Expert Opinion

Acyclovir delivery systems

Rita Cortesi† & Elisabetta Esposito
†*University of Ferrara, Department of Pharmaceutical Sciences, CoReS Techno Group, Via Fossato di Mortara, 19-44100 Ferrara, Italy*

Herpes viruses (herpes simplex, varicella zoster, cytomegalovirus) are the main cause of a wide variety of human infections. Although the development of successful antiviral agents against infections caused by herpes viruses had been slow until the last decade, the production of delivery systems for acyclovir are a promising alternative. The present review summarizes the principal advances made in developing carriers for the delivery of acyclovir by different routes of administration.

Keywords: acyclovir, drug delivery, drug formulations, herpes simplex virus, microparticles, microemulsions, nanoparticles, vesicles

Expert Opin. Drug Deliv. (2008) 5(11):1217-1230

1. Introduction

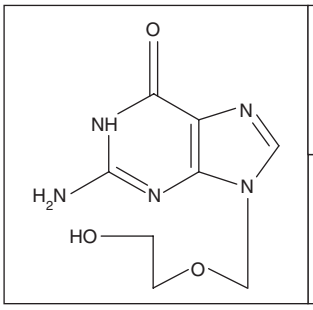
Acyclovir (9-[(2-hydroxyethoxy)methyl]guanine) (**Figure 1**) is a guanosine analog with an acyclic side chain at the 9-position. The activity of acyclovir is mainly directed against the herpes group of DNA viruses, such as herpes simplex virus type 1 (HSV-1), herpes simplex virus type 2 (HSV-2), varicella-zoster virus (VZV), Epstein–Barr virus and cytomegalovirus [1]. Acyclovir is characterized by poor physicochemical properties in terms of bioavailability and suboptimal formulations, thus the treatment is far from optimal. The fundamental pharmacokinetic properties of acyclovir are well established [2]. The i.v. administration of acyclovir is described as a two-compartment open model and the absorption of orally administered acyclovir is slow, variable and incomplete with an oral bioavailability of 15 – 30% [3].

Herpes simplex virus is highly contagious, spread by direct contact with infected individuals. The virus penetrates the epidermis or mucous membrane epithelium and replicates within the epithelial cells. After the primary infection, the latent non-replicating virus resides mainly within the dorsal root ganglia, whence it can reactivate, invade the skin and cause recrudescent lesions. HSV-1 is usually facial or non-genital, whereas HSV-2 commonly induces genital lesions. The pathological changes consisting in epidermal cell destruction by the herpes virus result in intra-epidermal vesicle and multi-nucleate giant cells. HSV-1 and -2 are sensitive to the inhibitory effects of acyclovir. The inhibitory doses range from 0.1 – 0.6 μM.

The mechanism of action of acyclovir is related to the inhibition of the HSV–DNA replication by the activity of viral thymidine kinases inhibiting viral DNA polymerases and thus blocking viral DNA synthesis. Acyclovir uptake and intracellular phosphorylation to monophosphate is mediated by viral thymidine kinase. It is further converted to triphosphate by cellular enzymes, which competitively inhibits viral DNA polymerases. The triphosphate is incorporated into elongated DNA chains as monophosphate, where it acts as chain terminator because of lack of 3′ hydroxyl group. Inactivation of viral DNA polymerase occurs following the formation of a complex between terminated DNA template and the enzyme. Since acyclovir is selectively converted to its active form in herpes virus-infected cells, it is not toxic to normal, uninfected cells [4-7].

It is well known that after administration, various acyclovir formulations are not able to ensure suitable drug levels in the target sites, probably because of their

10.1517/17425240802450340 © 2008 Informa UK Ltd ISSN 1742-5247



M.W.	225.2
λ max	254 nm

Figure 1. Chemical structure and some physico-chemical characteristics of acyclovir.

low water and lipid bilayer solubility [1]. Attempts have been made to improve the bioavailability of acyclovir by synthesizing new drugs or improving the characteristics of the existing pharmaceutical dosage forms to enhance the absorption of acyclovir.

The present review summarizes the main progress in developing carriers or formulations for the delivery of acyclovir by different routes of administration. A schematic view of the systems described in the present review is shown in **Figure 2**.

