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Drug Development in Cell Culture: Crosstalk from the Industrial Prospects

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Abstract

The molecular medicine and technology undoubtedly can accelerate the new pharmacophores industrial development. Even though there are many highly-efficient screening methods can be applied in the research, but we still have problems on how to explain these data qualities in the early experimental designs. The researchers recently are just focusing on the animals studies for the drug registration but this method is time-consuming, costly and harmful to animals. Therefore, we need not just focus on the biological parameters and combine them together with the absorption, distribution, metabolism, excretion and toxicity (ADMET) property, permeability and solubility for the optimization study, the cell culture system also play crucial role in the drug candidate development. In conversely to the animals study, the *in-vitro* cell culture methods are characterized by the low compound requirement and a short duration. Hence, the cell model is suitable for providing us an efficient support for selecting the most promising drug candidate. In this review, we would like to briefly discuss the permeability screening in different stages of drug development, the evaluation on the intestinal permeability of cell culture, the automated Caco-2 cells model determination, the quality control and standard method for the Caco-2 cells determination, the correlation with oral administration absorption fraction and others.

Keywords: Cell culture; ADMET property; Intestinal permeability; Solubility; Caco-2 cells model; Absorption fraction

Abbreviations: ADMET: Absorption, Distribution, Metabolism, Excretion and Toxicity; ATCC: American Type Culture Collection; BCRP: Breast Cancer Resistance Protein; BSA: Bovine Serum Albumin; CD: Candidate Drugs; CFTR: Cystic Fibrosis Transmembrane Conductance Regulator; CNS: Central Nervous System; CYP: Cytochrome; DMSO: Dimethyl Sulfoxide; ECACC: European Collection of Animal Cell Cultures; ER: Extended Release; HEPES: Hydroxyethyl Piperazineethanesulfonic Acid; HTS: High Throughput Screening; LC/MS: Liquid Chromatography/Mass Spectrometry; MDCK: Madin-Darby Canine Kidney; MES: 2-N-Morpholinoethanesulfonic acid; MDR: Multi-Drug Resistance; MRP: Multidrug Resistance-Associated Protein; MTT: 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; NCE: New Chemical Entities; NPSA: Non-Polar Surface Area; LDH: Lactate Dehydrogenase; TEM: Transmission Electron Microscope; TEER: Transepithelial Electrical Resistance; SPR: Structure Property Relationships; SDS: Sodium Dodecylsulfate; QSPR: Quantitative Structure Property Relationships; PSA: Polar Surface Area; PG: Propylene Glycol; PePT: Dipeptidetransporter; PEG: Polyethylene Glycol; LLC-PK1: Porcine Proximal Tubular Cells; LNAA: Large Neutral Amino Acids; LI: The Determination of the Candidate Drugs Stage; LO: Lead Optimization Stage, 2/4/A1: The Cell Lines Obtained from the Fetal Rat Intestinal Without Apoptosis; P-gp: P-glycoprotein; BBB: Blood Brain Barrier.

Introduction

The molecular medicine research and technology can accelerate the new pharmacophores industrial development. Recently, there are a lot of efficient screening methods can help us in accelerating the drug development process. Even though there are different types of "quick and easy" ways can provide us different biological parameters data (such as drug efficacy, ADMET properties), but we still have a lot of problems on how to explain these data qualities in the early experimental designs. In spite of the new chemical monomers biological parameters do not follow the quantitative structure property relationship (QSPR) rules, we should optimize the molecular structure changes to satisfy the different biological parameters requirements. If the fragment molecular structure changes are closey- related to the some valuable biological parameters, we cannot just consider on the biological parameters, but we have to combine them altogether such as the combination of the ADMET property [1], permeability and solubility with the drug effects for optimization study [2-4].

Undoubtedly, the drug absorption is a main limiting factor in the clinical study application of new drug development. For the example, the drug molecules can penetrate through the intestinal mucosa and bind with the target receptor or enter into the central nervous system (CNS). In order to achieve high quality and efficient evaluation of the transport process, a highly-standard *in vitro* model is required for screening a large number of molecules. The absorption process normally is affected by several factors such as the solubility, distribution, metabolism and the transport proteins [5-7]. Even though we know that the complex methods of the animal body is difficult to evaluate the oral absorption of a large number of drug molecules, therefore the oral bioavailability of compound can be determined by these "two steps process" Figure 1. The first step briefly explains that using the *in silico* method or directly applying the permeability method to predict the natural biological materials permeability. The second step, which is the important step, suggests that the permeability values can be used to predict the absorption percentage (F abs %). This step consists of several factors such as the solubility, the drugs dissolution rate, the difference of the permeability position, the difference of the gastrointestinal transit time and the different parts of the blood flow.

The first step evaluates the intestinal permeability (for the example:

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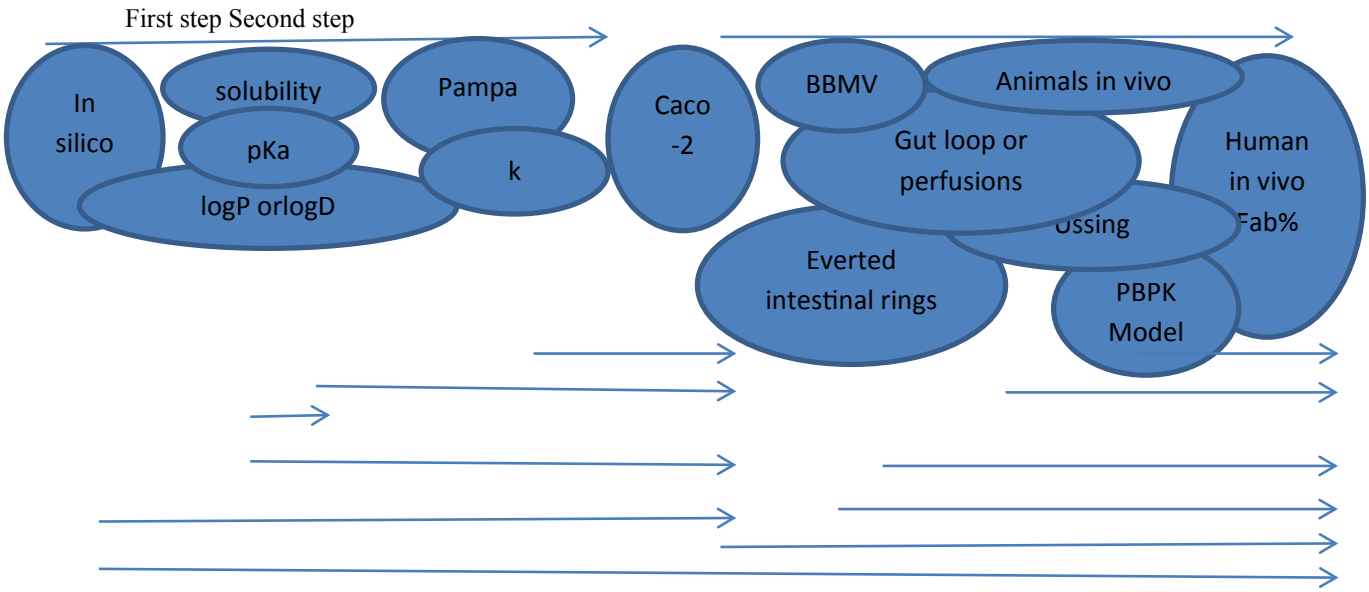

Figure 1: Evaluation on the intestinal drug absorption process-"two steps process".

the Caco-2 cell monolayer) from the physical chemical properties. This first step consists of some physical chemical properties such as the solubility, the p Ka, k, log P or log D and the *in silico* method and Pampa method are conducted to evaluate the intestinal permeability in the Caco-2 cell monolayer. The second step explains the different *in vitro* and *in vivo* techniques to obtain the intestinal permeability values to evaluate the human absorption fraction. This second step consists of some different *in vitro* and *in vivo* techniques such as the animals *in vivo*, BBMV, gut loop or perfusions, everted intestinal rings, Ussing, PBPK model and human *in vivo* Fab% to evaluate the human absorption fraction. The arrows represent the evaluation on the intestinal drug absorption process-"two steps process". Abbreviations: pKa, the acid dissociation constant; log P, logarithm of the partition coefficient, usually measured in the n-butanol/water system; log D, the number of n-butanol/water partition coefficient performance in the system; Pampa, parallel artificial membrane permeability analysis; Fab%, the oral administration descendants *vivo/in vivo* absorption in percentage; BBMV, brush border membrane vesicles; PBPK Model, physiologically based pharmacokinetic model.

The screening method can provide us many possibilities rapidly and accurately in the experimental study on the absorption of oral drug administration. The low solubility, plastic products and the cell adsorption during the metabolism process will cause the compounds existing the erroneous results. Hence, the more attention should be focused on the in vitro study to obtain the high quality data. Each different method can satisfy the different screening phase requirements, but all these must be optimized. The screening technique usually can be used to predict the intestinal permeability. Apart from that, this method can be facilitated to predict the molecular structure dynamic behavior.

The Permeability Screening Method in Different Stages of Drug Development

At the early stage of the drug development, the evaluation on the drug intestinal mucosal permeability possesses its special requirements. The LI stage is the phase that we can determine the candidate compound while the LO stage is the phase that we can optimize the candidate compound. When the efficient screening results reveal that many different compounds have their own target activity, the leading compound can provide us the basis for further molecular structure modification at the LI stage during the early permeability selection. Even though there are some compounds are lipophobic and difficult for analysis, but also have cytotoxicity. Therefore, we could not attain the high quality data at this stage. Even though we are still hoping that a large number of molecules can be obtained at the LI stage and providing us more structural information's, but the experimental problems risk may affect the high losing efficacy of the compound. The low permeability coefficient and the uncontrollable mass balance (recovery rate) will bring the false negative results. At this stage, we can exclude compounds as much as possible without economical drug development. Hence, the low permeability compound evaluation is more important than in high permeability compound.

