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Evaluation strategy of drug permeability in new drug discovery and development stage

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Abstract: Drug permeability is an important factor determining the effectiveness and safety of drug treatment. In the early stage of new drug research and development, testing drug permeability can improve research and development efficiency and reduce research and development costs. Because of its advantages such as rapidity and simplicity, drug permeability has gradually attracted attention. This paper introduces the methods of two-phase distribution, chromatography, capillary electrophoresis, optical sensor and parallel artificial membrane permeation test at home and abroad in recent years.

1. Introduction

In the process of drug research and development, there are only one or several potential active ingredients among 80000-10000 chemical ingredients. It is obvious that most of the research funds and time have been invested in the initial failed experiment. In the early stage of new drug development, screening safe and effective candidate drugs can effectively improve the efficiency of development and reduce the cost of development. The pharmacokinetic characteristics of drugs are directly related to their pharmacokinetic characteristics in vivo, which is also related to their transmembrane transport process in cells. Therefore, many mathematical scholars believe that drug permeability is the key factor affecting the efficacy and safety of drug treatment.

Although the existing drug permeability evaluation technology represented by live ileal perfusion can better simulate the physiological conditions of human body, its high cost, complex operation and long-time are not suitable for large-scale detection of the permeability of candidate drugs in the early stage of new drug development. Because of its simple and rapid characteristics, in vitro evaluation of drug permeability is a hot topic. This article reviews the research progress of drug permeability in recent years.

2. Two-phase distribution method

At present, the most commonly used method is two-phase distribution method to predict drug permeability. This method takes the biofilm containing hydrophobic substances as the research object, obtains the drug content between the two phases through the distribution of hydrophobic substances and drugs between the two phases, and characterizes the drug permeability through the distribution coefficient between the two phases. At present, organic solvents such as n-octanol, cyclohexane and n-butanol are mainly used, but these organic solvents can only simulate the

hydrophobicity of biological membrane, and the phospholipid membrane modified by phospholipid bilayer is more in line with the real physiological environment. At present, this method has many problems, such as cumbersome operation, time-consuming and laborious, which limits its practical application.

In view of the shortcomings of the existing two-phase separation methods, it is planned to develop a new efficient and stable separation method that can be separated and desorbed. First, polydimethylsiloxane (PDMS) is used as the coating agent to extract the free components in the coating agent. On this basis, move the extraction needle to the GC/MS syringe and put it into the gas chromatography-mass spectrometer to complete the desorption and content analysis of the sample. In this paper, the membrane water partition coefficients of 30 drugs, such as atrazine and chlorazoline, were determined by optical fiber membrane technology and gas chromatography-mass spectrometry. The results showed that there was a good correlation between the above results and logarithmic K/W (r=0.9539).

Riviere et al. studied the lipophilicity and polarity of drugs using PDMS, polyacrylate and solid PEG fiber films. The previous study found that there was a good correlation between the transdermal permeability of 25 aromatic components and the traditional diffusion cell method (r>0.9110), suggesting the possibility of applying this technology to drug permeability experiments. Guo et al. improved this process and used a new hollow fiber membrane liquid phase microextraction method (HFMSME). They used HFMSME and HPLC to detect the logP of 10 aromatic substances, and compared their experimental methods with the original literature. It is found that the calculation results of these two methods are relatively close. However, HFMSME method is a simple and low-cost detection technology. Due to the poor stability of hollow fiber membrane, its application in drug transmembrane detection is limited.

3. Chromatography

In the evaluation of drug permeability, there are two phase separation and component analysis problems in the two-phase separation method. Therefore, it is easier to detect the distribution of drugs in the water/lipid ratio by using chromatography to simulate biofilm. According to the chromatographic theory, k=(tR-t0)/t0, where tR is the chromatographic retention time of the sample and t0 is the stagnation time of the chromatographic system. The retention time of the substance to be tested in the chromatographic system can directly reflect the permeability of the drug film. At present, high performance reverse phase liquid chromatography with octadecyl silica gel as the stationary phase has become a research hotspot. Although reversed-phase high performance liquid chromatography (RP-HPLC) has the advantages of fast, no raw materials and good reproducibility, its application in biomembrane is limited.