2. Routes of administration of acyclovir

For the treatment of herpes virus infections, acyclovir can be administered through different routes. Particularly, topical application is widely used for the treatment of various herpes simplex infections [8], such as ocular (herpes simplex keratitis), vaginal and lip pathologies. In contrast, oral or i.v. administration of acyclovir has been extensively studied in both immunocompetent and immunocompromised hosts for both prophylaxis and treatment of herpes virus infection [9-11]. For instance, the i.v. formulation is used for the treatment of herpes simplex encephalitis in normal hosts and varicella zoster virus infections in immunosuppressed subjects and is shown to be superior to vidarabine. Moreover, parenteral acyclovir is the agent of choice for serious mucocutaneous, visceral or central nervous system disease due to HSV or VZV, unless resistance is suspected.

However, the currently available therapies are characterized by many limitations. For instance, as previously reported, the gastrointestinal absorption of acyclovir is slow, dose-dependent, highly variable and incomplete (approximately 80% of the oral dose is excreted through the feces) [2,9]. Nevertheless, the oral route is preferred to parenteral administration since this obviates the risk of local toxicity at the injection site due to the use of needles. The mean plasma half-life ($t_{½}$) of acyclovir is 3 h in adults with normal renal function. Hence, repeated administration of high doses (200 mg five times daily for 10 days) for the effective management of HSV infections is required [12]. On topical application the absorption of the drug is very slow and needs a permeation enhancer, whereas bolus rapid injection causes renal precipitation of the drug.

None of these regimens reproducibly reduces the risk of recurrent genital lesions [13]. Frequently recurring genital herpes can be suppressed effectively with chronic acyclovir dosage regimens [14]. The dosage requirements are very high for immunocompromised patients and recurrence is common after discontinuation of therapy [15]. Topical acyclovir ointment is not associated with clinical benefits in recurrent herpes labialis. Furthermore, oral acyclovir has been associated infrequently with nausea, diarrhea, rash, or headache. The topical treatment of cutaneous HSV infections using acyclovir offers several advantages over systemic therapy: the drug can be directly targeted to its site of action, reducing circulating drug levels and hence, attendant adverse effects. However, topical acyclovir creams have demonstrated only modest efficacy, partly due to a number of pathophysiological parameters, such as the type and phase of infection, severity of infection and the patient's immune status [16,17]. Moreover, ointment formulations have shown little or no clinical benefit in the treatment of cutaneous lesions (i.e., herpes labialis) [18,19], probably attributable to the slightly improved permeation of acyclovir from creams. Although the inability of acyclovir to efficiently penetrate the stratum corneum (SC) barrier has been proposed as one of the principal reasons for inadequate topical acyclovir therapy [16,20], a dermatopharmacokinetic study has shown that while total epidermal concentrations of acyclovir subsequent to topical delivery are superior to those attained after oral administration, the latter appears to deliver more drug to the basal epidermis, the site of infection [21]. Formulation strategies to enhance cutaneous acyclovir permeation have included the incorporation of enhancers [19,20] and the use of polymeric vehicles [22].

3. Acyclovir: prodrugs and delivery systems

3.1 Prodrug polymer conjugates and SADDS

Prodrug design strategies have been employed to improve the delivery of drugs with undesirable pharmacokinetic properties such as chemical stability and lack of specificity. Targeted prodrug design represents a new strategy for site-directed and efficient drug delivery. Targeting of drugs to transporters and receptors to aid in site-specific carrier-mediated absorption is a clinically significant approach. The term 'prodrug' or 'proagent', first introduced by Albert [23], indicates pharmacologically inactive chemical derivatives that could be used to temporarily alter the physicochemical properties of drugs, to increase their usefulness and/or to decrease associated toxicity. This term usually implies a covalent link between a drug and a chemical moiety, but sometimes it is used to indicate some forms of salts of the active drug molecule [24].