At the LI stage, the oral drug permeability coefficient is selected for the purpose of: (1) the high throughput screening is applied to establish a structure property relationship database(SPR) or the establishment of the common permeability model in each selected group; (2) to evaluate the molecular structure compounds at the later stage that will be optimized for determining the penetration potential problems; (3) to exclude the poor absorption of the compounds; (4) to provide the effective data for the target cells.

At the LO stage, it begins in the optimization of the compound and ends in the selection of the candidate drugs (CD). The main purposes of the oral drug permeability optimization are: (1) to establish a dedicated SPR of each class that is related to the compounds and permeability; (2) to optimize the metabolism, drug dosage, drug efficacy and solubility of the permeation requirements; (3) to establish the animal *in vitro/in vivo* correlation to predict the absorption fractions; (4) to evaluate the absorption mechanism and pathways by active or passive transport , or via the cell bypass transport; (5) to evaluate the possible transporters involvement(a specific plan is required for the selection or rejection close to the carrier for establishing the SPR); (6) to evaluate the possible drug-drug interaction(need to master the new chemical monomers

and drug interactions knowledge); (7) to evaluate the drugs dosage linearity; (8) using a possible extended release, ER to evaluate the different permeability conditions.

As the different purposes outlined above, the cell culture model can provide us the main knowledge of the screening and bio-pharmaceutics evaluation as well as the transport mechanism. Next, we would like to discuss briefly about each type of the cell culture models.

The Evaluation of the Intestinal Permeability in the Cell Culture

For the cell culture based model, it is normally applied to study the drug distribution, the drug absorption mechanism, and the carrier protein and enzymes involved in the epithelial cells interaction. These methods are simple, rapid and can illustrate the absorption mechanism. The most commonly used to evaluate the intestinal barrier of the cell systems are Caco-2 cells and MDCK cells. These two cell models can provide us a very useful tool for the pre-clinical screening a large number of the compounds and the mechanism research. On the other hands, these two cell models have also been applied to analyze the cassette dosage and the combinatorial library [8,9].

Caco-2 cells monolayer model

The development of the Caco-2 cells model: The Caco-2 cells monolayer models method originated in the early 1980s. The highly-efficiency screening *in vitro* of the candidate compound absorption, metabolism, toxicity and other oral administrations have become essential parts of developing new drugs in the early stages. With the peptide synthesis, high throughput screening and the computer aided design for the new drugs development will bring the tremendous new drugs evaluation workload. When applying this method in the animals study, this will cause a lot of drawbacks such as high consumptions in drugs development, the delayed cycle duration time, require a large number of animals due to affect the big correlation in the animals variability, time-consuming and others.

The Caco-2 cells model can be classified as the new model in which it can screen in vitro rapidly the active compounds of the orally drugs administration. The main reason for widespread application of the Caco-2 cells model are the cells can be easily survived and can be naturally high-differentiated in the standard cell culture conditions. In fact, the Caco-2 cell lines are the only differentiation cell lines that have found in the spontaneous human intestinal epithelial cells. These cell lines exhibit good reproducibility, good robustness and human intestinal epithelial cells characteristics. This cell models can be used to predict the oral absorption of the various compounds [10].

The Caco-2 cell lines which are derived from the human colon adenocarcinoma cells [11] can be preserved in the ATCC library or ECACC. These cells line possess good homology and strong vitality, easily cultured and operated, only require a small amount of the drugs dosage, can be accurately controlled and well reproducibility. They are polyclonal cells populations and consist of different kinds of cells formation. They can change within the incubation time. Therefore, these cell lines should be used in the specific generation, especially are suitable for the long time screening. These cell lines not only can be used for the uptake of the drugs determination, transmembrane transport and the transport mechanism, but also can be applied to study the drugs metabolism. This feature needs to be taken into account because they can provide us the information about intestinal membrane transport, the intestinal absorption and metabolism and the drugs toxicity without considering the correlation between different species. The heterologous cells can be conducted to explain the cell morphology, the cell permeability and bypass transport pathways that have been reported by different researchers groups on the differences of transporter protein expressions [12-16]. Therefore, the cell culture solution must be standardized and evaluated effectively in the course of screening.

The Caco-2 cell lines characteristics: The Caco-2 cell lines which are the human colon adenocarcinoma cells line [17], their physiological and biochemical characteristics are quite similar to the human intestinal epithelial cells. They contain enzymes that are associated with the intestinal brush border epithelium [18,19]. If the normal mature intestinal epithelial cells are carried out in vitro cultivation process, this will bring different adverse differentiation. The Caco-2 cells can grow onto the porous permeable polycarbonate membrane to achieve the fusion under the traditional cell culture conditions. After the cells fusion process is done, the cells will appear into the columnar bulge that quite similar to the microvilli structure. Then, all these cells will proceed to the one side to form the brush border and well-differentiated tight junction complex [20].

The Caco-2 cell lines ultrastructure was studied with the electron microscope and the results showed that the Caco-2 cell lines not only have similar morphology with the human intestinal epithelial cells, but also possess the same cells polarity and tight junctions characteristics. This phenomenon will cause the corresponding substances like the fluorescent yellow CH, fluorescein sodium, mannitol and polyethylene glycol cannot penetrate through the intestinal epithelial cells barriers. Especially for producing the Caco-2 cell monolayers [21]. The detection of the pinocytotic function also has revealed that the Caco-2 cells have the same characteristics with the human intestinal epithelial cells [13]. There are several kinds of the transport systems and metabolic enzymes that involved in Caco-2 cells such as glutamine trans-peptidase, alkaline phosphatase, sucrose, glucuronic acid enzyme, the cytochrome P450 iso-enzyme, sugar, amino acid, vitamin B12 and other active transport systems that are expressed in Caco-2 cells [20]. Therefore, they can simulate the drugs *in vivo* study by the intestinal epithelial cells absorption process. Borchart and his collaborators [13] early proposed that Caco-2 cell models can be used to study the intestinal absorption of the drug molecules. There are many normal intestinal brush border enzymes that involved in the formation of tight junctions in Caco-2 cell lines such as alkaline phosphatase, transferase and amino-peptidase [22-25]. All these enzyme activities are time-dependent. The cytochrome P450 iso-enzyme and the phase II enzymes such as glutathione-S-transferase, sulfo-transferase and glucuronidase [25-28] can be identified in these cells. The CYP levels in the primary cells expressions (for the example CYP3A4) are lower [29] under the standard cell culture conditions. The CYP1A1 levels are mostly expressed in the Caco-2 cells differentiation, but are rarely expressed in the differentiated cells aggregation [27]. There are some literatures proving that the CYP3A5 activity involved in the TC7 clone [30,31]. The CYP3A4 plays a decisive role in human intestinal as drug metabolizing enzymes that can increase its activity in Caco-2 cell lines. For the example, the CYP3A4 c DNA is transfected into the Caco-2 cells [32]. There is a report stating that the 2-hydroxyl-Vitamin D3 can be used to induce the increase of enzyme activity process in the Caco-2 cells [33,34]. Other articles also reveal that the CYP3A4 is transfected with 12-O-14 phorbol-13-acetate and butyrate into the cultured cells [35], the results find that the CYP3A4 activity is significantly expressed.

In the recent years, the researchers have identified a lot of efflux transporters. Among of these efflux transporters, the MDR-1 gene

product,P-glycoprotein(P-gp), which is the most extensive efflux transporter can affect the drugs absorption and distribution [36-38]. There are 9 different efflux transporters that being identified in Caco-2 cells at the m RNA levels expressions [39]. Out of them, there are 3 efflux proteins are expressed in the protein functional levels [40]. Interestingly, Taipalensuu's study has shown that the normal Caco-2 cells do not overexpress the P-gp efflux transporters when the Caco-2 cells compared with the human jejunal biopsy. In the recent studies, the researchers have discussed a genetic binding protein BCRP, but this protein is less expressed in the Caco-2 cell lines rather than in the jejunum [39]. The multi-drug resistant binding protein, MRP, which belongs to the CFTR protein, has been identified in the Caco-2 cell lines [39-41] and indicating that it plays an essential role in the II reaction (enzyme binding reaction) metabolites transport process [42,43]. With all these reports, the researchers have shown the existence of 8~10 different MRPs. Out of them, there are only 6 MRPs existing in the Caco-2 cell lines , which are designated as MRP1~6 [39,41,44].

The active transport system expression is time dependent, and may change as the nutritional environment changes [45,46]. We can discover the obvious biological and transport characteristics changing in the Caco-2 cells monolayers [47-50] during the transport process. The para-cellular transport pathway (tight junctions) permeability can be measured without allocating the hydrophilic marker molecules into the cell membranes (such as polyethylene glycol or mannitol). The hydrophilic marker molecules in Caco-2 cell monolayers permeability are relatively low, implying that the presence of tight junctions in the epithelial tissues [51,52]. The mannitol is the reagent that most commonly used in the cells labeled molecular pathway. After culturing the Caco-2 cells monolayers within 14~21days (the passage algebra: 25~45) in our laboratory, we find the mannitol permeability is about $(0.3\sim0.5) \times 10^{-6}$ cm/s. Based on the tight junctions characteristics of the molecular mass screening, the cell bypass Caco-2 cells permeability is quite similar to the rat and human colon [53,54]. The trans-epithelial electrical resistance (TEER) measurement is the another evaluation method for the molecular cell integrity in the para-cellular transport pathway. When the TEER values of the Caco-2 cells monolayers compared with the intestinal epithelial cells, we find that the colonic epithelial cells properties are quite similar to the Caco-2 cells monolayers [13,51,55-57]. In our laboratory, we find that TEER mean values of the Caco-2 cells monolayers are about 300 Ωcm^2. Different laboratories reporting the different TEER values, normally in the range from 70 to several thousand $\Omega\ cm^2$ [14-16]. All these changes may be due to the different temperatures and different types of the instruments being used for determination as well as different kinds of the cloning or the passage process.

