Discussion on the Development of New Ophthalmic Dosage Forms of Ganciclovir and Its Pre-Development Evaluation

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Abstract

To explore the new ophthalmic dosage forms of ganciclovir which are suitable for development, and to evaluate its physical and chemical experiments before development. The absorption of drugs in the eye was discussed, and the suitable dosage forms that can increase the solubility of drugs, prolong the retention time and increase the corneal transmittance of drugs were discussed. The physical and chemical properties needed for drug development were evaluated, and the methods for determining ganciclovir by ultraviolet spectrophotometry were established. The ultraviolet methods for determining the solubility and oil/water partition coefficient of ganciclovir were established. The solubility of ganciclovir in different aqueous media and the partition coefficient of ganciclovir in oil/water system composed of n-octanol and different aqueous media were determined. To determine the theoretical basis for the next dosage form development experiment. The liposome in situ gel developed by ganciclovir can significantly prolong the retention time of the drug in the eye and increase the corneal permeability of the drug. The development of microemulsion gel can also play a better sustained-release effect. Liposome in situ gel is more advantageous than microemulsion gel for the corneal transmittance of ganciclovir in the eye, which is more conducive to improving the effect of ganciclovir and reducing the waste in the use of ophthalmic preparations. Ganciclovir is more suitable to be developed into liposome in-situ gel. The established ultraviolet spectrophotometry method for determining Ganciclovir can accurately and quickly determine Ganciclovir. The established ultraviolet method for determining the solubility and oil/water partition coefficient of Ganciclovir can be used for drug in vitro determination. The determination of the solubility of Ganciclovir in different aqueous media and the partition coefficient of Ganciclovir in oil/water system composed of n-octanol and different aqueous media provide theoretical basis for the next development of prescription screening.

Keywords: *ganciclovir, ophthalmic preparation, pre-development study*

I. Introduction

CMV is a herpes virus, and maternal infection by CMV can lead to congenital fetal malformation. When human immunodeficiency virus (Hw) and CMV are mixed infection, retinitis, encephalitis, local pneumonia, hepatitis, gastric ulcer and skin injury will occur, which will seriously affect human health [1, 2]. Cytomegalovirus retinitis (CMVR) is a full-thickness retinal necrosis disease with slow progress, with symptoms such as glitter, floating objects, blurred vision or blind spots, which may or may not be asymptomatic, but the fundus has exudation and hemorrhage.

Ganciclovir (GCV), a derivative of guanosine, has broad-spectrum antiviral activity and is the first effective antiviral drug to treat human cytomegalovirus (CMV) infection [3, 4]. It has the advantages of definite curative effect, low toxicity, less adverse reactions and so on, and it has remarkable curative effect on CMV retinitis. Ganciclovir is often used to treat herpes simplex keratitis in ophthalmology. Ganciclovir has a relatively low bacteriostatic concentration, its IC50 in vivo ranges from 20.0 to 3480.0 ng/ml [5], and its side effects are relatively small, so it is the first choice for treating CMV infection in eyes [6]. Its structure is shown in Figure 1:

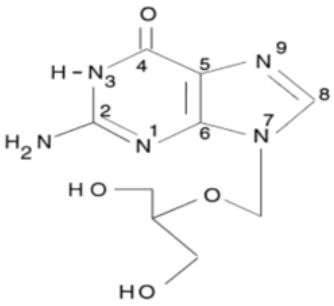

Fig.1: Chemical structure of ganciclovir

This product is a white crystalline powder, slightly soluble in water, easily soluble in acidic or alkaline solutions, but hardly soluble in ether or chloroform. GCV is an amphoteric substance with pKa1=2.2 and pKa2=9.4, respectively. The molecular weight is 255.34, and the melting point is 248-249℃.

II. The Development Direction of Ganciclovir Ophthalmic New Dosage Forms

2.1 Eye structure and drug absorption in the eye

It is the optically transparent tissue of cornea, the main refractive element of the eye and the biggest barrier of drug absorption. It consists of epithelial layer, Bowman layer, stroma, corneal posterior boundary layer and endothelial layer. See Figure 2 for cornea structure. Corneal epithelial layer has lipophilic cell membrane, which is the barrier of absorption of many water-soluble drugs.

