-releasing hormone [71]. In our experience and that of others, the properties of Caco-2 monolayers also vary with time within a laboratory, e.g. with the passage number [72], the time in culture [54, 73] the extracellular (filter) support [74] and the cell culture medium [75]. All sources of variability have to be taken into consideration when results from different laboratories are compared. Repeated investigation of the transport of reference compounds at regular time intervals could be one way to facilitate such comparisons [76]. The variability in Caco-2 monolayer permeability between laboratories is illustrated in Fig. 5. It is clear that although the drug permeabilities predicting incomplete or complete drug absorption varied considerably between laboratories, qualitatively similar correlations with the fraction absorbed in humans were established in all laboratories [10, 66, 77, 78]. The comparison in Fig. 5 also indicates a need for standardisation of Caco-2 cultures. Direct comparisons of drug permeabilities obtained in different laboratories will only be possible if the same Caco-2 population and cell culture conditions are used.

In summary, the results obtained to date indicate that Caco-2 monolayers can be used to predict drug transport by different pathways across the intestinal epithelium but that the best correlation with the absorbed fraction in vivo is obtained for passively transported drugs. Therefore, it is our view that Caco-2 monolayers can be used as a simple reference model in predictions of passive drug absorption. We also conclude that it may sometimes be hazardous to compare results on drug permeabilities obtained in different laboratories due to variabilities in experimental conditions and in the cell line itself.

3. Caco-2 monolayers as reference model in predictions of drug absorption from molecular properties

Many attempts have been made to explain and predict passive drug absorption directly from the properties of a drug molecule. In these studies single physico-chemical properties of the drug molecule, such as octanol/water partitioning coefficients [79], hydrogen bonding capacity [80, 81] or desolvation energy [82, 83], have been correlated to intestinal absorption rate or cell membrane permeability. Other, less frequently used, physico-chemical properties for the prediction of transcellular transport include molecular surface area [84, 85] and surface activity [86]. One advantage of using single physico-chemical factors for this purpose is that they are relatively easy to determine

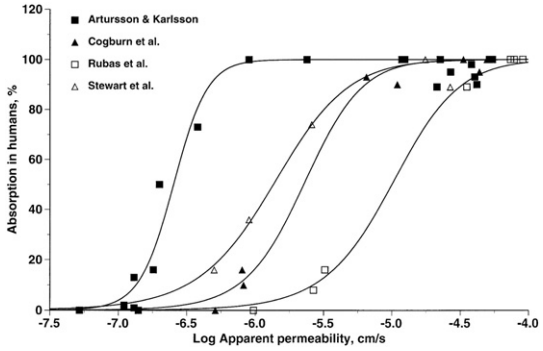

Fig. 5. Correlation between absorbed fraction in humans after oral administration (expressed as % of the administered drug dose) and permeability in Caco-2 monolayers obtained in four different laboratories. Qualitatively similar correlations were established in all four laboratories but the data are not directly comparable between the laboratories due to quantitative differences in the permeability of the Caco-2 monolayers. Data were compiled from [10, 66, 77, 78]. The equation: Fraction absorbed (F.A.) $= (0-100)/(1+(\log P_{app}/a)^b)+100$ was fitted to the data using non-linear regression analysis $a = \log P_{app}$ at F.A. equals 50% and b is the slope factor. One outlier, polyethylene glycol (m.w. 900), was excluded from the data set taken from [78].