MDCK cells model

MDCK cells model development: The MDCK cells (Madin-Darby canine kidney) have the tight connection properties. These cells can express the low transport proteins levels and the low metabolic activities. These cells are ideal in the lipid genetics and cell protein compositions study [58]. The MDCK cells exist in the cloning of the MDCK type I cells and type II cells. In the present studies, the MDCK type II cells are probably used in the transport experiments. After the cells inoculation to reach the fusion state in 2 days, the transmembrane resistance values, TEER are about 180~250 Ωcm^2. The MDCK cells can form a polarized monolayer and forming tight junctions at the AP side. When the mannitol is used to determine the cell leakage and permeability coefficient, the Papp value is 4×10-7 cm/s, proving that the MDCK cells monolayer has good compactness. After the cells monolayers grow in 20 days, we find that the P-gp protein expressions can be detected. The P-gp substrate, Rhodamine 123 can be conducted to detect low P-gp activity [58] in the certain period of growth time (more than 25 days). Although the MDCK cells monolayer has good compactness, but the P-gp expression is less. Therefore, early in 1988, Pastan et al. [59] facilitated the human mdrl gene transfected into the MDCK cells, then establishing a large number of P-gp expressing cell lines, named as MDCK-MDR1 cell lines. These cell lines possess their own advantages such as having a short culture cycle and can achieve the uniformity between the cells generations and high human P-gp expressions. The P-gp expressions are mainly located at the top side of the cell membrane. Therefore, the MDCK-MDR1 cells can be used as rapid screening model [60] in the intestinal mucosa and drugs penetrating through the BBB.

After culturing within 3~7 days, these cells monolayers can achieve the complete differentiation. These cells monolayers have two distinct sub-clones with different TEER values. The MDCK type I cells have the high TEER values, about 4000 Ωcm^2 while the MDCK type II cells have the low TEER values, about 200~300 Ωcm^2. When the mannitol is applied in the MDCK cells monolayers, the permeability is low, less than 0.5×10-6 cm/s. For the example, Irvine and his co-authors [61] studied 55 kinds of compounds in the Caco-2 and MDCK cells model systems and found that both of these cells models permeability are same in the oral absorption. Hence, we state that the transport protein expression in MDCK cell monolayers is different from the human enteral. Although the MDCK cells monolayers can be used to determine the passive transport of intestinal epithelial cells, but the intestinal epithelial cells active uptake or efflux is invalid to study the mechanism. In generally, the low P-gp levels [62] can be expressed in MDCK cell lines. There are some articles reporting that some uptake transporter proteins such as OCT and steroid hormone could play important role in regulation [63]. The MDR1 transfected LLC-PK1 cells [64] are another cells models to evaluate the P-gp leves expressions. But, the MDCK-MDR1 cells are like other transfected cell strains that have tendency to form multi-molecular layer and imperfect polarization [65,66]. The CYP3A expression that transfected in Caco-2 cells is low and unstable [45,67] but the CYP3A expression transfected in MDCK and LLC-PK1 cells have good stability and high activity.

MDCK-MDR1 cell lines characteristics

The MDCK-MDR1 cells are normally cultured in the polycarbonate membrane at the drug transport experiment. After the cells reaching fusion state, the cell monolayers can obtain higher TEER values. There are several articles [68] reporting that the TEER values of the mature MDCK-MDR1 cells can reach to 1000 Ωcm^2 or even higher. As the TEER values reaching to the maximum values will appear big wave changes, then existing the down-warding trend phenomenon. These cell lines have similar characteristics to Caco-2 cells. There are greater differences of the TEER values in each laboratory and may be influenced by the different temperatures and the cellular algebras. In recent studies, there is an article demonstrating that the MDCK-MDR1 cell lines have the same characteristics to the MDCK cells, but some studies [69] have discovered that after 4 days in culturing MDCK-MDR1 cells will form multicellular layers. The actin distribution of MDCK-MDR1 cells is quite similar to the wild type cells, but its stress fibers are totally frequent. The MDCK and MDCK-MDR1 cells have the similar cells growth curves under the same conditions, but the growth period time of the MDCK-MDR1 cells are longer and stable than MDCK cells, thus no contact inhibition will occur. These cells have a large number of tight junction protein ZO-1 expressions and there are no obvious changes of these protein expressions.

The MDCK cells can slightly express the dog P-gp, while the MDCK-MDR1 cells can highly express the human P-gp. By applying the RT-PCR analysis, immune-prescription and Western-blot assay detection methods to measure the P-gp levels expressions, we find that the P-gp expression levels of the MDCK-MDR1 cells are higher than the wild type MDCK cells, Caco-2 cells, ECV304 and BHK21. The Western-blot determination results indicate that the P-gp level expressions of the MDCK-MDR1 cells are 25 times higher than the MDCK cells, 10 times higher than the Caco-2 cells [70]. Some studies [60] have found that by using the Western-blot assay to determine the P-gp expression levels of the MDCK wild type cells, the molecular of 170 KDa is higher than that of 150 KDa P-gp protein. But the MDCK –MDR1 cells are mainly expressed in 150 KDa of the P-gp and inter-related to their algebra. The low algebraic cell expression of the 150 KDa P-gp is higher than the high algebraic cell expression.

In the early stage, the immunizing method and the drugs transport experiments have shown that the P-gp levels of the MDCK-MDR1 cells are mainly expressed on the surface of the monolayer [60]. In the recent years, the application of the fluorescent confocal microscopy also has proven this feature. Within the two phases of culturing MDCK cells for 4 days and 10 days, there are only specific regions that can express P-gp that detected by the fluorescent confocal microscopy. Within 25 days, the P-gp expression levels slightly increase, but the P-gp are not distributed on the top side of the surface cells. When culturing the MDCK-MDR1 cells for 4 days, a large amount of P-gp can be detected onto the top side and some of them distributed in the cytoplasm, but are rarely distributed at the bottom side. As the cell culture prolongs, the P-gp distribution also maintains its state. The P-gp distribution on the top side of the upper layer cells showing the significant characteristics than before within 10 days and 25 days [69].

2/4/A1 cell line: Recently, there are a lot of researchers try to improve the intestinal epithelial cell culture by conducting the unlimited cell proliferation or oncogenic virus. The limited proliferation of the intestinal cell lines, 2/4/A1 is being used for the intestinal drug transport study [71]. These cell lines are derived from the fetal rat intestinal and dependent on the sensitive mutation temperature to perform the limited proliferation. The mutant that produced can be used to promote the ape-oncogene virus 40(SV 40) in T cells. When the cells culture in the liquid form under 33°C, the cancerous gene becomes activated and rapidly involve in the cell proliferation. When the cells culture at 37°C, the oncogenes function will be terminated, hence the cells stop growing and producing surface type partially differentiated cells. There are some reports revealing that the 2/4/A1 cells monolayer TEER values are about 25 Ωcm^2. The mannitol permeability coefficient is 15.5×10^{-6} cm/s, which is 65 times higher than the values measured in the Caco-2 monolayer cells. Thus, the 2/4/A1 cell model has proved that having better penetrability than in Caco-2 cells. The relationship between the coefficient of the permeability model drug in 2/4/A1 monolayer cell (Papp) and the oral and fractional absorption (Fa) are presented in the S shape [72]. According to the relationship, the drugs apparent permeability coefficient which is greater than 55×10^{-6} cm/s, the absorption fraction is greater than 90%, while the drugs apparent permeability coefficient which is less than 10×10^{-6} cm/s, the absorption fraction is less than 10%. This relationship results can be comparable with the permeability coefficient in the human jejunum, but are different from the Caco-2 cell models. Taken together, we can draw the conclusion that the 2/4/A1 monolayer cells can provide us an alternative and more valuable model because it can simulate the para-cellular transport barrier of the intestinal drug transport rather than the Caco-2 cells models. But however, we need to finger out that the 2/4/A1 cell lines have the poor differentiation, lack of enzymes and transporters involved when compared with the Caco-2 cell lines and the normal intestinal epithelial cells. Therefore, this transport system can only be used to study the drug effects which are not affected by the transport mechanism or the passive transport. In addition, these cells culture are more demanding and more specific if compared with the Caco-2 cell lines and the MDCK cell lines. Since till now, their applications are limited in the pharmaceutical industry.

HT29: HT29 is another study of the human colon cancer cell lines [24,51]. Under the standard cell culture conditions and the presence of the glucose, these cells can form multilayer undifferentiated cells. These cell lines can differentiate into the polar molecules with the mucus secretion and /or absorption cell layer. The differentiation degree can be adjusted by culturing the cells in the free glucose medium or by the addition of the sodium butyrate inducing the differentiated agents. This method can establish the clonal cell lines with an infinite differentiation number. When the HT29-18-C1 clones compared with the Caco-2 cell lines, they can form the low TEER values. Thus it has become the alternative model to study the cell bypass transport pathway [72,73]. The drug, for the example atenolol when is applied in the para-cellular transport, the TEER value that measured in the HT29-18-C1 monolayer cell is about 100 Ω cm^2 and significantly higher than in the Caco-2 monolayer cells about 300 Ωcm^2. The Caco-2 cells model only consist of the absorptive epithelial cells, but do not contain the mucus layer that acts as drug absorption barrier. But however, the HT-29 cell lines have mucus that can produce the subtype clones, such as HT29-H and HT29-MTX (methotrexate induced cell). These cell lines have proved that they can be applied in the development of the mucosal cells model [74-77]. The co-culture of the Caco-2 cells with the HT29-H and HT29-MTX, can improve the cells bypass transport permeability [74,76,78].

Other cell lines types

T84: Another type of the cell lines are colon cancer cell lines, T84. When these cells grow on the permeable supports, they will create high TEER values, about 1000 Ωcm^2, but these cell lines are not well -differentiated. Therefore, these cell lines are appropriate applied in the intestinal epithelial ion transport, but cannot apply these cells to the drug transport study, especially for the carrier mediated transport [10,76,79-81].

IEC-18: The rat intestinal strain IEC-18 has been reportedly used as a study of the intestinal epithelial cell permeability model. These cell lines can form the strong single molecular layer. The TEER value is about 50 Ωcm^2, the mannitol permeability is 8×10^{-6} cm/s. There are some researches illustrating that the hydrophilic molecules permeability of the intestinal cell bypass transport in the IEC-18 cell model is better than in the Caco-2 monolayer cells. The strong permeability of the cell bypass transport pathway is closely-related to the low cell differentiation levels. On the other hands, the lack of its peripheral gap which is connecting to the actin will not develop the cell barriers. Due to the low expression of the transport proteins, hence we cannot conduct them to study the carrier mediated transport [79,81].