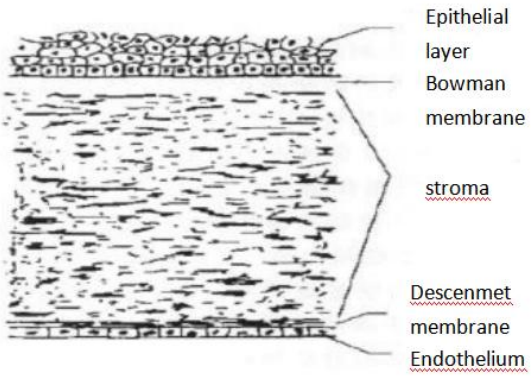

Fig.2: Corneal Cross Section

The conjunctiva covering the eyelids and eyeballs is a continuous, thin and vascularized mucosa with a surface area of 18cm2. Conjunctiva can form and maintain the tear film in front of cornea and protect eyes. Conjunctival epithelium is divided into three different types of epithelium: bulbar conjunctival epithelium adjacent to cornea, Fornix epithelium, Eyelid epithelium adjacent to eyelid epidermis. The tight junction of surface conjunctival epithelium is the main barrier for drugs to pass through conjunctiva.

Drug dripping into conjunctiva is mainly absorbed through cornea and conjunctiva [7]. Because the drug dripping method must ensure that the drug dripping into the eye first penetrates into the cornea and the cornea-anterior chamber-iris, the permeability of the drug to the cornea is particularly important. For most drugs, cornea is usually the main barrier of drug penetration [8]. See Figure 3 for the diameter of absorption chart of eye administration.

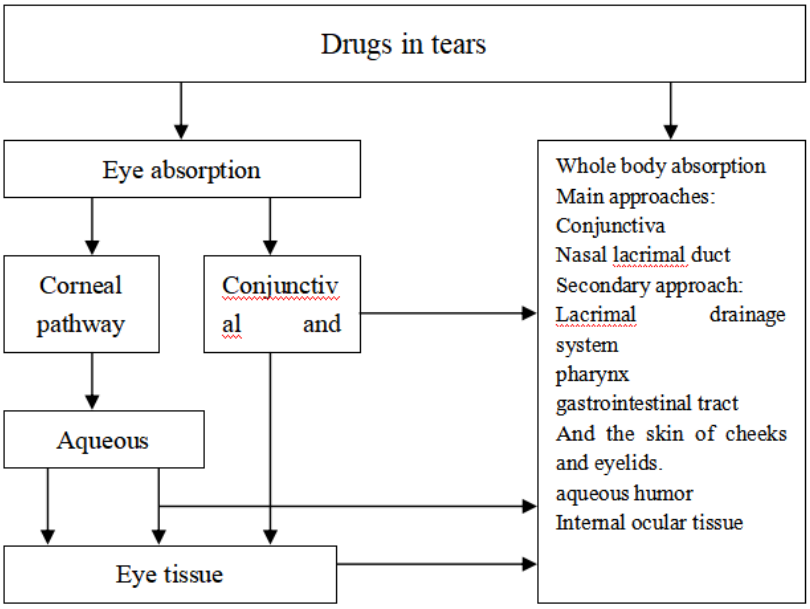

Fig.3: Ocular penetration routes for topical ophthalmic drugs administration

Corneal epithelium is the largest diffusion barrier layer, and Bowman membrane, stroma, Descenmet membrane and endodermis are the biggest barriers for lipophilic drugs. The hydration degree of cornea is as high as 78%, which is much higher than that of skin. Corneal epithelial cells are closely arranged. Although intercellular spaces can be channels for water-soluble drugs, it is difficult for water-soluble drugs to enter corneal stroma. On the other hand, fat-soluble drugs can easily penetrate the corneal epidermal cell layer, which reduces the permeability of some drug matrices.