Among the many acyclovir prodrug synthesized [25], the most famous is valacyclovir. Valacyclovir is an esterified

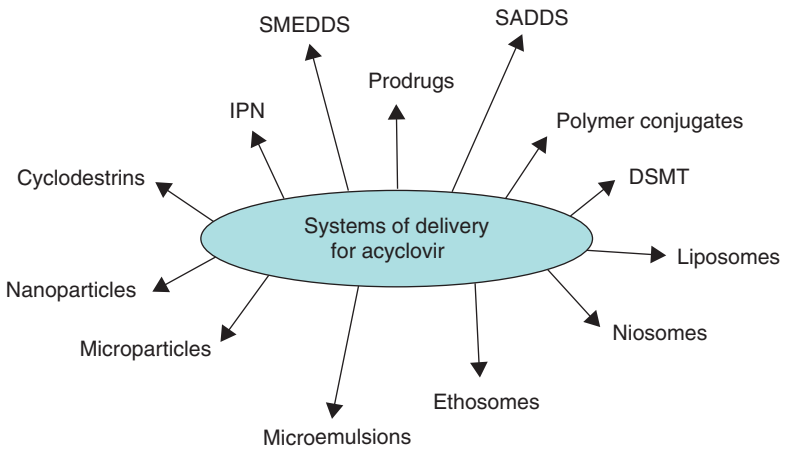


Figure 2. Schematic representation of the systems used for the delivery of acyclovir described in the present review.

version of acyclovir obtained by the addition of a naturally occurring amino acid, l-valine to acyclovir. This prodrug has greater oral bioavailability (about 55%) than acyclovir (10 – 20%). It is converted by esterases to the active drug acyclovir via hepatic first-pass metabolism. Valacyclovir results in the achievement of plasma acyclovir concentrations superior to those obtained with oral acyclovir, while requiring less frequent administration. Valacyclovir is at least as effective as oral acyclovir for a number of indications [26-28].

Anand *et al.* [29] studied the feasibility of improvement of ocular bioavailability of acyclovir by designing amino acid prodrugs targeted to the amino acid transporters on the rabbit cornea. Particularly, two water soluble amino acid ester prodrugs of acyclovir, namely γ-glutamate-ACV (EACV) and L-tyrosine-ACV (YACV) were studied across freshly excised rabbit cornea. It was found that EACV inhibited the uptake of [^{3}H]L-Arg in rabbit primary corneal epithelial cells and exhibited longer half-life in cornea in comparison to YACV. In contrast, the amino acid transporter did not recognize YACV as its transport across the cornea, and was not inhibited by arginine. However, YACV and EACV exhibited excellent antiviral activity against HSV-1 and 2 and VSV in comparison to acyclovir.

Amidon and colleagues [30] have broadened the application of Peptide Transporter Associated Prodrug Therapy (PTAPT) to non-peptidyl type prodrugs, such as amino acid ester prodrugs. They synthesized several amino acid ester prodrugs of the nucleoside antiviral drug acyclovir and examined their intestinal absorption characteristics in three different experimental systems [31,32]. Amino acid ester prodrugs significantly (three to tenfold) increased the intestinal absorption of their parent drugs via a peptide transporter mediated mechanism, even though they do not have a peptide bond in their structures. Following the membrane transport, this prodrug was rapidly converted to the active parent drug by intracellular hydrolysis [32]. These studies also demonstrated that the hPEPT1 transporter can recognize various amino acid groups with stereoselectivity and that the nucleoside component can be varied, providing a new rationale for non-peptidyl prodrug design targeting peptide transporters. Other research groups [33,34] have independently reported the peptide transporter-mediated membrane transport of valacyclovir and confirmed the high potential of PTAPT in non-peptidyl as well as peptidyl drug design.

The group of Palmberger [35] has developed a novel oral delivery system for the efflux pump substrate acyclovir using thiolated chitosan as excipient able to inhibit P-glycoprotein (P-gp). The inhibition of P-gp might be a promising strategy to improve intestinal uptake of acyclovir [36] since it has been demonstrated that *in vitro* acyclovir absorption could be increased in the presence of P-gp-specific inhibitors [37,38]. Permeation studies on rat intestinal mucosa and Caco-2 monolayers were performed using three chitosan-4-thiobutyl-amidine (Chito-TBA) conjugates with increasing molecular mass. Additionally, drug release behavior from tablets comprising the conjugates and acyclovir were tested. It was found that the secretory transport of acyclovir was 2.5-fold higher in the case of the Caco-2 monolayer and 2.3-fold higher in case of rat small intestine with respect to the absorptive transport. Transport studies across Caco-2 monolayers showed that P-gp inhibition is dependent on the average molecular mass of thiolated chitosan. The higher the molecular mass of Chito–TBA, the more sustained was the release of acyclovir.