Other cell type: Calu-3 cell model

The Calu-3 cell model development: From the beginning of 20 century 1980's, there are more researchers focusing on the pulmonary inhaled drug delivery system. Recently, there are many experimental techniques applying to study the pulmonary drugs absorption and sedimentation, including the sedimentary study, the isolated lung

perfusion, the *in vitro* cell model study and the *in vivo* pharmacokinetics analysis [82].

The *in vitro* cell model can be used to study the drugs absorption and metabolism process in the respiratory epithelium and drugs positioning, its retention time has special advantages because it can be used to explain the drugs absorption and metabolism mechanisms from the molecular aspects [83]. In the recent 20 years, there are different types of the lung epithelial cell models that simulating the respiratory tract into the blood biological barrier, including the human and murine cell models are also shown in the following Table 1 [84]. On the other hands, the differentiation and transformation of the tumor tissues and cell lines such as Calu-3, A549, 16HBE14-ð-(cells transformed human bronchiol epithelial cells of the epidermis), BEAS-2B(the permanent biochemical normal human epithelial cells) and other cells are also being developed. It is due to the fact that the Calu-3 cell models can be continuously cultured and possess good repeatability, therefore there are more researchers applying these cell models to carry out the experiments.

The primary rat type-II alveolar cell model can be used to simulate the human respiratory epithelium. These cell models have been widespread applied in the pharmacology studies, the chemical transport studies and the transport and toxicity studies of the nanoparticles inhaled into the blood circulation. But the simulation of the lung epithelial cell models which are derived from human cells can form the tight junctions. The A549 cells, which are the human carcinoma transformation cells that manifesting various characteristics of the type II alveolar cells. They can be used to study the drug absorption [85] and metabolism [86] by the alveolar way, but are difficult to reach the satisfactory in the alveolar toxicity study, tight junction formation and the drug detection. Thus, they are not the ideal cell models that can be used to simulate the alveolar epithelium cells.

By carrying out the treatment with aerosol inhalation drug, the drugs are mainly deposited in the respiratory tract. The respiratory epithelium is the place where the drugs involving in the inhalation and the intracellular drugs metabolism. Therefore, the Calu-3 cell model can be used to simulate the drugs inhalation by the delivery process. Meanwhile, it also can indirectly reflect the drug transport characteristics and metabolic process [87,88] of the airway epithelial cell model.

The Calu-3 cell model characteristics: These cells can differentiate into multiple closely-connected cells. These multiple closely-connected cells are the main characteristics of the respiratory epithelial and the drug absorption and metabolism, but these closely-connected characteristics (tight junctions), but these tight junctions can limit the drug intracellular transport. The inoculation of the Calu-3 cells can differentiate into the polarization form onto the porous membrane. These cells are made of the ciliated cells and secretory cells. They have the closely-connected cell monolayers, the cilia and mucous cell monolayer characteristics. The one side of the porous membrane is generally coated with collagen and the diameter is about 0.4 μm. After the cells inoculation, they can grow and merge into single layer [89], then reach the highest transmembrane resistance growth (TEER) within 10~14 days. These cells are polygonal in shapes, with cilia and tight junction characteristics. Generally, the Calu-3 cells are inoculated at the density of $5\times10^5/cm^2$, but a lot of experiments have shown that even if their inoculation densities reduce at $1\times10^5/cm^2$, they do not affect the highest TEER value and the highest resistance value of time in the transmembrane cell model [90]. Due to the difference of the trans-epithelial electric resistance measurement conditions and cell culture model resistance, therefore causing each laboratory measured values are different. The maximum resistance of the Calu-3 cell model in some laboratories measured values are in the range of 400~600 Ωcm^2 [91], but some of them are reportedly measured in the range 700~2500 Ωcm^2 [92]. Some studies have indicated that the trans-epithelial electrical resistance value which is greater than 300 Ωcm^2, the permeability marker of the fluorescent yellow does not change obviously and still maintain at 0.05~0.1 μg/(ml.min) [91].

The mature Calu-3 cells monolayer possesses the obvious polarization, microvilli characteristics and the asymmetry transferrin receptor expression. The microvilli are mainly distributed at the AP side. The transferrin receptor expression at the AP side is greater than at the BL side. This is consistent with the physiological characteristics of the bronchial epithelial microvilli. The P450 metabolic enzymes are widely present in the lungs. The CYP1A1 and CYP2B are expressed in the Calu-3 cell monolayer. There is only 20% of the CYP3A4 expressed in the in vivo lung epithelial. Therefore, the Calu-3 cell monolayer still can be used for the drugs oxidative metabolism study [91].

The application of the Calu-3 cell model for the drug absorption study has the following advantages and disadvantages. The advantages are: (1) only small amount of the sample is required in the experiment. The experimental conditions such as temperature, pH can be precisely controlled and time-saving; (2) these cells models can be used to study the drug absorption and metabolism mechanism in the respiratory epithelium; (3) these cells which are derived from the human sub-mucosal adenocarcinoma cells possessing good homology, easily cultured and strong life span; (4) the sample analysis is simple than the *in vivo* samples, therefore is easy for determination. The disadvantages are: (1) these cells models are made of single cell and lack of the cell heterogenety ; (2) the tight junctions causing the differences in the barrier characteristics and lung epithelial cells; (3) there are lack of some metabolic enzymes involved in [91].

The Intestinal Permeability Screening

The Caco-2 cell culture and the transport experiments

The screening method is very important at the pre-culturing stage of the cells model. Hereby, we list the experience and the experimental plan of the Caco-2 cells culture model. Under 37°C conditions, these cells can culture in the DULBECCO modified EAGLE medium, 1% non-essential amino acid, the 1.5% 200 mmol/L glutamine solution, 9% fetal bovine serum and antibiotics(penicillin and streptomycin), then incubated at the air containing 5% CO_2 [51,93,94]. The antibiotics are only added to the growth in the filter core cells, but are not included in the culture flask cell culture medium. The liquid medium should be routinely checked that has no mycoplasma contamination. The EDTA

Epithelial cells	Acquisition mode	Source	Animals source
Primary cultured cells		The trachea, bronchus,alveolar(II)	The bronchus, alveolar(II)
Continuous cultured cells	The cancer cell transformation	Calu-1,Calu-3,Calu-6 H441,HBE1,A427,A549	
	The normal lung tissue transformation	9HTE16ð-,16HBE14ð-,1HAEð-,BEAS-2B,CF/T43,AK-D	SOPC1

Table 1: The lung epithelial cells *in vitro* model of pulmonary drug delivery research.

with trypsin (0.25% tripsin+0.2% EDTA) are needed for carrying out the trypsin digestion. Next, the precision of trypsin treatment process is very important because it will directly affect the cells monolayers properties [50]. The Caco-2 cells can be inoculated into the specially designed cell culture insert filter to study the transport process. Some different filter membrane materials and aperture can be bought such as TRANSWELL(polycarbonate, derived from the Corning Life Sciences) and BD Falcon insert filter (polyethylene terephthalate, PET, derived from BD Bioscience). The Caco-2 cells can be directly cultured in the polycarbonate filter with the absence of extracellular matrix such as collagen. Other cell lines may require matrix package supports, such as collagen or the collagen gel, so as the cells can combine appropriately and carry out the differentiation process. Some reports have revealed that when using the PET filter to culture the Caco-2 cells can form multi-layers [95], therefore it is necessary to conduct it for the full test on the insert filter.

In our lab, we use 0.4 μm aperture without the matrix surrounding the polycarbonate membrane insertion filter. The large aperture may result in the both membrane sides, thus affecting the cells depolarization and misleading the drugs transport results. Some literatures have reported that the inoculum density is in the range of (0.5~5) cells/cm^2 but in our lab we use the density at 2.5×10^5 cells/cm^2. The most suitable density is achieved when the rapid fusion process occurs without forming multi-molecular layers. After growing 2~3 weeks, the cells will be attached on the filter and form the completely differentiated epithelial cells monolayers [10,13,51,55]. When the cells fusion is totally completed, the permeability of the monolayer cells is continuously kept constant for 2 weeks [51]. This means that at this stage, when the same number of single cell used to study the drugs passive transports, this feature will appear different results [13,19,51,52,55]. The vector expression is often dependent on the culture time, therefore it is necessary that the more definite intervals should be carried out in the active transport [45,96]. The pre-planning worksheet cell culture is very important. For the example, when we are in charge of the trypsin digestion function/passage in Monday, then following the Wednesday, Friday and Monday, we must replace the medium.

The cells model can be easily applied for the drug absorption study. The drugs can be added at the apical membrane (mucosal) side, then the drugs will appear at the basement membrane side. The cells model permits the opposite direction happening such as the basolateral membrane side to the apical membrane side when carrying the experiments.

The experiments are usually carried out under 37°C. The TEER values are measured to verify the single molecular layer integrity. The single molecular layer cells must be stirred during the experiment. This phenomenon not only can help us to obtain the repeatable results, but also can reduce the epithelial membrane adjacent aquifer effects [97]. The experiment does not maintain the proper agitation, thus this will significantly underestimate the permeability of the fast transport compounds. In order to create the correct establishment of the prediction cell model and the SPR model, the accurate permeability values must be determined.

In order to avoid compounds from receiving a considerable number of inverse error diffusion and keep the constant concentration gradient during the experiment, the experiments can be carried out at the "leakage" conditions(such as the drug concentration in the donor side is 10 times above the receiving side drug concentrations). Generally, the apparent permeability coefficient is calculated by using the following equation (Papp):

$$Papp=\frac{d\,Q/dt}{A\times Cd0}$$

In this equation, d Q/dt is the rate of the drug in the receiving side; Cd0 is the donor side of drugs initial concentration; A is membrane surface area.