2.2. Characteristics and existing problems of ocular administration

Eye is an important organ of human body, and eye drops in conjunctival sac are the most common drug treatment for eye diseases. According to statistics, the proportion of solution in ophthalmic preparations is over 60% [9]. Pharmacokinetic studies show that conventional eye drops have some problems, such as short retention time and low bioavailability (only 1%-10% of the administered dose). Less than 5% of drugs reach the eye through cornea to play a therapeutic role [10, 11]. Generally, about 70% of the liquid medicine of eye drops overflows directly from the eyes, and 90% of the liquid medicine will be lost if you blink [12]. This is because the eye has a very effective protection mechanism such as tears and blink reflex, so that the liquid medicine dripping into the eye can be quickly eliminated from the anterior cornea area. In addition, a large number of drugs enter the nasal cavity or digestive tract through nasolacrimal duct and are eventually absorbed by the whole body, which increases the risk of induced side effects and toxicity. On the other hand, the biological barrier of cornea also limits the drug from reaching the intraocular target tissue.

Especially for patients with hypertension and diabetes, under the influence of chronic diseases, it is easier to induce eye diseases, and the eyes are more prone to swelling and itching. As a result, the loss of liquid medicine in the form of liquid preparation to the eyes is serious, and the expected therapeutic effect cannot be achieved [13-15].

The congenital deficiency of ophthalmic liquid preparation in curative effect urges people to explore and develop ophthalmic drug delivery system with long retention time and sustained and controlled release capability. Researchers have designed various drug delivery systems to prolong the retention time of drugs in cornea and increase the absorption of drugs in eyes. Pharmaceutical scholars believe that increasing the viscosity of the solution can prolong the retention time of the drug in the conjunctival sac. The research results show that adding water-soluble polymer materials to eye drops can delay the elimination of the drug [16, 17] and improve the local

bioavailability. Increasing the permeability of drugs in the eyes can make more drugs reach and enter the eyes more effectively.

2.3. Selection of dosage forms

2.3.1 Application of in-situ gel in ophthalmic preparations

In situ gel drug delivery system refers to a semi-solid dosage form which is non-chemically cross-linked immediately after administration in solution. In situ gel has the advantages of simple preparation, convenient use, strong tissue affinity and long retention time. It combines the accurate dosage of solution, convenient administration and long retention time of gel, which makes in-situ gel especially suitable as the carrier of ophthalmic drug delivery system. In-situ gel drug delivery system has become a research hotspot in the field of pharmacy [18].

The mechanism of in-situ gel formation is to make use of the response of polymer materials to the external environment, so that the polymer can undergo reversible changes in dispersion state or conformation under physiological conditions, and realize the transformation process from solution to gel [19]. According to the mechanism of phase transition, it can be divided into pH sensitive type, temperature sensitive type and ion sensitive type.

2.3.2Application of liposomes in ophthalmic preparations

Liposome is one of the research hotspots of ophthalmic preparations at present. It has the advantages of increasing corneal permeability, slow release and reducing toxic reaction [20]. Liposome consists of phospholipid bilayer membrane, which is easy to be bio-fused, promotes the penetration of drugs into biofilm and improves the trans-corneal transport efficiency of drugs [21]. Liposomes (1iposmoe) were first proposed by Banghan et al. as a biofilm model in 1965. In their research, Bnahgna et al. found that when phospholipids are dispersed in water, they form multi-layer vesicles, and each layer is a lipid bilayer, and each layer is separated by water. Liposome can encapsulate water-soluble and fat-soluble drugs. It can not only improve the stability of encapsulated drugs, increase the therapeutic index, reduce the therapeutic dose of drugs and reduce the toxic and side effects, but also has certain targeting.

Liposomes are usually liquid solutions, which are not convenient for direct eye drops. It can be considered to disperse it in gel, which can not only improve the stability of liposomes, but also delay the release of drugs from liposomes, thus playing the role of sustained release.

2.3.3 Application of emulsion in ophthalmic preparations

Emulsion usually consists of oil phase, emulsifier, co-emulsifier and water phase. The particle size of ophthalmic emulsion is 10 ~ 1 000 nm. The oil phase and emulsifier in the prescription can improve the solubility of fat-soluble compounds and increase the utilization rate of fat-soluble drugs. In addition, the ophthalmic emulsion is submicroemulsion or nanoemulsion, which is formed by dispersing micron or nanometer oil droplets in viscous aqueous phase. This structure is very similar to the tear film that normally exists in the eyes, so it has good physiological compatibility [22-24]. With the deepening of research, it is found that it can guarantee the power of the drug in lipid emulsion to continuously transmit to corneal cells, prolong the drug effect and play a sustained release role, which makes the advantages of topical ophthalmic drug use play a better anti-inflammatory effect [25-27].