Tallury *et al.* [39,40] have studied the release of antiviral drug acyclovir and antibacterial drug chlorhexidine diacetate from synthesized copolymers of ethyl methacrylate and hexyl methacrylate of different molecular weights. The effect of the copolymer molecular weight and the effect of drug loading into the copolymer on the release of the drugs were studied. Copolymers (I-IV) of ethyl methacrylate (EMA) and hexyl methacrylate (HMA) were synthesized by free radical solution polymerization with a yield of 76 – 82%. Copolymers I and II were of higher molecular weight with

respect to copolymers III and IV. The copolymers were impregnated with 2.5, 5.0 and 7.5 wt.% of acyclovir and chlorhexidine diacetate individually and the release rate of these drugs in water at 37°C was examined. The *in vitro* rate of drug release showed a sustained release of drugs over an extended period of time. Particularly, the acyclovir release rate increases with a decrease in copolymer molecular weight from copolymers I to IV, while chlorhexidine diacetate release rate was higher from copolymers I and II with respect to that from copolymers III and IV. Acyclovir release rate increases steadily with increase in drug load. Taken together, these results suggest that the variation of the copolymer molecular weight as well as the drug concentration alters the drug release rates, and thus it is possible to control the release rates to desired values.

Kalachandra and co-workers [41] studied the performances of the biocompatible ethylene vinyl acetate copolymer on the release of acyclovir and gancyclovir at oral therapeutic levels over extended periods of time. Dry square films were prepared from the sheet obtained by solvent evaporation of polymer casting solutions of ethylene vinyl acetate and drug (40:1 by weight). The drug release characteristics from the drug-loaded films were examined for 14 days. The rate of drug release of gancyclovir was almost threefold with respect to acyclovir. This difference in rate values may be explained due to its relatively greater solubility in ethylene vinyl acetate copolymer, facilitating faster diffusion of the molecules through the channels of the cast polymer. In the case of acyclovir, the molecules may undergo molecular associations, perhaps dimerization or trimerization, in addition to its lower solubility in ethylene vinyl acetate copolymer. The diffusion of acyclovir tended to be slower under these circumstances as compared to gancyclovir, resulting in a lower rate value. Biological studies revealed that acyclovir exhibited a remarkable decrease in a number of viral organisms present in a virus infected cell culture system using reverse transcriptase polymerase chain reaction (RT–PCR).

Self-assembled drug delivery systems (SADDS) are defined as the self-aggregates of amphiphilic prodrugs [42]. Knowledge of the self-assembly of amphiphilic prodrugs and the formation rules of SADDS is very limited. Jin and co-workers [43] have synthesized a series of cholesteryl derivatives of antiviral nucleoside analogs, such as acyclovir, didanosine and zidovudine, and the different acyl linkers, such as succinyl, adipoyl and phosphoryl. The unique structure of the nucleoside-derived amphiphiles determined the type and strength of intermolecular interaction between themselves or between them and their surroundings, and the physicochemical properties, including self-assembly behavior. The molecular self-assembly of derivatives preferentially occurs in the non-competitive solvents (i.e., chloroform, tetrahydrofuran) based on intermolecular hydrogen bonding. The derivatives formed nanosized vesicles based on hydrophobic interaction after injecting their non-competitive solvent solutions into water. This paper provided some basic rules about the physicochemical properties of lipid derivatives of nucleoside analogs. More importantly, the antiviral nucleoside analogs were derived to amphiphilic prodrugs and then the obtained SADDS might act to deliver themselves *in vivo* and release active agents in macrophages, the targets of SADDS. Macrophages are the reservoirs of many viruses [44-46], thus the targeting of the antiviral SADDS would be very useful.

3.2 Vesicular drug delivery systems

In recent years, vesicles have become the vehicle of choice in drug delivery. Lipid vesicles were found to be of value in immunology, membrane biology, diagnostic techniques and genetic engineering [47-49]. Vesicles can play a major role in the transport and targeting of active agents. Vesicular drug delivery systems delay drug elimination of rapidly metabolizable drugs functioning as sustained release systems. This system solves the problems of drug insolubility, instability and rapid degradation. Consequently, a number of vesicular systems carrying acyclovir, such as liposomes, niosomes, ethosomes, etc, were developed.