At present, the internationally mature cell membrane chromatography technology based on phospholipid molecule (IAMC) is an orderly phospholipid membrane chromatography system constructed on solid surface, which is more in line with the real physiological environment. Barbato et al. found that there was no significant correlation between the IAMC-based Logk value and the reported Logk value, which explained the impact of electrostatic field on the Logk value, while the traditional Logk value was only hydrophobic, so it could not be accurately determined. Because of its good repeatability and simple operation, IAMC method has been widely used in the study of drug permeability, and has achieved good results in the study of drug permeability. Kotecha et al. used the IAMC method to determine the kIAM (including acid, base, neutral and amphoteric) of 28 compounds with different characteristics, and compared it with the existing population absorption experiment (HOA) results. The study found that k'IAM and HOA data have good correlation when the mobile phase pH value of r=0.9255 is 4.5~7.4, and k'IAM data have low correlation when the

pH is greater than or equal to 7.4 (r=0.8604). Therefore, as long as the chromatographic conditions are reasonably selected, IAMC method can well predict the oral absorption after administration. Yoon et al. used 23 compounds with significantly different structures to determine the optimal reaction conditions of IAMC method, and obtained 7 new phosphodiesterase-4 inhibitors. The previous study of the research team showed that the pharmacodynamic ratio of the seven components in the body was positively correlated with the kIAM/Mr4 value in the body (r=0.9590), indicating that the IAMC method can be used as an indicator to evaluate the permeability of the passive transport drug BBB[1].

4. Capillary electrophoresis

When the biomimetic membrane used in chromatography is modified on its surface, its structure may change, resulting in some false positive or false negative when testing its permeability. Capillary electrophoresis method constructs a "quasi stationary phase" by adding micelles, micro lotion, phospholipids and other bionic structures to the operating solution of capillary electrophoresis to achieve the purpose of experimental conditions. In capillaries, the retention time of drugs can well reflect the interaction between drugs and biofilms. Compared with conventional chromatographic methods, capillary electrophoresis has the advantages of good separation effect and small sample size, which is more suitable for the early detection of candidate drugs. Diniz et al. determined the retention coefficient of polyphenols in rats by micellar capillary electrophoresis ($r \geq 0.85$), Ornskov et al. detected 22 model compounds (including 10 β Receptor blocker), the intestinal absorption coefficient of rats was compared with the oral absorption coefficient found in the literature. The results showed that the correlation of capillary electrophoresis was the highest, with a correlation of more than 0.8. Wang et al. measured the retention coefficients of 27 neutral substances by liposome capillary chromatography, and analyzed logPo/W by Shake Flask method. The results showed that the r value was 0.9798. Then, they compared the results with their absorption coefficient in the oral cavity, and obtained r=0.91. Therefore, capillary electrophoresis can not only accurately determine the lipophilic properties of drugs, but also better predict the oral absorption of drugs after administration[2].

Generally, similar membranes need to be added to the solution when screening by capillary electrophoresis, and the loss of such membranes is very large. Compared with the forward analysis method (FACE), this method has the advantages of low cost and wide application range. FACE uses the commonly used buffer solution as the separation medium, and injects the sample after the balanced mixing of the drug and biological tissue. On this basis, the molecular probe is used to analyze it quickly and accurately to realize the effective recognition of target molecules. On this basis, the peak height method is used to determine the content of drugs in the biofilm, and then the force of drugs in the biofilm is calculated, and then the drug permeability is predicted. Franzen et al. measured the apparent partition coefficients of six cationic compounds and eight anionic compounds using the FACE method. The results show that the data obtained by FACE method is consistent with the data measured by equilibrium analysis. The biggest advantage of FACE method is that it can directly analyze samples containing drugs or phospholipids, but it needs high-purity test substances.