If the concentration of donor side (Cd) changes, we can improve the Papp equation calculation because the Cd affects the concentration gradient and the passive diffusion driving force [71]:

$$Papp=\frac{k\times Vr}{A}$$

In this equation, k is the drug concentration of the receiving room in unit time change (Cr-ti/Cd-ti). Cr-ti is referred to the receiving side drug concentration at the end of each interval time; Cd-ti is the donor side of the average drug concentration at the beginning of each interval time; Vr is the receiving chamber volume; A is the surface area membrane. This calculation method can help us to obtain more accurate Papp values , especially for the Papp values which are greater than 10×10^{-6} cm/s the fast transport drugs.

There are some cultured programs have been published in an accelerated differentiation process. For the example, the monolayer cells can be cultured after 3~7 days [98-100]. We can buy the system named as BD Biocoat intestinal epithelial cell differentiation from the BD Bioscience. The drugs permeability of the monolayer cells in this system is quite similar to the Caco-2 cells properties. But however, the high permeability and low TEER values of the mannitol suggesting that the cell bypass transport barrier function is low.

The automated Caco-2 cell determination: The trend of the industrial development is using the semi-automatic or fully automatic program to determine the Caco-2 cell permeability. When using this system, about 400~500 compounds can be screened out per week. The automatic Caco-2 measurement system can be bought from Tecan/BD Bioscience and Bohdan Mettler Toledo. We can buy the automatic system to maintain the cell culture. There are some companies maintaining and developing the culture growth in permeable filter supporting the cell automatic system like Automation Co Ltd.

Here, we briefly explain Astra Zeneca R&D Molndal using the automation method for determination Caco-2 cell models. Before using the Caco-2 cell models for determination, the predicted solubility compounds must be measured. In order to measure the permeability coefficient accurately during the transport experiments, the drugs must dissolve into the cell monolayer. Thus, it will cause the inadequate determination of the solubility compounds. We usually use about 10 μmol/L of the concentration for determination. But in the specific project or in some cases, some researchers use about 1 μmol/L of the concentration. The determined compounds firstly must be soluble in DMSO (1 mmol/l) solution and the transport buffer salt is used for dilution to 10 μmol/l (the solution containing 1% DEMO).

For the automatic determination, the cell culture thermal insulation, shaking and suction are completed by the automatic micro-culture processor Plato 7(Rosys). The drug donor solution preparation including the standard solution must be completed in the multi-probe. This measurement method can deal with 4 cell monolayers in 24 well culture plates. Each drug is measured 2 times so as to gain the differences of the measurement results time/passages between different generations. We select the internal standard drug metaprolol, zomig and melagatran, their Papp values are about 15×10^{-6} cm/s (Fa=100%), 1×10^{-6} cm/s (Fa=5%).

For the automatic measurement, we use the 0.33 cm^2 polycarbonate filter on the Caco-2 monolayer cells for growth about 14~18 days. Before the automatic work starting, the TEER values must be determined. The automation system can be used to measure TEER values, such as by using the filter World Precision Instruments Inc 24 liquid storage tank culture plate system. The TRANSWELL plate base concubine is firstly filled with the new transport buffer solution. The drug solution is added into the top side of the Caco-2 monolayer cells. Then, the experiments begin. Next, we can determine the initial donor concentration from the donor side sampling. When the time reaches to 45 min and 120 min respectively, we can carry out the sampling process and determine the concentrations of the receiving pool side compounds. Finally, the changes in donor side sampling concentration is measured and the mass balance (recovery rate) is calculated in the transport experiments. Generally, the samples are collected in the liquid storage tank with 96 deep holes in culture plates. After diluting with acetonitrile, the LC/MS analysis is used for determination the sample contents. This method can measure accurately the low and high permeability compounds [Papp values are in the range of (0.05~200) × 10^{-6} cm/s]. In order to ensure the high quality and accurate Papp values, the recovery rate needs to be kept at a limited range. The recovery rate is limited in the range of 80% ~120%. The recovery rate can be calculated by the following equation:

$$\text{Recovery rate (\%)} = \frac{\text{Mr-120+Md-120}}{\text{Md-0}} \times 100\%$$

In the following equation, Mr-120 is the accumulation of drugs into the receiving side (including the removal amount of drug samples); Md-120 is the donor side drug residual amount; Md-0 is the total amount of donor side drugs at the beginning of the experiment. When the recovery rate exceeds the limitation, the Papp value is impossible to reach the high reliability, thus we need to do a further research to prove it. However, if the recovery rate is low and the Papp value is high, the Papp value may be underestimated but the high permeability material explanation is still valid. Due to the drugs binding to the low recovery filter and/or binding to the plastic surface automatic measurement system, this phenomenon causes the low recovery rate. The optimization of the solubility and bovine serum albumin (BSA) conditions will be further discussed by using the different methods to overcome the low recovery rate problem.

From the industry prospects, it is necessary to establish good data that can be downloaded into the local and global database system. The evaluation and determination of these data quality whether can be downloaded into the standard database is very important. There are different approaches that can be used to overcome these problems. The first method is to download each database. For the example, the original results and the experimental set, the experimental settings include the evaluation of data quality and important parameters (such as TEER and mass balance). Another method is only to download the specific quality standard data.

In spite of there are some articles reporting that using the automatic absorption to measure the both sides of polycarbonate membrane of Caco-2 cells, but only little public data illustrating the evaluation of automation system [101]. The bottom side membrane of the cell culture can be used to study the transport process of the secreted basolateral side to the upper side. But however, this approach leads to increase the variability and weaken the cell monolayer active transport properties, therefore this method is not desirable.

The quality control and standard method for the Caco-2 cells determination: In order to obtain the high quality repeated results, each new batch of the monolayer cells must measure their TEER values and the para-cellular transport pathway markers. For the example, the 14C-mannitol is used to determine qualitatively the permeability. We can conduct the microscope such as the transmission electron microscopy (TEM) and fluorescence microscopy to observe the morphology and integrity of the monolayer cells. If we neglect the cell culture generation, this may lead to the in migration of Caco-2 cells [47,102]. Therefore, we should take in account to check the cell passage algebra.

By going through the following standard parameters, we can improve the data quality and also enhance the data comparability:

(1) Defining the cell generation

(2) Determining the filter using the support and the growth medium

(3) Determining the culture methods, inoculation density and trypsin treatment process

(4) Micro-proving that single molecular layer morphology

(5) Regularly repeating a set of reference drug Fa(%) and Papp.

(6) The internal standard/markers and the acceptance criteria.

(7) The specific experimental conditions(such as p H, stirring , drug concentration and Papp values results)

(8) The important enzyme and transporter system function and expression characteristics.

The above factors that contribute to the differences of the permeability values can be obtained from different laboratories [94]. The different test plan documents or lab without the prior coordination of the data should not be placed together. The Caco-2 cell lines with the passage algebra in the range of 20~110 can be used for the study. Generally, the Caco-2 cell lines which are derived from the ATCC and ECACC, the passage algebra are usually about 20~40. But some laboratories use the high passage algebra of Caco-2 cell lines, such as 90~105 [94]. In our lab, we often use a low algebraic (20~45) cells. There is a significant difference between the cell bypass transport which has different cell permeability and transport proteins expression. For the example, when carrying out the cell bypass transport, the mannitol permeability are in the range of (0.1~2.0)×10^{-6} cm/s. The different P-gp substrate efflux rates also have significant changes [49,102]. Although the high generation cells are probably undifferentiated, but there are still many instances revealing that the high passage algebraic Caco-2 cell lines have good morphology and transport properties. Whether the application of the passage algebraic in the Caco-2 cells is low or high, we are recommended to use the limited transmission range to determine the cells algebraic properties.

The Correlation between the absorption fraction of the oral administration: In the recent years, there are many universities and industrial laboratories showing that the Caco-2 cells monolayer permeability can be used to predict the oral drug absorption. Therefore, the different database [93,94,103] relationships between the Caco-2 cells monolayer permeability and the human oral absorption fraction can be established. Generally, there is a great difference between the correlation curve obtained from different laboratories [94], but these studies show that Caco-2 cells model has good predictability. These studies emphasize the need to establish the standard correlations in each laboratory procedures.

The Caco-2 cells model not only can be used to study the passive transport mechanism, but it also can be used to study the transport mechanism which involves transporters. If compared with other simple artificial systems, these cell models only can be explained by the passive diffusion, which is the main advantage of Caco-2 cell model.

Another limitation of the Caco-2 cell monolayer is that they origin from the colon and the closely-connected cell structure, this will underestimate the permeability of the compound during the cell bypass transport [17]. To our knowledge, no literature reporting that the reason causing the false negative results of the fractional absorption which induces the low permeability estimation in the Caco-2 cells bypass transport. But in our experiments, when using the drug para-cellular transport pathway, the Caco-2 cells permeability cannot be used to predict the fractional absorption in the *in vivo* study.

We have established the Caco-2 cells penetrating research and drug absorption in the human body. A set of 25 model drugs can be applied for the study, as shown in the following table. We have studied the importance of the concentration and pH conditions that affecting the transport process from top side to basolateral side (absorption) and from the substrate side to the top side (secretory). At the concentration of 10 μmol/L, 50 μmol/L and 500 μmol/L, and the pH values are 6.5/7.4 and 7.4/7.4, the apparent permeability coefficient can be measured. The selected compounds which show the difference in molecular structure and transport surface properties, including the body absorption degrees like low (≤20%), middle (20%-80%) and high (≥80%) compounds are shown in the following Table 2.

The active uptake mechanism compounds (such as glucose and glycerol acid-proline) and efflux transporter substrates (such as digoxin and verapamide) are also included in this following table. For the 10~500 μmol/L concentration application, there is only little influence on the permeability value. Therefore, the concentration of this group compounds is not critical. The top donor side of the pH value changes may significantly affect the Papp values of the compounds. These effects are closely –related to the acid-base properties of the compounds. When the basic drugs pH is 7.4, the Papp values measured is higher than pH 6.5, but for the acidic compounds are in the contrary when compared with the basic compounds. As expected, when the proton couples with the transporter substrates on the top side reaching the pH value to 6.5, the permeability value is higher. The permeability values change when the pH changes. At the two different pH conditions, the compound can be divided into low, medium or high absorption fraction but the classification is same. At the drug concentration of 50 μmol/L and pH7.4/7.4 for determination of the bidirectional transport, there are obvious outflow following compounds which the outflow rate is greater than 2: acyclovir, gancyclovir , digoxin, chlorothiazide and sulfasalazine.