3.2.1 Liposomes

Liposomes have shown great potential as versatile drug delivery systems to deliver many bioactives, including proteins, peptides, antineoplastic agents, antibiotics, and antiviral drugs [50-53]. Conventional liposomes (unilamellar and multilamellar liposomes) have certain limitations, such as low entrapment efficiency for water-soluble drugs, stability problems and release of drugs after a single breach in the external membrane [50,54,55]. This challenge has been successfully met by the use of a multivesicular liposomal drug delivery system [56,57]. Multivesicular liposomes are multiple non-concentric aqueous vesicles surrounded by a network of lipoidal membranes. Multivesicular liposomes contain a neutral lipid (triolein, tricaprylene, trilaureine, tributyrine, etc) as an integral component, which is responsible for its unique multivesicular structure [56,58]. The multivesicular liposomes technology has successfully been used to deliver several small molecules, analgesics, antitumor drugs and antiviral drugs for prolonged therapeutic concentrations [58-61]. Jain and colleagues [58] have designed a depot delivery system of acyclovir sodium using multi-vesicular liposomes prepared by reverse phase evaporation method. The loading efficiency of the multivesicular liposomes (45 – 82%) was found to be three to sixfold higher than concentric multilamellar vesicles. In contrast, the *in vitro* release of acyclovir from multivesicular liposomes was sustained and 70% of the drug was released in 96 h, whereas conventional multilamellar vesicles released 80% of the drug in 16 h. The multivesicular liposomal delivery system as an intradermal depot offers the advantage of a very high loading and controlled release of acyclovir for an extended period of time. In fact, following intradermal administration to Wistar rats, multivesicular liposomes showed: i) effective plasma concentration for 48 h compared

with free drug solution (12 – 16 h); ii) C(max) values that were 1.4-fold lower with respect to the free drug solution; and iii) their area under the curve (AUC) value was threefold higher as compared to free drug solution. Taken together, these results suggest that multivesicular liposomes could reduce the toxic complications and limitations of conventional i.v. and oral therapies.

Law *et al.* [62] have studied an acyclovir-containing liposome system for the corneal penetration and absorption of the carried drug. *In vitro* corneal penetration experiments demonstrated that positively charged liposomes get through the cornea with a lower rate with respect to negatively charged liposomes and free acyclovir in solution. In contrast, an *in vivo* study indicated that the extent of acyclovir absorption from positively charged liposomes was higher than from negatively charged liposomes and free acyclovir. The acyclovir concentration in the cornea after administration of positively charged liposomes showed that an acyclovir deposition in the cornea was greater than those of negatively charged liposomes and free acyclovir. Morphological observation of the cornea surface treated with liposomes suggested that positively charged liposomes formed a completely coated layer on the cornea surface. The intimate bind of liposomes on the cornea surface would lead to an increase of residence time and thus an increase of acyclovir absorption.

Among topical applications, liposomes are widely studied for drug administration at dermatological level [63] and also for vaginal drug delivery [64-69] to have both local and systemic effects. For example, the group of Pavelic [64,65] has studied the production of a liposomal carrier system, and was able to provide controlled and sustained release for the local treatment of gynecological diseases, but also for the treatment of genital infection of herpes virus. Acyclovir was encapsulated in liposomes prepared by the polyol dilution method [69] and three different compositions were used, namely egg-PC/egg phosphatidylglycerol (9:1 mol/mol), egg-PC and egg-PC/stearylamine (9:3 mol/mol). To be closer to *in vivo* conditions and to further improve their stability, liposomes were incorporated in a bioadhesive hydrogel made from Carbopol 974P NF with adequate pH value and viscosity. *In vitro* release studies of liposomes incorporated in the hydrogel indicated their applicability as a vaginal delivery system with localized and sustained release of encapsulated acyclovir.