The combination of microemulsion and in-situ gel can form a microemulsion-in-situ gel system, which is liquid at room temperature (20 ~ 25℃) and triggers the formation of in-situ gel after eye administration. This kind of preparation has the advantages of prolonging the retention time of drugs on the ocular surface, increasing the corneal penetration of drugs, and no foreign body sensation in the eyes. According to the principle of triggered

phase transition, it can be divided into microemulsion-thermosensitive gel and microemulsion-ion-sensitive gel [28, 29].

2.4 Discussion

2.4.1 In-situ gel has the advantages of accurate dosage, long stay time in the eyes, improved eye transmittance, etc., and can be used to prepare temperature-sensitive and PH-sensitive in-situ gel which is more suitable for the eyes, which is a hot spot in the development of new ophthalmic formulations at present.

2.4.2 Liposomes can be coated with fat-soluble and water-soluble drugs, which can improve the therapeutic index, reduce the therapeutic dose and side effects of drugs, and have certain targeting. However, because it is in liquid state, it may be seriously lost in use as an eye preparation. Combined with in-situ gel, it can significantly prolong the retention time of the drug in the eye, and increase the corneal transmittance and efficacy of the drug.

2.4.3 Microemulsion can also increase the solubility of fat-soluble drugs, prolong the efficacy and play a sustained-release role, so as to give full play to the advantages of local administration to the eyes. The combination of microemulsion and in-situ gel can also improve the solubility of ganciclovir, prolong the retention time of drugs in the eyes, and play a better role in sustained release.

2.4.4 Temperature-sensitive in-situ gel is more beneficial to the corneal transmittance of ganciclovir in the eye than microemulsion gel, which is more conducive to improving the effect of ganciclovir and reducing the waste in the use of ophthalmic preparations.

III. Pre-development Evaluation Experiment of Ganciclovir Ophthalmic New Dosage Forms

Pre-formulation evaluation is the basis of formulation development, and its purpose is to make the drug stable, effective and suitable for the requirements of formulation and preparation process in industrial production. The ideal pharmaceutical preparation should have high efficiency, low toxicity and stable quality. When designing the prescription of pharmaceutical preparation, it is very necessary to know the structure and physical and chemical properties of drugs, which can be used as the basis for selecting dosage forms, processes and quality control in prescription design and production development. Some basic physical and chemical properties of ganciclovir were studied, the solubility of GCV in different media, oil/water partition coefficient, ultraviolet absorption wavelength of GCV were measured, and an in vitro analysis method was established.

3.1. Experimental instruments and reagents

3.1.1 Instrument

Agilent 8453 scanning visible-ultraviolet spectrophotometer (Agilent Technology Co., Ltd.); UV-9100 ultraviolet spectrophotometer; Electronic balance (Beijing Sai Dolis Balance Co., Ltd.); Tp-5 intelligent transdermal diffusion instrument.

3.1.2 Reagents

Ganciclovir (Nanjing Haichen Pharmaceutical Co., Ltd.); Octanol (Shanghai Ling Feng Chemical Reagent Co., Ltd.); Pure water; 0.1mol/L HCl (self-made); Phosphate buffer with pH6.8 (self-made); Phosphate buffer with pH7.4 (self-made); PH5.0 phosphate buffer (self-made).

3.2. Experimental method

3.2.1 Methodology for determination of ganciclovir by ultraviolet spectrophotometry

3.2.1.1 Determination of detection wavelength

Take a proper amount of GCV, weigh it precisely, use purified water as solvent to prepare GCV aqueous solution

with appropriate concentration, and prepare the corresponding adjuvant solution according to the prescription ratio. With purified water as blank, perform ultraviolet scanning in the range of 200 ~ 800 nm to determine the maximum absorption wavelength of GCV.