Remarks: 1) Fa types: low (≤20%); middle (20%~80%); high (≥80%)

2) Fa- absorption fraction; F-bioavailability; CL_H-Liver clearance rate; Q_H-Liver blood circulation(the human body-21 $ml.min^{-1}.kg^{-1}$)

3) The corresponding results, referred to the literature references.

The Caco-2 cell penetration data in the study is in the S shape relationship [71,94]. There are two kinds of the p H conditions can achieve the relationship between the curve comparability and good predictability from the Caco-2 cells penetration and absorption fraction (Fa). When the p H value is 7.4, the whole curve is slightly moving to the higher Papp values. This is due to the fact that the model drugs in the database are decided by the basic model drugs. When the Fa values

Compound	Fa(%)	Fa types	Literature references	Remarks
Ganciclovir	3	Low	[176]	
Melagatran	5	Low	In-house	
Enalaprilat	10	Low	[177]	
Sulphasalazine	12	Low	[178]	Drain substrate
Acyclovir	17	Low	[128,180]	
Chlorothiazide	25	Middle	[179,180]	Dose-dependent F;saturated abs
Mannitol	26	Middle	[178,181]	
Atenolol	56	Middle	[178,182]	
Zomig	60	Middle	In-house	
Ranitidine	61	Middle	[183]	The Fa value is calculated from $Fa=F/(1-CL_H/Q_H)$; With transport protein interaction
Digoxin	81	High	[184]	Static urinary excretion data, Fa values can be calculated from the oral and intravenous injection ;P-gp substrate
Methotrexate	82	High	[185]	Static urinary excretion data, Fa values can be calculated from the oral and intravenous injection; a decrease in absorption at high doses
Quinidine	90	High	[186]	The Fa value is calculated from $Fa=F/(1-CL_H/Q_H)$; P-gp/MRP inhibitor/substrate
Antipyrine	100	High	[187]	
Caffeine	100	High	[188]	
Metoprolol	100	High	[178,189]	
probenecid	100	High	[190]	MRP inhibitor/substrate
Propranolol	100	High	[191]	
Testerone	100	High	[192]	
Theophylline	100	High	[193]	
Verapamil	100	High	[194]	P-gp/MRP substrate inhibitor
D-glucose	100	High		Glucose transport protein
Glycine-proline	100	High		Dipeptide transport protein
L-leucine	100	High		Amino acid transport protein
L-phenylalanine	100	High		Amino acid transport protein

Table 2: It shows the establishment of the human oral absorption (Fa) and labelled compound permeability relationship in Caco-2 cell monolayers.

reach at pH6.5/7.4, the predicted Fa values that are obtained from the Caco-2 cell model permeability is in the linear regression graph with r^2=0.97, that is fully compliant with the S shape relationship. When the pH values reach at 6.5/7.4, we can obtain the S shape relationship and determine the relationship between the following absorption fraction and Papp values (unit×10^{-6} cm/s): The Papp values ≤0.3, the Fa prediction is less than 20%; the Papp values are in the range of 0.3~2.5, the Fa prediction is at the range of 20% ~80%. The Papp values ≥2.5, the Fa prediction is greater than 80%.

Optimization of the experimental conditions: pH

There are two main factors affecting the optimization results in early screening technology. For the example, the use of cell monolayer method for rapid and high throughput determination in Caco-2 and MDCK cells; the other is can synthesize the insoluble lipid compounds. A large number of different parameters that applied in the cell analysis can cause the data obtained is different. In order to increase the model prediction and the screening speed, we need to optimize different parameters [104-106].

The pH of the gastrointestinal tract is an important factor affecting the ionized drug absorption. The pH conditions can affect the drugs.

The first is the ionization degree of the weak electrolyte (dissociation), thus can affect the compounds in passive diffusion. Another is to create the pH gradient. The general pH values of the stomach are at 1~2; the p H values of the duodenum and proximal jejunum are at 5~6.5, the p H values of the middle jejunum are at 6.5~7.5, and the pH value of the ileum is almost at 8 [7,107]. The p H values change from 6.5 to 8 at the large intestine, the colon to S sigmoid. The pH values can affect the compounds dissolution and the dissociation. Therefore, it can be used to absorb different position of the uncharged compounds to provide impetus [108]. However, the intestinal epithelial cell membrane transport is normally affected by the pH conditions. This is so-called as micro-environment or the p H surface, which is located at the front surface of the small intestine epithelial cells. This is due to the fact that the H+ is secreted by the mucus layer, therefore causing the pH value is lower than in the intestinal environment [109]. There are some articles reporting that the pH value of the human colonic inner surface is in the range of 7.1~7.4, indicating that it is more alkaline than cavity when determined by the radio telemetry [110]. The p H values of the top side solution has a direct impact on the application of the single cell model transport experiments because the solution is directly in contact with the membrane, therefore we can simulate the micro-environment [104]. In all the cases, the pH values of the basal side can reflect the submucosa extracellular pH. The submucosa extracellular p H is normally influenced by the blood pH.

The screening cell model not only can reflect the gradient under the physiological conditions but also can reflect the jejunum (mainly the absorption site of most drugs) absorption. Therefore, the pH values of the top side are in the range of 6.0~6.5/7.4 gradient [104]. When the pH value is 6, the researchers have found that some compounds (n=14) having good correlation with the Fa%. For the general screening, the pH conditions in the top side solution are worse. But when evaluating the asymmetric transport, there are differences in the position, therefore we must simulate the intestinal environment so that we can carry out the experiments in a non-gradient system. If we would like to study the small intestine absorption, such as ileum, the non-gradient system may be appropriate (for the example the pH 7.4/7.4). Similarly, when we study the colonic permeability, we should also use the non-gradient system to carry out the study.

The effects of rapid evaluation pH values on the different parts of the intestinal transport are recommended as follows:

1) Jejunal transport=6.0/7.4

2) Ileal transport=7.4/7.4

3) Colon transport=7.4/7.4

The weakly acidic drug, such as salicyclic acid is influenced by the proton concentration of the top side solution [54,57,104,111]. When the pH value decrease from 7.4 to 6.5, that means the transport from the top side to basolateral will increase by more than 20 times, therefore the relationship in the pH gradient is relatively complex. Similarly, the weakly basic drug, such as alfentanil or cimetidine, the pH value will drop to 6.5, that means the passive diffusion process will be reduced to the basal side [111]. According to the pH distribution hypothesis, the ionized compounds transport has the same traits with the pKa curve.

Neuhoff et al. [112] have reported the application of the p H gradient complexity. For the example, the weakly basic drugs used in the Caco-2 cell model will appear "false outflow phenomenon" when applied in the physiological pH gradient. This "false outflow phenomenon" is not caused by the like metaprolol and atenolol. The results show that the false negative results existing in the considerable outflow exclude compounds. Therefore, we should be careful to explain the early screening data obtained from the gradient system because the transport process is not clear at the early screening conditions. If we are going to carry out the screening process, it will become difficult because there are great differences appearing between the passive asymmetric (uneven) absorption or the pH effect on the ionization outflow and carrier mediated uptake or efflux. Therefore, we are recommended to use the following methods to overcome these problems:

1) If the screening of common intestinal absorption= pH gradient system, only reported the a→b direction of the program.

2) If any direction is selected for carrier mediated transport= application of non-gradient and pH gradient system, this should report the bidirectional transfer data.

The optimization of the experimental conditions: Solubility and Bovine Serum Albumin

In order to avoid plastic materials, filter absorption or the cell monolayer accumulation, we need to optimize the permeability measurement methods for the early screening of the high lipophilic drugs. This feature has great relationship with the increasing predictive screening model [104,105]. In addition, the low solubility compounds can cause the increase in permeability determination complexity and easy to produce the erroneous results. The researchers are mainly looking for the best solvent and/or prescription for the insoluble compounds. Normally, the 100% DMSO is used as the final solution in the combinatorial parallel synthesis because the DMSO solution is the most widely used solvent in the early time. Even though at this stage is related to the improved solubility prescription, but the solubility data to improve the data quality by the biological measurement method is very important. In order to make the high quality determination in the cell model, we need to point out the low solubility limitation. If the drug does not dissolve during the experiment time, the data quality evaluation and low permeability recovery will affect the measurement. The solubility limitations are affected by several factors such as the analysis method and the involvement of the enzymes and transporters. Therefore, before determining the lipophilic drugs, we should consider the solubility, analysis and permeability problems.

In order to obtain the best oral drugs, we need to carry out a lot of experimental evaluation on the human generated pharmacophore so as we can combine with the solubility and permeability to carry out the study [2,3]. For the example, there is about 1.0 mg/kg drug dose is required for human body, if the permeability is moderate (20%~80%), then the drug solubility is required about 52 μg/ml [3]. This is equivalent to the molar mass of 400 g/mol compound, the solubility compound is approximately 100 μmol/l. When the Caco-2 cell model permeability is determined, the solubility limitation will affect the experiment results, therefore the experiment only can be carried out at the concentration of 10 μmol/l or even low. If the compounds doses in the body are less than 0.1 mg/kg dose effect that means the drug possesses the oral absorption activity. Another meaning is that, the carrier mediated transport (uptake or efflux) shows the significant results at low concentration. Taken together, this may cause the low permeability coefficient when the compounds combining with the efflux transporters at the small intestine membrane. When the compounds concentration is higher or higher doses, this will cause the efflux transporters becoming saturated or no correlation relationship.

The compounds at the plastic surface absorption and the cell membrane accumulations have the closely-connection to the lipophilic

compounds. The high lipophilic drugs with high endogenous permeability in vitro system can influence the low recovery rate, therefore we must specify the acceptable limitation rate to improve the data quality. Some researchers suggest the application of BSA for increasing the leakage conditions and reducing the absorption phenomenon [104,113]. But however, BSA has a concentration dependent relationship and being determined at 0%~4% [104]. The BSA's role can be determined by measuring the protein drug binding ability and permeability. The high protein binding response and high permeability value will increase the base's BSA [104,105].