Again, Jain *et al.* [70] demonstrated that elastic liposomes bearing acyclovir sodium are able to enhance both the permeation of the formulation to the deeper layers of the skin and transdermal flux, decreasing lag time. The obtained flux was nearly 2.0 and 6.3-fold higher than conventional liposomes bearing acyclovir sodium and drug solution, respectively. The studied elastic liposomal formulation for acyclovir provides better transdermal flux, higher entrapment efficiency, ability as a self-penetration enhancer and effectiveness for transdermal delivery as compared with conventional liposomes. *In vivo* studies showed that the concentration of acyclovir sodium in plasma was found to be about 4.2-fold for elastic liposomes compared with conventional liposomes. Thus, elastic liposomes may be a promising vehicle for the transdermal delivery of acyclovir sodium.

Among liposomal delivery systems, the work of Kajiwara *et al.* [71] on long-circulating liposomal ganciclovir for i.v. and intraperitoneal injection has to be mentioned. In this study, PEGylated ganciclovir-liposomes based on egg-PC:cholesterol:stearylamine (5:5:1 mol/mol/mol) were prepared by the freeze–thawing method that allows significantly high entrapment efficiency of ganciclovir with respect to others' techniques [72,73]. Ganciclovir is an acyclovir prodrug, characterized by short biological half-life and dose-limiting toxicity for i.v. injection. Although the liposomes' *in vitro* cytotoxicity, tested on HeLa-TK cells, was similar to that of ganciclovir solution, the *in vivo* intravenously administered PEG-ganciclovir-liposomes were able to significantly inhibit the tumor growth of KB xenografts expressing HSV-TK, suggesting that these liposomes could be confirmed as interesting for their use on gene therapy.

3.2.2 Niosomes

Niosomes are non-ionic surfactant vesicles with bilayered structures, which can entrap both hydrophilic and lipophilic drugs either in an aqueous layer or in the lipid membrane [74]. Niosomes are widely studied as an inexpensive alternative of non-biological origin to liposomes. Studies have shown that the function of niosomes *in vivo* is similar to that of liposomes [75,76]. They have all the advantages of liposomes together with low cost, greater stability and ease of storage. These features make niosomes attractive for industrial manufacturing [74,76,77]. Theoretically, niosome formulation requires the presence of a particular class of amphiphile and an aqueous system. Cholesterol is added in order to prepare less permeable vesicles. Stabilizers may be included to prevent vesicle aggregation by repulsive, steric, or electrostatic effects.

Unfortunately, there is not enough research conducted to investigate the toxicity of niosomes. Researchers measured proliferation of keratinocytes in one of the topical niosome formulations [78] investigating the toxic effect of surfactant type. It was determined that the ester type surfactants are less toxic than the ether type surfactants [78,79]. This may be due to enzymatic degradation of ester bounds. In general, the physical form of niosomes did not influence their toxicity, as was evident in a study comparing the formulations prepared in the form of liquid crystals and gels. In some cases, encapsulation of the drug by niosomes reduces toxicity, as was demonstrated in the study on preparation of niosomes containing vincristine [80].

Attia *et al.* [81] have studied acyclovir niosomes to improve the poor and variable oral bioavailability. The non-ionic surfactant vesicles, consisting of cholesterol, span 60 and

dicetyl phosphate (65:60:5 mol/mol/mol), were prepared by the conventional thin film hydration method. The vesicles entrapped approximately 11% of acyclovir. The *in vitro* drug release profile exhibited significantly retarded release for the drug from niosomes with respect to the free solution. The *in vivo* study revealed that niosomes improved more than twofold the oral bioavailability of acyclovir in rabbits after a single oral dose of 40 mg/kg as compared to the free solution. Moreover, niosomes showed significant increase in the mean residence time of acyclovir, reflecting sustained release characteristics and suggesting that they could be a promising delivery system for oral administration of acyclovir.

Another study, conducted by Mukherjee *et al.* [82], has considered the use of acyclovir-loaded span 20-niosomes to sustain the release of the drug by increasing residence time and its dose-related systemic toxicity as compared to liposomes based on cholesterol and soya L-alpha-lecithin. The percentage of drug loading of niosomes was higher as compared to that of liposomes. Moreover, it was found that the *in vitro* drug release of the liposomes was about 90% in 150 min, whereas niosomes released just 50% of the drug within 200 min. Taken together these results suggest that niosomes could be considered as a good choice for i.v. delivery of acyclovir.

