Drug absorbed administration

Introduction

The oral route is still the most desired route for the administration of medicinal products [1] due to the ease and lack of inconvenience associated with this administration route, in comparison to others such as the pulmonary route or the more invasive intravenous route.

The pharmaceutical industry has developed considerably over the past 40 years with respect to the rate at which new chemical entities are being discovered. This increased rate is primarily due to the invention of high throughput screening, but there is no correlation between the rate of synthesis of these novel compounds and the release of new drugs on the market due to the high failure rate during the development process [1] .

In order to minimise cost and resources associated with this loss, effective screening methods for both pharmacological action and bioavailability have to be used.

The most important process that influences bioavailability of the drug is absorption and the necessity of creating and using suitable models that can predict the in vivo absorption profile of a drug is absolutely critical in achieving the desired reduction in cost associated with the pharmaceutical development process.

There are two primary phases of absorption for orally administered drugs; the first is dissolution of the drug in the aqueous media present at the site or sites of absorption [1] the second is permeation of the drug particles in

solution through predominantly the small intestinal membrane into the hepatic portal vein [1] .

The main factors affecting dissolution of a drug in the gastrointestinal (GI) system are the pH of the environment, volume of dissolution media and the presence of food by either encouraging or delaying the passage of the dosage form into the small intestine where many drugs are absorbed.

Permutation of the drug through the small intestinal membrane is influenced by several variables. The presence of influx and efflux pumps on the apical surface is a main consideration [2] . There are three main routes of absorption that drugs can take; transcellular absorption through the cells, paracellular absorption by passing thorough the tight junctions between cells or by using influx transporters present on the apical surface [3] . Efflux transporters are also present which act to eject the drug molecule out of the cell and limit bioavailability [1] .

All of these processes and scenarios need to be considered in developing an in vitro model to accurately predict gastrointestinal drug absorption. The extent to which a particular model represents the results seen in vivo can be conveyed through a mathematical relationship known as the in vitro- in vivo correlation (IVIVC) [2, 4] . The predictive power of this correlation ultimately depends upon the capacity of the in vitro method used to simulate and reflect what occurred in vivo. The fact that different models are able to do this to different degrees has been appreciated as different levels of IVIVC

have been defined; levels A, B, C, multiple C and D with A being the highest level [5] .

There are many factors to consider and appreciate when looking at IVIVC made from drugs absorbed from the gastrointestinal tract, as models are either based on the dissolution of the drug within the GI media at the absorption site or permeability of the drug across the intestinal membrane.

This review primarily considers models used to simulate and predict drug permeability, with a discussion of the ability of each technique to reflect and predict the in vivo environment and response; which would allow a representative IVIVC to be formed.

In silico permeability models

These models are computer programs that aim to predict the absorption and permeability of a drug. One review [6] gave a very good summary of the programming process and highlighted the specifications against which the physicochemical properties of drugs are judged.

An advantage of using such a model is that a high turnover of compounds can be tested within a short period of time [6] , a property that makes it very practical in industry.

But in terms of developing an IVIVC, this model has limited use [7] . One major argument against the use of this model highlighted by another review [1] is that absorption predictions are based only on the physicochemical properties of the drug. This assumption is false as there are other factors to consider

such as drug – membrane interactions through active transporters and efflux pumps [1]

Parallel Artificial membrane permeability assay (PAMPA)

This technique is based on the formation of an artificial membrane by using a hydrophobic filter material as support upon which lecithin and organic solvents are placed upon to produce an artificial lipid [1] .

One recent review [8] greatly criticised the use of this technique in the drug discovery process. It was stated that there was no real benefit in using this technique over the cell culture methods such as caco-2 and MKCD cell lines because it was just as time consuming with less informative data being obtained [8] .

One of the main advantages of using this technique was that it was less labour intensive and quicker to do [9] , but this was a main focus of the argument against use of the technique by this review. Due to the different manipulations such as testing in various pH that need to be carried out, the process was deemed just as labour intensive as the caco-2 or Ussing chamber method.

An attempt to debate against the points raised by this review was done by another [9] which highlighted the ability to use this technique to obtain various information such as the partition coefficient and apparent permeability (P_{app}) of a drug.