The application of BSA in the basal side has many positive feedbacks. Firstly, it can simulate in vivo environment because albumin (serum protein content and α-glycoproteins), serum albumin and others because it can provide good foundation of leakage condition in the blood circulation [114]. Secondly, the serum protein can block the drug absorption in the plastic surface and a filter to avoid the loss compounds in the experimental system. Thirdly, the leakage worse conditions will cause the lipophilic drug accumulation decrease. The above feedbacks that improve the recovery rate and permeability of the large compound values can be applied to the experimental program. These data do not have great impact explanation for the high permeability compounds. The increase of the recovery rate can change the low "yes drug" potential compound. The SPR information that obtained from the structural fragments can provide us the necessary conditions and good permeability, therefore this phenomenon has a great influence for further synthesis. If the obtained information lost, these data will mislead chemists in the synthesis of new analogues work. Hence, we need to exclude the "wrong" from the "real" not good absorbing compounds [112].

The BSA sample provides a complex biological analysis matrix, which can inhibit the response analysis, especially in the LC/MS analysis. Before the analysis, we should separate the BSA, but this will lose some polar drugs [104]. Therefore, the cold acetonitrile solution can be used for prescription and dilution to improve the analysis, but this method depends on the analysis of compound. When using the efficient screening automation system, we are not recommended using BSA in the transport experiments and analysis.

The application of the cell model in the transport research

Paracellular transport pathway: The cell bypass transport pathway in the epithelial cells not only is size-dependent (molecular weight, volume), but also is charge-dependent [53,115,116]. In general, the active small absorption area and tight connection restrictions will cause the cell bypass transport compounds cannot be effectively absorbed. The molecular weight cut-off points of the small and large intestine are 400 g/mol and 300 g/mol respectively. The molecular weight cut-off points of the Caco-2 monolayer cells is 300 g/mol [53], indicating that the Caco-2 cell monolayer model has a lot of similar colon characteristics. The molecular radius which is larger than 0.35 nm, showing that the molecules coefficient of permeability is similar to mannitol, the corresponding absorption percentage is about 15%~50% [93]. For the neutral compounds their conditions are same as shown above, but for the charged molecules, the cations are better than anionic and easier to penetrate the tightly coupled systems [115]. This means that the cation size is larger than the neutral or anionic, but if the molecular radius is not more than 0.4~0.5 nm can also pass through the cellular membrane.

For the application of Caco-2 cell lines or other high tight junction resistance cell line screening compounds, this will mislead the low Papp values. The *in vivo* permeability data obtained from rats are high, but in the Caco-2 cell lines data is low. These differences are caused by the intercellular components. The Caco-2 cell lines can be used to evaluate the cell bypass transporter permeable intercellular constituent effects. For the example, the use of EDTA or medium without the addition of Ca^{2+} [117-120] or the application of opening tight junctions by the pharmacological tools such as cytochalasin D and other methods to open the tight junctions in the cell culture. This method is also applicable for MDCK cell monolayer [118]. The polar compounds which are tightly-connected to system will penetrate into the cell membrane to open the channel, but will not affect the trans-cellular lipid compound transport. The mannitol can be used as positive control cells at the bypass transport pathway [121]. Therefore, the Caco-2 cells are recommended to be used for evaluation the dependent transport assay, the flow of the external research for the continuous fluorescent cell bypass transport and the cell transport route direction [117]. When the Caco-2 cells in vivo is compared with the small intestine, the polar molecule permeability is lower because the single molecular layer has the smaller effective surface area than the small intestine villi and also is closely-connected with their resistance [57,72,122].

The cell transport pathway

The high molecular weight or size of large molecules can be taken consideration in the cell transport. This transport is closely-related to the hydrophobicity of molecules. The carrier mediated transport and passive diffusion compositions have been studied for many years. They are main ways for studying the drugs absorption. There are many main requirements for the passive diffusion process occurring such as the molecules are hydrophobic form. The relationship between the permeability coefficient and the inter-cell monolayers have been identified, such as determining the Caco-2 cell lines with lg P and lg D 7.4 or lg D 6.5 relationship (109,124). But however, this relationship is non-linear. When the lg P is 2, the higher lipo-philicity leads to lower the permeability [108,123,124]. For this reason, the molecular lipo-philicity parameters are paid more attention [94,125-131]. The Caco-2 cell lines have been used to evaluate different charges [116,118] and sizes of compounds [119] in the cell bypass transport and the relative compositions at the cell membrane.

The low permeability can limit the high protein and peptide oral absorption. These compounds are mainly involved in the cell bypass transport pathway, but they will be highly restricted by the channels close connection system because of their polarity and molecular size characteristics. Therefore, these peptides can use another cell transport pathway, namely transcytosis pathways that the Caco-2 cells receptor involved in mediating endocytosis [132].

Carrier mediated transport

On the other hands, the passive diffusion process can be occurred by the lipid membranes or between the cells. The drugs can be carried out by the specific system transport such as the active transport and facilitated diffusion across the biological membranes. Until now, the active transport is just discussing the nutrition or endogenous substances (such as amino acids, glucose, bile acid, small peptide), but does not play a major role in the drug absorption. However, there is enough evidence to prove the *in vivo* drug transporters involved in the configuration [42,133].

The correlation between the human body transporter system and the new drugs selection have been discussed in the industry, but also possibly to decide whether choosing a fundamental reason for the new compounds. These findings are mainly attributed to the increase

of effective "old" drug research and also the increase of transporter interaction knowledge. These transporters can be applied to explain the drug pharmacokinetic behaviors in vivo. In addition, we can gain the interaction knowledge about cytochrome P450 enzyme system. In the recent years, the transporter inhibition, induction and polymorphism are paid more attention in this study. . Furthermore, it has become the main screening tool to evaluate the transport proteins so as we can get more information on the selection of the potential candidate drugs.

There are researchers often using the Caco-2 cell lines, MDCK cell lines [31,39,41,49,63,134-141] and other cell lines to study the gastrointestinal tract different transporters. Normally these cell lines can be used for evaluation the absorption and secretion of the transport direction. In addition, these cell lines can also be used in the special transporter system transfected into new clones [27,35,66,76,77,141] or co-culture [142]. Some organic cation transporter (OCT) uptake transporter series have been identified in the porcine kidney cell lines, LLC-PK1 and MDCK cell lines [134]. In fact, we need to further elucidate the Caco-2 cell lines, as some reports pointing out the existence of this transporter in the transporter series [143-145].

Some organic anion transporters (OAT and OATP) have been identified and cloned into the kidney cells, such as LLC-PK1, MDCK, HK-2 and Caco-2 [136]. The most famous uptake transporters are amino acid transport proteins [96,134,146] and peptide transporters (PEPT1 and PEPT2) [146-149]. These two transporters series can be expressed in most of the animal small intestines to involve in the drug absorption process. The PEPT1 is also expressed in Caco-2 and HT-29 cell lines [147-149].

From the industry prospects, the existence of different transport proteins in cell system is very important in the course of screening because it can be used to explain the impact prediction values of permeability coefficient and the quantitative results. On the other hands, the drug development of animal or human, a species difference, similarity or difference between animal models and cell culture information can provide us the important basis for the data extrapolation [40].

The active and passive transport occur simultaneously in different concentration gradient, but their quantitative effects are different. The active transport plays a major role at low substrate concentration, but when the substrate concentration is higher than the saturated concentration, the passive diffusion is the main transport process. These simple rules can be used to study the cell model. There are two factors such as the compound transport dependence concentration and the cell membrane asymmetric transport can be used for evaluation the transport protein effects. The previous data have shown that, the lack of some uptake transporter expression in the Caco-2 cell model may underestimate the permeability of the active absorption compounds. However, for those the transporter experimental data which have no effects are obtained in the saturated concentration conditions. On the contrary, if the concentration conditions of 10 μmol/L or lower, then the transport will not become saturated, the Papp values may have better relationship with the in vivo absorption fraction. When the compound is the outflow transport substrate, the use of concentration is very important.

The information obtained from the cell experiment can provide us the following data:

1) Transporter interaction/binding SPR
2) Affinity and compounds on the transport protein sorting
3) The correlation between the body's internal environment and evaluation the prediction ability
4) The drug interaction may exist.
5) It can be used to determine the compounds are substrates, inhibitors or inducers.

From the industry prospects and importance, they can provide us the information in determining the subject possibility compounds, transporter interaction and the structure information. The aim is to inhibit or promote interaction (establishing a SPR system). Therefore, we need the functional and specificity for determination.

In general, we can conduct the Caco-2 cells monolayer for the standard screening model. The Caco-2 cells model applications are shown as follows:

1) By measuring the bidirectional transport of cell membrane(a to b, b to a), from these data we can calculate the absorption(a to b/b to a) or outflow rate(b to a/a to b);
2) To determine whether the concentration dependent is in the range of 1~5000μmol/L ,then we can calculate the Km and Vmax values;
3) By using the known transporter of different inhibitor or substrate(usually in 2 different concentrations determination) ,we can determine the Ki value;
4) To investigate whether the Na^+, K^+, Cl^-, H^+ are dependent ions[ion dependent on and dipeptide carrier(PEPT1)(H^+)or large neutral amino acid transporter(LNAA)(Na^+) interaction with special relativity];
5) To evaluate the dependent temperature(in 4°C still exist asymmetric transport);
6) In order to study the absorption/outflow illusion, the acid and alkali p H dependent should be monitored.