3.2.3 *Ethosomes*

A further vesicular system used to carry drugs is typified by ethosomes [83]. Ethosomes are multilamellar vesicles composed of phospholipid (soy phosphatidylcholine), ethanol and water. Ethanol is known to be an efficient enhancer of permeability [84,85]. Ethosomal systems have a high entrapment capacity for molecules of different lipophilicity [43,44,85-87]. An *in vivo* study in rabbits demonstrated that ethosomal systems are more efficient to deliver topical agents to the skin, in terms of quantity and depth, than either liposomes or hydro-alcoholic solution [85]. It is thought that ethosomes enhance permeability by their fusion with skin lipids releasing the drug at various points along the penetration pathway, perhaps via a follicular transport mechanism. It is reported that the replication of virus takes place at the basal dermis [86]. To overcome the problem of poor skin penetration to the dermal layer associated with conventional topical preparation of acyclovir, Horwitz *et al.* [87] prepared an acyclovir ethosomal formulation. They clinically evaluated its performance in a double-blind, randomized study with a marketed formulation of acyclovir (Zovirax, GlaxoSmithKline, Glaxo Wellcome UK Ltd, Middlesex, UK). Significant improvement in the evaluated clinical parameters was observed when the disorder was treated with ethosomal formulation in comparison to the marketed formulation. The observed clinical progress could be associated with the efficient delivery of the drug into the deep skin strata and the production of acyclovir skin reservoir at therapeutic levels.

3.3 Particles

Targeting the delivery of drugs to diseased lesions is an important aspect of the drug delivery systems. To convey a sufficient dose of drug to the lesion, suitable carriers of drugs are needed. In this view, micro and nanoparticle carriers have important potential applications for the administration of therapeutic molecules.

3.3.1 *Microparticles*

Duvvuri *et al.* [88,89] have developed a formulation for intravitreal delivery by dispersing ganciclovir-loaded PLGA microspheres in thermogelling PLGA–PEG–PLGA. Ganciclovir-loaded PLGA microspheres were prepared by the solvent evaporation method from two kinds of PLGA polymers and their blend (1:3), namely Resomer RG 502H and PLGA 6535. The amounts of ganciclovir entrapped in the microspheres were sufficient to administer therapeutically relevant doses in 60 µl. The formulation maintained mean vitreal concentrations of ganciclovir at approximately 0.8 µg/µl for 14 days, whereas direct injections maintained drug levels above 0.8 µg/µl for 54 h.

To administer acyclovir at the ophthalmic site, Cortesi *et al.* [90] studied the production and characterization of polyacrylic microparticles (Eudragit RL, RS and NE) by the spray drying method. The spray drying method allowed the production of microparticles with acceptable encapsulation efficiency and appropriate dimensional characteristics for ophthalmic administration. Release profile data, determined by dialysis, indicated that acyclovir is released from microparticles in a controlled manner. The release pattern of the drug is influenced by Eudragit type used for microparticle production. In the most number of cases, the plaque reduction efficiency of acyclovir containing microparticles is comparable to that displayed by the drug solution.

Genta *et al.* [91] have studied acyclovir-loaded chitosan microspheres with the aim of promoting the prolonged release of drug and increasing its ocular bioavailability. The microparticulate drug delivery systems obtained have been characterized by their morphology and physicochemical characteristics by *in vitro* dissolution tests and *in vivo* ocular administration to rabbits. The results show that the microspheres obtained are always quite small and physicochemical characterization shows that the drug is homogeneously dispersed in an amorphous state inside the microspheres. The *in vitro* dissolution profile of acyclovir from chitosan microspheres is slower than that for the free drug. *In vivo* ocular administration of acyclovir-loaded microspheres to the rabbit eye showed prolonged high concentrations of acyclovir and increased AUC values.

Rokhade [92] has produced semi-interpenetrating Polymer Network (IPN) microspheres of acrylamide grafted on dextran and chitosan by the emulsion–crosslinking method using glutaraldehyde as a crosslinker. The IPNs are a combination of two or more polymers in a network form that are held together by topological bonds without the

formation of covalent bonds between them [93,94]. Thus, an IPN structure can be obtained when one polymer network is synthesized and/or crosslinked independently close to another. Acyclovir was successfully encapsulated into IPN microspheres and successfully extended release up to 12 h, demonstrating the good potential of this system as a controlled release system for acyclovir.