experimentally. In addition, they can sometimes be derived from theoretical models. For instance, octanol/water partitioning coefficients can be calculated using the fragment constants methods of Hansch and Leo or Rekker [87]. Recently, alternative more generally applicable methods for estimation of octanol-water partitioning coefficients have been proposed, e.g. atom/fragment contribution methods [88] and molecular lipophilicity potentials that take into account steric and conformational effects [89]. The disadvantage of using single physico-chemical factors is that they are only roughly correlated to passive drug absorption. Good correlations can be obtained for series of homologous compounds, but the correlations are generally impaired when structural diversity is introduced [10, 79]. One reason for this shortcoming is that established single predictors of drug absorption, such as octanol/water partition coefficients, only model drug absorption by the passive transcellular route and do not take the paracellular route of absorption into consideration. To establish more general correlations between molecular properties and passive permeability, several physico-chemical properties have sometimes been combined into one expression, often with the aid of multiple linear regression analysis [85, 90]. However, multiple linear regression models are controversial as they are often empirically deduced and, as more parameters are included, statistical fits may improve [91, 92]. Furthermore, many of the commonly included physico-chemical parameters are interrelated (that is, they are incompatible with multiple linear regression analysis) and the methods can be time consuming since they require many experiments. Some measure that reflects several of the important physico-chemical parameters would therefore be interesting as a predictor of passive drug absorption. Recently, van de Waterbeemd et al. attempted to correlate a variety of molecular descriptors of lipophilicity, molecular size and hydrogen bonding capacity to published data on drug permeability in Caco-2 monolayers [10, 93]. The results suggested that Caco-2 permeability is reasonably well described by log D (sigmoidal relationship) or alternatively, by a linear combination of molecular size and hydrogen bonding descriptors. Since both molecular size and hydrogen bonding descriptors can be obtained by calculations (without experiments), this approach could have potential as a theoretical method to predict drug absorption [93]. In the first part of this section, we review some characteristics of the two molecular properties which are most commonly used to predict drug absorption: octanol/water partitioning coefficients (P_{oct}) and hydrogen bonding capacity (Δ log P). Studies in which these parameters have been correlated to drug permeability in cell monolayers are discussed. In the second part, we present a new theoretical model for the prediction of drug absorption based on molecular surface properties using Caco-2 monolayers as reference model.

3.1. Single physico-chemical properties and permeability coefficients

The octanol/water partitioning coefficient (P_{oct}) is the most widespread predictor of drug absorption and P_{oct} is routinely determined for new chemical entities. P_{oct} describes the ability of a drug molecule to partition into the lipophilic phase, octanol, which is assumed to have a lipophilicity comparable to that of a cell membrane. In the case of ionizable drugs, the apparent distribution coefficient at pH 7.4 (D_{oct}) is often used instead of P_{oct}. D_{oct} is a function of the P_{oct}- and pK_a-values of the molecule. The permeability in the intestinal epithelium increases roughly with the lipophilicity of the drug molecule until it reaches a plateau at a log P_{oct} value of about two [79]. Drugs displaying log P_{oct} values close to two are generally predicted to be completely absorbed in humans. For log $P_{oct}>4$ the permeability starts to decrease with log P_{oct} [94], since very hydrophobic drugs generally have low aqueous solubility and partition at a slower rate from the (lipophilic) cell membranes to the extracellular fluids [95]. A high hydrophobicity and/or low aqueous solubility may complicate transport studies both in situ and in cell monolayers. Very hydrophobic drugs may adsorb to the walls of the transport chambers during the experiment with a large non-specific loss of the drug as a result. Further, the proportional loss of the hydrophobic drug will be larger when the starting concentration is low, e.g. because of a low aqueous solubility. An elegant biophysical kinetic model for the study of very hydrophobic drugs in cell monolayers was recently presented by Raub et al. using lazaroids with calculated log P_{oct}-values of ≈8 as examples [95]. In a related study, it was speculated that not only the slow rate of partitioning from the cell membrane to the extracellular fluid, but also protein binding in the extracellular submucosal tissues, may influence drug permeability [96].

It has been found by experience, that P_{oct} and D_{oct} are rough predictors for the (transcellular) absorption in vivo of a homologous series of conventional drugs (small organic molecules) although there are exceptions to this rule [97]. Several contributions also suggest that reasonably good correlations exist between these parameters and permeability coefficients in cell monolayers [44, 98–100]. How can a simple organic solvent such as octanol model the much more complex lipid bilayer of the cell membrane? One possible explanation has been provided by Franks et al., who made X-ray diffraction analyses of octanol [101]. The results suggested that hydrated octanol molecules are arranged in roughly spherical aggregates with a polar centre and an apolar outer region, with a polar-apolar interface between them. Thus, hydrated octanol consists of a range of localised environments mimicking the diversity of binding sites in the more complex lipid bilayers.

Could measurement of drug partitioning into membrane lipids be a better predictor of passive drug transport than log P_{oct}? Recently, two methods based on the chromatographic retention of drugs in membrane lipids have been introduced. In one of the methods, a phosphatidylcholine analogue immobilised on silica particles was used as a solid phase [102]. In the second method, a gel bed with immobilised liposomes was used [103]. Both methods gave relatively good correlations between capacity factors (a measure related to the retention time or volume on the column) and published P_{app}-values in Caco-2 monolayers [10]. Further studies are needed before it can be concluded whether any of these new methods is an alternative to octanol/water partition coefficients in predictions of drug permeability.