3.2.1.2 Drawing of Standard Curve

Accurately weigh the right amount of ganciclovir and prepare the standard solution of ganciclovir with the concentration of 20μg/mL with purified water. Suck 0.5,1,2,3,4,5,6 ml into a 10ml volumetric flask, add purified water to constant volume, and obtain ganciclovir standard solution with concentration of 1,2,4,6,8,10,12 μ g/ml, and measure absorbance A at 252nm. The standard curve of linear regression of drug concentration C with absorbance A was obtained.

3.2.1.3 Precision examination

Arrange ganciclovir standard solutions from three groups, 4.0, 8.0, and 12.0μg/mL. Then, estimate the absorbance (A) at a wavelength of 252nm, five times a day and five days, individually. And determine the intraday and interday precision of ganciclovir purified water.

3.2.2 Determination of drug oil/water partition coefficient

Octanol and buffer solutions of various pH values are mixed and left for 24h, so that they are mutually saturated. 10.0μg/mLGCV solution was prepared with phosphate buffer solution saturated with n-octanol with pH 5.0, 6.8 and 7.4. After adding GCV, the above pH values were measured. Take 5ml of each buffer in a conical flask, mix it with saturated n-octanol in equal volume, plug it, put it in a constant temperature water bath at (35 ± 1)℃, place it for 24 hours under electromagnetic stirring to reach equilibrium, and centrifuge the two phases [19]. The drug concentration in the solution before and after equilibrium was measured at the wavelength of 252nm. The oil/water partition coefficient (P) is calculated by Formula 1:

$$P = (C - Cw) / Cw \quad (1)$$

C: initial concentration of drug in n-octanol saturation; Cw: concentration of drug in aqueous medium at equilibrium.

3.2.3 Determination of drug solubility in different media

Put distilled water, artificial tears, Ringer's reagent, 0.1mol/LHCI, 0.1mol/LNaOH in a penicillin bottle, add excessive drugs, put the penicillin bottle in a constant temperature water bath at (35 ± 1)℃, stir electromagnetically for 24 hours to reach equilibrium, filter, dilute the filtrate, measure the absorbance at 252nm, and calculate the solubility of drugs in different media.

3.3. Results and discussion

3.3.1 Determination of maximum ultraviolet absorption wavelength of GCV

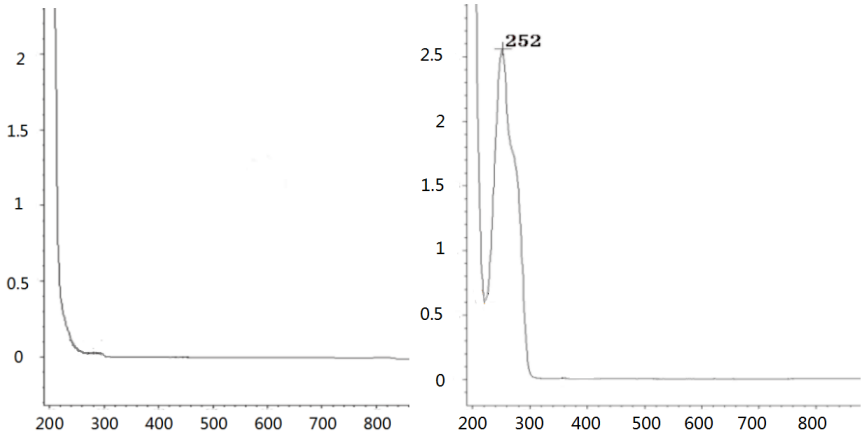

Fig.4: The UV spectrum of excipients (a) GCV (b) (200~400nm)

The ultraviolet scanning results are shown in Figure 4. From the ultraviolet scanning chart, it can be seen that GCV has the maximum absorption at the wavelength of 252nm, and the auxiliary materials have no interference here, so 252nm is selected as the detection wavelength.

3.3.2 Standard curve

Linear regression of absorbance A to C(μg/mL) gives the standard curve equation formula 2:

$$A = 4.32\times10^{-2}C \ -1.91\times10^{-4} \tag{2}$$

r = 0.9999, There is a good linear relationship in the range of 1 ~ 12 μ g/ml.