5. Optical sensor measurement method

At present, the evaluation of drug permeability is mainly based on the determination of drug distribution ratio in oil-water phase, which indirectly reflects the drug permeability of biomaterials. In recent years, the technology based on SPR (surface plasma resolution) has been widely used to study the interaction between substances and biofilms.

Surface plasmon resonance (SPR) is a physical and chemical effect based on the complete reflection of fluorescent molecules at the phospholipid/liquid interface, which can characterize the interaction between drugs and biofilms. The biomolecule is combined with the fluorescence on the metal film to change the intensity of the generated fluorescence, so that it can generate the detection signal and reflect it back directly and quickly. Lombardi et al. measured and analyzed the cell membrane affinity of salmeterol and propranolol by SPR technology. The blood relationship data obtained by the two methods are basically the same. The results show that SPR method can better evaluate the membrane permeability of drugs. Although surface plasmon resonance (SPR) technology has the advantages of high sensitivity, rapid stability and no labeling, it has a high cost in the detection of cell drug permeability[3].

Considering the screening cost, fluoroliposome method may be a feasible method to evaluate drug permeability. The project plans to use phospholipids as carriers to carry phospholipids, build phospholipids targeted to phospholipids, and evaluate the penetration ability of phospholipids under the targeting action of phospholipids. Parry et al. used the fluorescent phospholipid method to quantitatively analyze the cellular drug permeability of simulated biological cells. They believe that this new detection technology may be used to evaluate the permeability of small drugs, but the heterogeneity of hydrophobic fluorescent molecules in liposomes cannot be ignored. The use of cheap polymer materials as sensing materials is the most promising new optical sensing technology at present. Polydiacetylene (PDA) has been widely concerned because of its color change characteristics in the visible light region. By introducing phospholipids and other substances into the polyacetylene group, a kind of liposome with fluorescence property was prepared, and the liposome was introduced into the polyacetylene group[4]. On this basis, the fluorescence properties of polyacetylene group were studied. At present, chromosomal liposomes have been widely used in the study of the interaction between biomembranes and biomacromolecules. Katz et al. studied the binding of polymyxin B derivatives to cell membrane and the permeation of cell membrane by fluorescence quenching test, electron spin resonance spectroscopy and chromotropic phospholipid method. It is found that lipoprotein plays a key role in protein-cell membrane interaction. Groysman et al. modified the chromatin liposome and found that the binding strength of chromatin lipid and coating depends on the phospholipid composition in liposome and living cells, which can accurately predict the permeability of coating. At present, this method can only qualitatively detect drug permeability, while the study of drug quantitative analysis has not been reported.

Compared with chromatography, capillary zone electrophoresis and other methods, spectrometry can be used for on-site analysis, and is compatible with microporous plate reactor, which is easy to realize automatic screening. Therefore, the use of spectral technology for high-throughput detection of cellular drug permeability will show greater potential.

6. Parallel artificial drug permeability determination method

PAMPA (Parallel American Pulse Association, PAMPA) is a new concept first proposed by Kancy in 1998. The device of the invention mainly comprises a filter disk with holes and a receiving disk. A filter material with a certain porosity is adopted, and a high-strength artificial filter material is carried out on it. The procedure of PAMPA method is simple: add buffer solution containing sample on the receiving plate, and then put the filter plate and acceptance plate together. After the equilibrium phase, measure the concentration of active substances in the donor solution and the receptor solution, and calculate the apparent permeability coefficient Papp, Papp=(- c) using the following formula × Ln (concentration of active substance in 1-receptor solution/concentration of balancing agent) × 107, where c=VD × VA/（そ VD+VA A） × t) (VA represents the volume of the recipient space, VD represents the volume of the donor space; A represents the effective area of

the artificial membrane, t represents the equilibrium time).