Nevertheless, both reviews failed to specifically highlight the strengths or weaknesses of the technique in creating IVIVC. It appeared that the capacity of this technique to do so is limited as there is a gross underestimation of active transport of hydrophilic compounds with low molecular weights [1] .

Ussing Chambers

This cell technique involves the isolation of intestinal membrane and cutting the tissue into strips. These strips are clamped onto a suitable clamping device to produce a flat sheet between two chambers, the donor and receiver chamber [1] . The measurement is taken as the amount of drug that appears in the receiver chamber [1] . To monitor the viability of the intestinal tissue, electrical resistance is measured by placing a current across the membrane [1] .

Only few studies have used this technique to reflect its capability but this has only been used to show a level D IVIVC, where drug candidates during the development process are placed in rank order. One such study [10] presented this technique as being equally capable of ranking drug candidates when compared to caco-2 cells and the in situ technique of a perfused jejunum loop.

One article [11] opposes the use of this technique and presents the counter argument to the method being used to create such a correlation. The paper identified the ability of this model to be biologically representative but clearly stated that the technique is not robust enough to incorporate as a method which is routinely used in early development, due to the complexity

associated with setting up the instrument. This is a good observation and highlights an impracticality of the method.

Caco-2 cell lines and separated clones

The method that has been supported in recent studies is the Caco-2-cell culture model that has been shown to effectively mimic intestinal absorption. These cells are human colon adenocarcinoma cells that undergo proliferation when in culture [1] which are grown on small porous membranes that fit in the wells of welled plates. The sample of the drug being tested is placed on top of the membrane with the amount of drug that passes through being calculated and the P_{app} is determined.

Arguments in favour of this method state that the ability of this model to reflect in vivo conditions is very good as not only can transcellular and paracellular diffusion occur, both influx and efflux transporters are present, allowing active transport processes to be considered [1, 12] . Such transport systems are those for sugars, bile acids, the efflux transporter P-glycoprotein [11] and the more recently discovered multiple drug resistance protein (MDRP) [11] .

This view is supported by many whom consider this model to be very representative of the prediction of intestinal absorption. A study by Yee [13] analysed 36 drugs and observed the correlation between the apparent absorption (P_{app}) obtained from the cells and the percentage absorbed determined from in vivo testing.

A correlation coefficient of 0. 90 between percentage absorbed in vitro and in vivo was obtained, showing that the technique is capable of reliably predicting in vivo results [13] . Another study [14] confirmed the predictive ability of this model using 20 compounds and also established a correlation coefficient of 0. 92 between P_{app} and the percentage of dose absorbed

To further support the use of caco-2 cells, some studies [10, 11] have highlighted the ability of this method to be used in early stages of development in order to produce level D IVIVC where drug candidates are placed in rank order.

But despite all these positive aspects some [13, 15-16] remain critical of this technique because of an associated low level of reproducibility with gross variability in results from different labs [15] . This has been attribute to differing culture conditions within each lab [13, 16] . For example one study highlighted the importance of culture nutrients and duration of cell feeding as more L-methyldopa was absorbed as the feeding time increased [13] .

Another important limitation of the model that has been recognised is that as the number of cells within a cell line increases, the Trans epithelial electrical resistance (TEER), mannitol flux and cell growth changes [1] . The TEER is a validation tool used to quantitatively reflect the integrity of the monolayer as the viability of this cell culture diminishes [17] .

The cell line is unable to express mucus [17] which has been shown to act as a barrier to drug permutation in retarding drug contact with the apical

membrane of the small intestine and a fixed pH is used in the model [17] . This is not reflective of in vivo as the mucus layer has been shown to retard permutation and the pH of the small intestine changes.

A strong counter argument against the use of caco-2 cells is that the predictive power of the method differs depending upon the main absorption route that the drug uses. Two studies [14, 15] have indicated variability in the P_{app} for mannitol, polyethylene glycol (PEG) 4000 and fluorescein that have low paracellular permeability in various batches of caco-2 cells from different origins. Another study [17] clearly showed that caco-2 cells underestimated the absorption of amoxicillin – a passively absorbed drug and was not able to truly model the absorption of drugs that are absorbed using a carrier-mediated process due to the saturation or under-expression of these influx carriers and the over-expression of the efflux transporter P-glycoprotein.