The weakness of P_{oct} as a predictor of cell membrane permeability has been shown by Young et al., who found no clear correlation between the blood-brain uptake for 20 histamine H_2 receptor antagonists and log P_{oct} [80]. Rather, a better correlation was found for the difference between log P_{oct} and log cyclohexane-water partition coefficients ($\Delta \log P = \log P_{octanol\text{-}water} - \log P_{cyclohexane\text{-}water}$). A simple explanation for these findings was given by Burton et al. who also obtained good correlations between Δ log P and the permeability of peptide-like substances in Caco-2 monolayers [82]: In order to be transported (by the passive transcellular route) across the lipid bilayer, a molecule must pass through the outer hydrated polar part of the bilayer as well as through the much more hydrophobic membrane interior. Since octanol is a hydrogen bonding solvent, the desolvation energy associated with breaking the peptide-water hydrogen bonds can be balanced by the formation of peptide-octanol hydrogen bonds. Therefore, in terms of solvent properties, octanol mainly models the affinity of the drug for the more polar membrane interface rather than the transport through the entire lipid bilayer. In contrast, no hydrogen bonds can be formed with cyclohexane and therefore this solvent has properties more similar to the more hydrophobic membrane interior. Accordingly, Δ log P should model the membrane interface to membrane interior transfer of the molecule, i.e. the desolvation energy required for transcellular transport [82]. Therefore, Δ log P may be a better predictor of drug permeability than log P_{oct} in certain circumstances. For instance, hydrophobic peptides generally display a low permeability since they have many hydrogen bond forming groups. The low permeability can be predicted from the high Δ log P-values but not from the

high P_{oct}-values of these peptides. Burton et al. also reintroduced a related and straightforward, but less robust, theoretical model for the prediction of peptide absorption based on counting the number of potential hydrogen bond forming groups in the molecule [81, 104–106]. As pointed out by the authors, this measure fails to account for the steric/electronic influence on the solute-solvent hydrogen bond strength and intra- vs. intermolecular hydrogen bonding [82].

With the exception of the studies by Young et al. on the blood-brain permeability of histamine H_2 receptor antagonists [80] and Burton et al. on peptide-like molecules [34, 81, 82, 104], only a few comparisons between log P_{oct}, Δ log P and permeability have been performed. Buur et al. found that log D_{oct}, but not Δ log P, could be correlated with permeability of 5-fluorouracil prodrugs in Caco-2 monolayers [98] (Fig. 6). Similarly, ter Laak et al. found that the brain permeability of a series of structurally diverse histamine H_1-receptor antagonists was better explained by log D_{oct} than by Δ log P or hydration capacities [92]. At the present time, therefore, it is unclear under which circumstances log P_{oct} or Δ log P should be used as a single predictor of drug absorption.

In summary, many physico-chemical properties of drug molecules have been used to describe their passive transmembrane permeability. The relative importance of these properties will vary from one type of drug to another, so, only rough correlations can be obtained with single physico-chemical properties such as log P_{oct} and Δ log P. A theoretical model that incorporates several physico-chemical properties in one measure would, therefore, be more attractive in predictions of drug absorption. In the last part of this review, we present data suggesting that such a theoretical method based on dynamic molecular surface properties of drug molecules can predict passive drug absorption with high accuracy.

3.2. Dynamic molecular surface properties

Recently, van der Waterbeemd and Kansy established a relationship between the calculated polar molecular surface areas[1] of drug molecules and blood-brain uptake [85]. Although the criteria for the selection of the molecular conformations used in the calculation of the surface areas were unclear, and no consideration was given to the flexibility of the molecules, a relatively strong correlation was found. Furthermore, Barlow and Satoh have recently reported a similar relationship between percentage polar surface area of peptide-like molecules and log D_{oct} [84]. Together, these results suggest that molecular surface properties are of potential interest as predictors of drug absorption for conventional drugs (small organic molecules) as well as for somewhat larger peptide-like molecules.