The specific ligands of the transporter protein (usually a known substrate) can be labelled by a radioactive isotope method. Recently, there are some articles reporting the Caco-2 cell lines are used to determine the substrate of paclitaxel (TAXOL) and the performance of P-gp affinity(Ki value) [150]. This method can be simply described as:1) for determination the normal 3H-taxol in the untreated b→a transport Caco-2 cells; 2) for determination of the presence (0.2 mmol/L)Verapamil in the 3H-transport of pacilitaxel. These two differences exist between the two parts of the P-gp active transport. The two concentrations of the detected compounds are about 0.25×Ki and 4×Ki, but these concentrations can be used as the pacilitaxel transport inhibitor. Gao et al. [150] are firstly selecting the 16 μmol/L and 250 μmol/L as the preferred concentration for the detected compounds. The Ki values of verapamil, vinblastine, daunorubicin and etoposide are similar to the literature reported. This method usually provides a standard process and transport protein interaction. We only need to change the radioactive activity assay ligand when applying this method. We also can use the similar methods to study the LNAA and dipeptide carrier (PepT1) interactions with substrates which have been radiolabeled and Caco-2 cell model [134]. However, the analysis and identification of the specificity substrate intestinal transporters are needed to evaluate the drug-drug interaction and human pharmacokinetics study - which is special emphasized by this authority (administrative department) [133].

There is no doubt that, in the early stages of the drug development process, it is difficult to use the NCE-transport protein interaction information. Some companies have tried several external flow rate limits (such as ER=5) for evaluation the compounds. The main purpose is to eliminate them in the early stage. In contrast, if the high ER values of the structure group compounds are excluded in the early stage, this may limit the structure modification and synthesis of compounds. This phenomenon aims for obtaining the ideal compounds in the late stage.

Interestingly, the transporter expression like enzyme expression, can be adjusted at RNA and protein levels. Nuti et al. [50] have evaluated the P-gp transporter induction and they found that when the pancreatic proteolytic enzymes are used for the separation cells from the support, the MDR1gene can be positively regulated by these enzymes. The MDR1 gene can be outflew from the nuclear receptor for SXR regulation [151]. But the transporters and drug-drug interaction are still in their early stages of development. Before they are applicable in industrial for pre-screening, we need to do a great deal of research to form a specific cell model.

Evaluation of metabolism transport process

The cell model can be used for evaluation of intestinal metabolism, including the oxidative metabolism of cytochrome P450 and phase II reaction [15,31,106]. Recently, in order to make the oral drug bioavailability image graph model, we must determine the Caco-2 cell model permeability and metabolism *in vitro* stability rate [152]. These tools can provide us the information about the evaluation of bioavailability compound. There are several important enzymes (such as the combinatorial enzyme) which exist in the intestinal mucosa. For the example, the UDP-glucose pyrophosphorylase (UGPase), glutathione-S-transferase and sulfotransferase when using the Caco-2 cells model for study [43,106]. Therefore, these cell models can be used to study the combination of II phase in the intestinal epithelial cells during the transport process.

In addition to oxidative and combinatorial metabolism, the Caco-2 cells model also has other enzymes. The main peptidases which exist at the apical cell surface are aminopeptidase N,P and W dipeptidyl peptidase IV, endopeptidase-24,11 and ɤ-glutamine transferase [153]. In addition, there are some literatures reporting the esterase activity in the Caco-2 cells models [154,155]. The Caco-2 cells model has been successfully applied to study the biological activation of pro-drugs, such as double(POC)-PMPA ester pro-drug [154], the matrix metalloproteinase inhibitor ethyl and isopropyl ester [106], thrombin inhibitor pro-drug [46,139], four peptide gastrin acyl derivatives [153] and the fruit extracted for lipase activity inhibitor [154].

The existence enzymes in the cell model can make the permeation/transport model becoming more complex. The evaluation of the experimental data can be regarded as error when screening the large compound at the early stage. This is possibly affected by the recovery rate problems or the possible variability source. The first implication is that, the drugs with low permeability caused by the metabolism rather than low passive diffusion, it has the different structure activity relationship. If the human intestinal drug degradation enzyme does not exist in the cell model, it will cause the false negative results of the single molecule layer permeability data. In general, all these meanings are not too important. In fact, the Caco-2 cells model can serve as an excellent model to study the permeability and first pass metabolism study. Although the Caco-2 cells model also contains a lot of important transporters (such as P-gp efflux transporter), this cell model still can be used to evaluate the oxidative metabolism and the limited oral bioavailability of drug carrier mediated efflux relative contribution [106].

Toxicity evaluation

The intestinal epithelial cells can form a physical and enzyme oral drug barrier. The physical barrier of the intestinal cavity including the lipid bilayer cells to form tight junction complex. Therefore, we need to use the cell model for a fully functional barrier in all experiments. In general, before and after the experiments, the mannitol, sodium fluorescein or carboxyl- fluorescein, yellow fluorescein or Rhodamine 123 [4,156] cell bypass transport marker are applied together with the TEER values measurement to evaluate the integrity of the single molecular layer [106,157]. We can conduct the algebraic period to further determine the integrity and restriction of cell monolayers when carrying out the screening process. When using the fluorescent marker fluorescein and Rhodamine 123, we need to focus on the outflow transport process [143].

When the screening method is applied for determining permeability, the compound solubility will increase in solvents and prescription of excipients, thus affecting the barrier [106,158]. These compounds can modulate the barrier, through different mechanisms [4,156,157]. For the example, they can increase the tight connection way by suppressing the carrier mediated transport on enhancing the cholesterol leaching from the lipid bilayer, thereby increasing its liquidity. There are several articles reporting that various absorption enhancers are used for toxicity screening in the Caco-2 cells monolayers [159]. There are some commonly used excipients that influence the cell monolayer integrity including polyethylene glycol (PEG400), propylene glycol (PG), cyclo-dextrin, SDS, Tween-80 and the surface active agent for inhibiting the top polarization drain system [106,160]. Therefore, the use of high concentration surfactant in the test medium should be taken in account. When the drug absorption in vitro is compared with the in vivo model, the Caco-2 cells are sensitive to the different promoters. This feature indicates the obvious relationship when the concentration used in the single molecular layer cells and having great difference in the application of the pharmaceutical research and development.

The integrity of the assay can be used for: 1) the evaluation of toxicity compound; 2) the evaluation of toxicity formulation excipients; 3) selecting the test solution; 4) preparation of the intestinal permeability enhancers.

On the other hands, the cell bypass or the cell transport markers not only can be used in the epithelial cells, the MTT test, lactate dehydrogenase (LDH), morphology and confocal laser scanning technology can also be used to evaluate the toxicity [156,161,162]. The MTT test is based on the combination of the colored MTT and lactate dehydrogenase compounds. If the cell membrane is integrity and the cell is living, the compounds will appear fluorescent condition. The LDH method is the determination of LDH (an intracellular lytic enzyme) release. If the lipid membrane damages, the LDH leakage condition will happen thus resulting the increase of the enzyme levels [162].

There are some reports illustrating the application of the cell monolayer and excipients (for the example PEG400, DMSO) for screening process will cause the lower permeability coefficient of the compound [104]. The DMSO and ethanol solution will affect the cell metabolism, leading to overestimate the permeability [163].

The calculation and prediction model for the intestine permeability

The octanol/water (log P) and Δg P (octanol/water and heptane/ethylene glycol or heptane/octanol partition coefficient distribution differences) distribution coefficient can be used to demonstrate the study of the epithelial cells by passive diffusion [164,165]. The lipophilic drugs (log P) (or a p H value, log P) can be easily measured or calculated because it can be used as the drug permeability value predictor. In the recent years, the artificial membrane (PAMPA) method is established to describe the passive diffusion. This method is similar to the Caco-2 cells monolayer method [166].

The relationship between the permeability and the lipophilic, lg P is approximately 2 will reach to the platform [108,123]. The lipid bilayer permeability of the cell membrane depends not only on the access distribution, but also the diffusion process. Therefore, we need other molecular parameters to describe this phenomenon. These parameters may be the molecular weight and volume, polar surface area (PSA, nm2), non-polar surface area (NPSA, nm2), hydrogen bonding ability, the number of the hydrogen bonds, polarization and the number of rotatable bonds [94,125-131,167,168]. The cell monolayers (for the example Caco-2, MDCK) are usually used for the optimization permeability of drugs through the intestinal mucosa. The establishment of QSAR/QSPR is very useful in describing the transporter affinity SPR [1,2,125-131,139,168-173].

The transport system normally performs the narrow specificity (SPR) of the substrate. The transporter also exists in the wide specificity such as the oligo-peptide transporters (PepT1) [137,147,148,174] and efflux transporter MDR1 gene product, P-gp [175]. The SPR efflux protein P-gp is more appropriate in the thrombin inhibitor series of new compounds [48,139], especially suitable for a class of analog peptides (peptidomimetics) [164], but also more applicable for the known substrates and inhibitors. They are normally used to evaluate the general structural features of P-gp as substrate [169,175]. There are some articles reporting the substrate structure exchanging the transporter ion properties [136]. The SPR can be evaluated by the Caco-2 cells monolayers. The Caco-2 cells monolayer can be used as affinity to test data collection system, indicating that the cell monolayer is more valuable in this field.

In the early stage of drugs screening, the application of the computational methods for predicting the intestinal permeability has a great influence in the drug companies. Egan and Lauri have introduced several calculation methods for prediction of the intestinal permeability [168]. The early stage of screening not only can save the screening time and cost, but also can be used as the tools applied in the biological screening and optimization process. The QSPR infiltration model must be established for each specific compound enteral in the industry. Each of them has their own role at the LI and LO stages. The calculation models of the early stage usually have their own common characteristics, and are built from a number of different compounds. The more specific model for a series of compounds structure must be established at the LO late stage. The data obtained from the cells model should have high quality and low variability, but do not show good precision from the different laboratories.

Many companies are using the Caco-2 cells model for the study of the intestinal permeability. For the example, Vol-Surf (Tripos Inc.) and LION'S I DEA(LION Bioscience, Inc.). The Simulation Plus has produced the Gastro Plus Module system that has the potential to predict the plasma drug concentration. The intestinal perfusion data (Peff) permeability is considered as input at the LO stage, so there is the need for the conversion from Caco-2 data to Peff data. In general, these models can provide good prediction for the oral drug absorption, but also provide the molecular surface parameters. Other important factors are the permeability or dissolved and metabolic stability of the oral drug absorption.

Conclusion

In this review, we provide the information and experiment about cell culture model application. The cell model is suitable for efficient screening model, evaluation and also appropriate for the transport pathway or mechanism study. Meanwhile, the cell culture can provide us the useful and efficient support selecting the most promising drug candidate.

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