In PAMPA method, screening rate is an important parameter. In order to speed up drug screening, researchers have improved the materials of artificial films to reduce the proportion of drugs. Sugano et al. combined mixed phospholipids with PVDF hydrophobic membrane to obtain a new type of artificial membrane. Avdeef and others later found that in PVDF filtration membranes, artificial membranes are unstable and take a long time to reach drug balance. To solve this problem, Wohnsland et al. used micron hydrophobic polycarbonate filter membrane instead of 120 micron PVDF, which can reduce the effect of drug culture in vitro to 5 hours. Zhu et al. used hydrophilic polyvinylidene fluoride (PVDF) filter membrane as the support membrane of the artificial membrane, and its incubation time can be reduced to 2 hours. While improving the artificial membrane, it is also an effective method to screen it with faster detection methods. Mensch et al. introduced ultra-high performance liquid chromatography/mass spectrometry in the determination of PAMPA method, reducing the determination time of each sample to 1.5 minutes. Balimane et al. developed a fully automatic microfluidic chip tandem mass spectrometry (nanoESI MS) with microfluidic chips as the core. The process only took 49 seconds to complete the sample analysis. The high sensitivity and specificity of this technology is particularly suitable for high-throughput screening combined with PAMPA technology[5].

The method for determining the permeability of parallel artificial drugs is simple and has good reproducibility. In the process of new drug development, this technology is widely used in the detection of cellular drug permeability. In response to different needs of new drug research and development, researchers have established a series of PAMPA models from the aspects of membrane materials, membrane components, pH of the donor and recipient cavities, and culture time. Ottaviani et al[6]. used the PAMPA method of 70% silicone oil prop30% isopropyl myristate as an artificial membrane to determine the apparent permeability coefficient P app of a group of compounds (such as caffeine), and simultaneously determined the skin permeability coefficient KP of this group of compounds using the diffusion cell method. The correlation between various products was good (r=0.9). Their research shows that the PAMPA method used in this experiment can be used to rapidly predict the skin absorption of drugs. This method can also be used to establish the skin model of PAMAP. Han et al. established an animal model of PAMPA to measure the intestinal absorption of drugs. In addition to the above two models, PAMPA blood-brain barrier is also used to measure the blood-brain barrier permeability of drugs. Mensch et al. selected 88 representative compounds and measured their blood-brain barrier and apparent permeability Papp through brain dose and PAMPA in vivo. The analysis results show that there is a very significant correlation between the two samples (r=0.841).

At present, PAMPA has developed a variety of PAMPA simulation technologies, but because the artificial phospholipid membrane used in this technology lacks the active material transport system and corresponding metabolic enzymes, PAMPA simulation technology is only applicable to the study of the passive transport of intracellular substances. PAMPA method is usually combined with Caco-2 cell model, which can more accurately simulate the transmembrane permeation characteristics of drugs, thus providing a fast and reliable experimental basis for the development of new drugs.

7. Conclusion

Due to the advantages of short time and low cost, the in vitro cell membrane permeability detection method is particularly suitable for the early stage of new drug development. Two-phase distribution method is the earliest method to predict drug permeability, but it is not widely used because of its complex and time-consuming operation. Although the detection sensitivity of

capillary electrophoresis is very high, a large number of artificial membranes are needed, and the detection efficiency needs to be further improved. Capillary zone electrophoresis analysis is only used to determine lipophilic activity, and its results in vivo have not been reported. At present, photosensitive detection can only qualitatively detect drug permeability. The phospholipid membrane chromatography technology is relatively mature and widely used. Optical sensor measurement method has good repeatability and simplicity, but its detection mode is relatively simple, which cannot meet the diversity of current common detection methods. Compared with the traditional phospholipid membrane chromatography, the parallel artificial drug permeability determination method can obtain higher separation efficiency. In the process of new drug development, this technology is widely used in the detection of cellular drug permeability. At present, the study of drug permeability in vitro is limited to the evaluation of drug delivery in vitro, while most drugs are delivered in vivo, so the study of its mechanism in vivo will have a good development prospect.

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