The dynamic molecular surface properties can be determined from the (low energy) conformation(s) of the drug molecule obtained by molecular mechanics calculations of conformational preferences. The potential advantage is that the calculated surface characteristics determine numerous physico-chemical properties of (drug) molecules including lipophilicity, the energy of hydration and the hydrogen bond formation capacity [84, 92, 107, 108]. For instance, the surface properties of a molecule that forms an intramolecular hydrogen bond may be less polar resulting in an enhanced membrane permeability in comparison to a homologous molecule that exposes the (polar) hydrogen bonding group on its surface [83]. It can, therefore, be hypothesised that the relative importance of each of the physico-chemical factors will be reflected by a single measure such as the polar molecular surface area calculated from low energy conformations of the drug molecule. However, the influence of the surface characteristics on each of the physico-chemical properties may

[1] The polar molecular surface area of a drug molecule is defined as the sum of the parts of the surface area associated with polar atoms, e.g. oxygen, nitrogen and hydrogen attached to polar atoms.

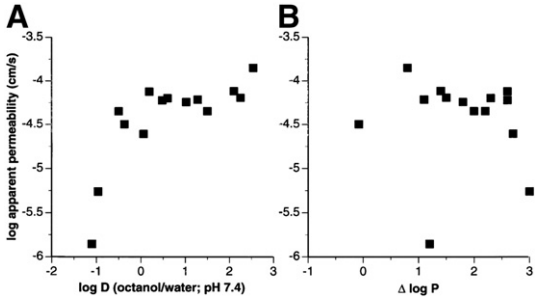

Fig. 6. Correlation between permeability of 5-fluorouracil prodrugs in Caco-2 monolayers and A. apparent octanol/water distribution coefficients at pH 7.4 or B. Δ log P (a measure reflecting the desolvation energy of the drug molecule). A classical relationship between permeability and log D_{oct} is seen in A while no obvious correlation with Δ log P is seen in B. Data were compiled from [98] with permission from the publisher.

also vary from one conformation of a drug molecule to another. Thus, it is inappropriate and also misleading to select a single conformer for the calculation of static surface area properties [109]. A dynamic method, which takes into account all preferred (low-energy) conformations, should give a better description of the surface properties than methods that consider only single conformations. Such methods have been available for a long time and are routinely used for the prediction of drug molecule-receptor interactions, i.e. in prediction of structure-activity relationships [110]. Using molecular mechanics calculations to assess the three-dimensional shape, various surface properties such as polarity and size can be calculated. To our knowledge, there is only one example of the use of such calculations for the prediction of passive drug absorption:

In this work, Palm et al. recently compared dynamic surface properties of a series of beta-adrenoreceptor antagonists and drug permeability in Caco-2 monolayers and rat intestinal segments, respectively [111]. Excellent correlations were obtained between the dynamic polar van der Waals' surface areas and the permeabilities in Caco-2 cells and rat intestine (Fig. 7). The correlations were stronger than those obtained with calculated log D_{oct}-values. Moreover, the permeability coefficients were ranked in the correct order in both models using the dynamic polar molecular surface areas, but not using the calculated log D_{oct}-values or the number of potential hydrogen bonds. These results suggest that Caco-2 monolayers can be used as a convenient reference model in theoretical predictions of drug absorption.

One limitation of using the polar molecular surface area as a predictor of drug absorption is that the number of required calculations increases rapidly with the flexibility of the molecules. This problem can be reduced by the use of more powerful computers, the development of automated calculation procedures and the introduction of analytical algorithms for the surface area calculations [112]. A second limitation is that no consideration is given to the influence of the charge of the molecule. Nevertheless, the results obtained so far indicate that the dynamic polar surface area is a better theoretical descriptor of intestinal drug absorption than calculated lipophilicity (log D_{oct}) or hydrogen bonding potential, thus motivating further studies of the predictive value of this new method. Studies on structurally more diverse molecules are therefore underway in our laboratory.