3.3.3 Precision test

Table 1 The result of Precision determination within-day (n=6)

C (μg/mL)	Found (μg/mL) 0h	1h	2h	3h	4h	5h	$\bar{C}$±SD (μg/mL)	RSD (%)
4.00	3.96	3.97	3.95	3.82	3.92	3.83	3.91±0.062	1.59
8.00	7.92	7.94	7.88	7.84	7.86	7.91	7.89±0.038	0.48
12.00	11.93	11.96	11.86	11.88	11.91	11.85	11.90±0.043	0.36

Table 2 The result of Precision determination between-day(n=6)

C (μg/mL)	Found (μg/mL) 0d	1d	2d	3d	4d	5d	$\bar{C}$±SD (μg/mL)	RSD (%)
4.00	3.95	3.97	3.85	3.81	3.82	3.83	3.87±0.070	1.81
8.00	7.94	7.96	7.93	7.87	7.84	7.85	7.89±0.051	0.65
12.00	11.97	11.95	11.96	11.85	11.87	11.84	11.91±0.060	0.50

The precision experiment results are shown in Table 1 and Table 2. The results show that the method has good reproducibility. The intra-day RSD of ganciclovir is less than 1.59% and the inter-day RSD is less than 1.81%. The precision results meet the methodological requirements.

3.3.4 Drug oil/water distribution coefficient

Table 3 Effect of various pH on the octanol-water partition coefficients (n=3)

pH	P
5.0 PBS	0.040±0.064
6.8 PBS	0.038±0.078
7.4 PBS	0.025±0.043

See Table 3 for the measurement results of oil-water partition coefficient of drugs. It can be seen from the measurement results that the oil-water partition coefficient of GCV is small. And the oil/water partition coefficient of GCV decreases with the increase of pH value. The oil/water partition coefficient of drugs is one of the main factors that affect the corneal penetration degree and penetration rate of drugs.

3.3.5 Drug solubility in different media

Table 4 solubility of GCV in diverse medium (35 ± 1)℃(n=3)

Medium	pH	Solubility(mg/mL)
Water	7	4.30±0.054
Simulated tear	7.5	15.67±0.083
GBR	8.2	11.56±0.067
0.1mol/l HCI	1	21.78±0.039
0.1mol/l NaoH	11	30.14±0.041

The determination results of drug solubility in different media are shown in Table 4. From the results, ganciclovir is slightly dissolved in water (S=4.3mg/mL), and the solubility increases under the condition of partial acid or partial alkali. The solubility in simulated tears is 15.67 mg/mL, which is about 4.12 times that in water. The solubility in Ringer's test solution is 11.56 mg/mL, which is about 3.04 times of that in water, and it can meet the conditions of leakage in vitro.

3.4 Discussion

3.4.1 A method for the determination of ganciclovir by ultraviolet spectrophotometry was established in this part. After UV scanning, the excipients did not interfere with the determination. Ganciclovir had good linear relationship in the range of 1~12μg/ml, high precision and good reproducibility. This method can be used for accurate and rapid determination.

3.4.2 A UV method for determining the solubility and oil/water partition coefficient of ganciclovir was established. The method is simple, sensitive and reproducible, and can be used for drug determination in vitro.

3.4.3 The solubility of ganciclovir in different aqueous media was determined. It can be seen from the determination results that the solubility of drugs in acidic and alkaline aqueous solutions is relatively high.

3.4.4 The partition coefficient of ganciclovir in oil/water system composed of n-octanol and different aqueous media was determined. The Oct/Water system was used to study the oil-water partition coefficient of drugs with constant temperature stirring method. The results showed that the drug had poor lipophilicity and pH had little effect on the oil-water partition coefficient of drugs, which provided a theoretical basis for the next prescription screening.

IV. Summary

Ganciclovir developed into liposome in-situ gel can improve the solubility of Ganciclovir drug, increase the retention of drug in eyes, and has certain targeting, which can selectively penetrate cornea and further improve the drug utilization rate. The established ultraviolet spectrophotometry, the ultraviolet determination methods of ganciclovir solubility and oil/water partition coefficient are all used for drug determination in vitro. The solubility of ganciclovir in different aqueous media and the partition coefficient of ganciclovir in oil/water system composed

of n-octanol and different aqueous media also provide theoretical basis for the next development of prescription screening.

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