This limitation of the caco-2 cell line is where the calu-2 cell line proves to be superior. This is a sub-clone of the caco-2 cell line that is isolated at a late passage number and has been shown to express different levels of sucrase isomaltase and glucose transporters [17] .

Arguments in favour of this model claim that it is more representative of the in vivo situation [17] as it expresses levels of sucrase isomaltase similar to that seen in the human jejunum [17] . UDP-transglucoronyltransferase, an enzyme involved in conjugation metabolic reaction is also seen at a level that is more representative of that in vivo and also an IVIVC has been formed using the in-vitro data obtained from this model [17] .

Another sub-clone of the caco-2 cell line is the HT29-18-C $_1$. A study [18] used this cell line and the information obtained was used to calculate a permeability coefficient (P $_C$) for a particular compound. A relationship between the percentage absorbed and the P $_C$ was formed much in the same manner as that created using P $_{app}$ and was shown to be a good model to use in the early development process.

Although this method possesses a significant flaw which is that the tight junctions established in this cell line were not as tight as those seen in vivo [18] , therefore allowing passive diffusion to occur to a greater extent than would normally occur. This was shown in the same study [18] where the P $_c$ of mannitol was ten times less than that seen in caco-2 cells, which is not reflective of in vivo conditions.

Madlin Derby Canine Kidney (MDCK) cells

The progressive changes in TEER seen in caco-2 cells have led to the use of Madlin Derby Canine Kidney (MDCK) cells as a model to predict intestinal absorption [14] . These are differentiated epithelial cells that form tight junctions when cultured in semi-permeable membranes [14] that also possess transporters, but not as many as seen in the caco-2 cell line [14] .

One study [19] highlighted both opposing arguments and those in favour of the technique by comparing the ability of the model with not only in vivo data but also with the caco-2 cell line. The predictive power of the model was similar to that of the caco-2 cells for passively absorbed compounds that showed good permeability [19] . For those that were poorly permeable or were

actively transported, the model was unable to accurately present the degree of absorption; for the latter this is due to the minimal transporters expressed by the MDCK cells [19] , resulting in a poor IVIVC

2/4/A1 cell line

This cell line which originated from fetal rat intestine was reported to mimic the permeability of the small intestine to drugs absorbed via the paracellular route to a greater extent than the caco-2 cell line [1] .

One paper [20] clearly advocates the use of this cell line because of this point as the tight junctions seen are more representative with the extent of passive absorption being similar to that in vivo. In this study this cell line was transformed in order to improve viability and a sigmoid relationship between fraction of drug absorbed in vivo and permeability coefficient obtained in vitro was obtained.

The predominant argument against the use of this model also presented by the same study [20] , was that the shape properties of the cell line were not similar to that of the small intestine. The cells are cuboidal as oppose to columnar and there was a lower number of villi present on the apical surface. This limits the model's capability of reflecting transcellular or carrier mediated absorption, which are major routes for many drugs which negatively impacts the IVIVC created.

Conclusion and the Future

In examining the arguments for and against the different cell culture techniques, the caco-2 cell line appears to be the most reflective of *in vivo*

absorption. This is because the cell line can express transporters, allow all routes of absorption, has an associated low operating cost, high reliability and throughput capacity. All these advantages make it a very practical and useful model to routinely use in industry.

Nevertheless, there is still room for improvement as the in vivo environment is not completely shown with this cell line. One significant aspect omitted is the dissolution of the drug and the impact that this process has on amount of the dose of drug available for permutation.

Therefore the next step in producing a completely reflective model that can be used to form a good IVIVC is the combination of methods to take into account the many aspects influencing bioavailability [1] with an ultimate goal of creating an in vitro gastrointestinal system model.

Incorporation of a modified caco-2 cell line that has been co-cultured with other cells such as MDCK cells with an artificial digestive system model such as the TIM-1 model is an example of such steps that can be investigated into attaining the ultimate goal. Within the TIM-1 model there is still room for improvement but it does provide a foundation to build and develop upon. The incorporation of the newly created PBL dynamic gastric model to replace the gastric compartment of the TIM-1 would be a combination that would shed more insight into actual food effects on drug absorption and permutation. Developments similar to this would eventually lead to the creation of a very reliable and reflective in vitro model.

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