As discussed above, the decreased permeability of very lipophilic (log $P_{oct} \geq 4$) compounds is generally related to their high solubility in the lipophilic cell membranes. Although these drugs distribute rapidly into a cell membrane, their transcellular transport is decreased by a slow distribution from the cell membrane into the extracellular (aqueous) fluids [95]. However, Wils et al., who observed a parabolic relationship between permeability in HT29-18-C_1 monolayers and log D_{oct}, could not correlate the decreased permeability of the most hydrophobic drugs to an increased cellular uptake [94]. Thus, other factors, such as polarity could have contributed to the low permeability of the hydrophobic drugs. We therefore investigated the dynamic

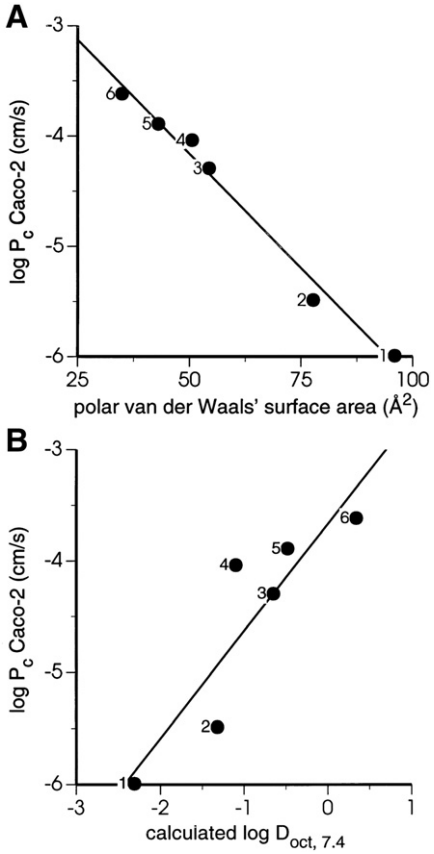

Fig. 7. Linear correlations between log cellular permeability (P_c; determined from apparent permeabilities at two different stirring rates) in the Caco-2 model, and: A: dynamic polar van der Waals' surface area ($r^2=0.99$) and B: calculated log D_{oct} values ($r^2=0.80$). The numbers refer to: 1, atenolol; 2, practolol; 3, pindolol; 4, metoprolol; 5, oxprenolol and 6, alprenolol. Each point represents mean ± one standard deviation ($n=4$). Data taken from [111] with permission of the publisher.

surface properties of these drugs. It was found that some of the very hydrophobic drugs had larger polar van der Waals' surface areas than expected [113]. This finding provides an alternative explanation for the low permeability of the drugs in HT29 cell monolayers [94]. Thus, while the low permeability of some lipophilic drug molecules undoubtedly results from retention in the lipophilic cell membranes [95, 96], the low permeability of other lipophilic drugs may be related to their polarity [113]. To summarise, the results obtained so far suggest that the dynamic polar surface area is a new promising alternative model for the theoretical prediction of drug absorption. For instance, it may have applications for predicting the influence of structural modifications on drug absorption prior to the exploration of new synthetic schemes.

4. Conclusions

Comparison of drug transport in Caco-2 monolayers with intestinal drug transport in vivo indicates that the monolayers can be used to predict drug transport by different pathways across the intestinal epithelium. The best correlation to the in vivo situation is obtained for drugs that are transported by the passive transcellular route. The passive paracellular route is less permeable in the cell monolayers than in vivo, but the data obtained so far indicate that the selectivity of this pathway is comparable to the in vivo situation. From these results, we conclude that Caco-2 monolayers can be used to identify drugs with potential absorption problems, and possibly also to select drugs with optimal passive absorption characteristics from series of pharmacologically active molecules generated in drug discovery programs. The absorption of drugs transported via carrier-mediated mechanisms can probably also be predicted in some but not all cases. However, to confirm this, a more extensive characterisation of each active transport mechanism needs to be performed.

Theoretical methods for prediction of drug absorption usually rely on a single physico-chemical property of the drug molecule such as lipophilicity or hydrogen bonding capacity. Initial studies with a new theoretical method that is related to several physico-chemical properties of the drug molecule — the dynamic polar molecular surface area — suggest that this new method may be an interesting alternative for the prediction of drug absorption. The dynamic polar surface area gives excellent correlations with drug permeability in Caco-2 monolayers and excised intestinal segments, suggesting that Caco-2 monolayers can be used as a convenient reference model for theoretical predictions of drug absorption. Very powerful methods have recently been developed for the combinatorial synthesis of large libraries of peptides and organic compounds as have methods for high throughput screening of pharmacological activity. As a result, large numbers of compounds with promising pharmacological activities are being obtained. This has increased the demand for screening methods for oral drug absorption, suggesting that the interest in cell culture models for experimental and theoretical predictions of drug absorption will continue to increase.

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