3.3.2 *Nanoparticles*

Nanoparticles are defined as submicronic colloidal systems generally made of polymers (biodegradable or not) [95]. Nanoparticles generally vary in size from 10 to 1000 nm. Depending upon the process used for the preparation of nanoparticles, nanospheres or nanocapsules can be obtained. Nanocapsules are vesicular, reservoir systems in which the drug is confined to a cavity (an oil or aqueous core) surrounded by a unique thin polymeric membrane. Nanospheres are polymeric matrix systems in which the drug is physically and uniformly dispersed throughout the particles. Nanoparticles can be prepared using different polymers, such as polyesters and their copolymers. In contrast, natural macromolecules, such as proteins and polysaccharides, non-polar lipids, and metal oxides and silica, can also be used. Giannavola *et al.* [96] have demonstrated that acyclovir could be entrapped in a PLA polymeric colloidal drug delivery system, providing a sustained ocular drug release and increasing the acyclovir levels in aqueous humor. The ocular pharmacokinetics of acyclovir-loaded nanoparticles was evaluated *in vivo* and compared with an aqueous suspension of the free drug. PEG-coated and uncoated PLA nanospheres showed a sustained acyclovir release and were highly tolerated by the eye. Both types of PLA nanospheres were able to increase the aqueous levels of acyclovir and to improve the pharmacokinetics profile. In particular, PEG-coated PLA nanospheres were much more efficient in improving the ocular bioavailability of acyclovir. The biologic results on acyclovir bioavailability, as well as ocular carrier tolerability, lead us to propose PLA nanospheres as a potential ophthalmic dosage delivery system for the treatment of ocular viral infections.

Recently, Cortesi *et al.* [97] have produced solid lipid nanoparticles containing acyclovir. It was found that the antiviral activity of acyclovir–SLN is comparable to that of the free acyclovir solution until 1 – 0.5 μM on Vero cells. In particular when a multiplicity of infection (MOI) of 0.1 was used, the acyclovir–SLN formulation is slightly more efficient with respect to the free drug.

3.4 Cyclodextrins

Cyclodextrins are cyclic oligosaccharides useful as pharmaceutical excipients [98-100]. The molecular structure of these glucose derivatives, which approximates a truncated cone, generates a hydrophilic exterior surface and a nonpolar cavity interior. Cyclodextrins can thus interact with appropriately sized molecules resulting in the formation of inclusion complexes. These noncovalent complexes offer a variety of physicochemical advantages over the unmanipulated drugs including the possibility for increased water solubility and solution stability. Further, chemical modification to the parent cyclodextrin can result in an increase in the extent of drug complexation and interaction [100,101]. Following this strategy, Bencini *et al.* [102] have produced a poly(amidoamine) (PAA) copolymer with beta-cyclodextrin (beta-CD). This beta-CD/PAA copolymer bears beta-CD units along the macromolecular chain, is water soluble and non-cytotoxic. The complexing capacity of beta-CD/PAA with respect to acyclovir demonstrated that beta-CD/PAA can solubilize up to 11% w/w of acyclovir notably increasing the aqueous solubility of the drug. The *in vitro* release studies showed the dependence of acyclovir release rate on the solution pH. The antiviral activity of acyclovir beta-CD/PAA complex, evaluated against HSV-type I in cell cultures, exhibited a higher antiviral activity than the free drug.

3.5 Other delivery systems

3.5.1 *Microemulsions*

Microemulsions are homogeneous, transparent, thermodynamically stable dispersions of water and oil (o/w), stabilized by a surfactant, usually in combination with a cosurfactant (typically a short-chain alcohol) [103]. As pharmaceutical drug delivery systems, microemulsions have many advantages, including clarity, high stability and ease of preparation. This drug delivery system has been reported to improve the rate and extent of absorption of lipophilic drugs [103,104]. Ghosh and colleagues [105] have developed an oral microemulsion formulation for enhancing the bioavailability of acyclovir. Particularly, a microemulsion Labrafac-based formulation with Labrasol as surfactant and Plurol Oleique as cosurfactant was developed. The *in vivo* oral absorption of acyclovir from the microemulsion was investigated in rats. Acyclovir displayed high solubility in the microemulsion constituted by Labrafac (10%), Labrasol (32%), Plurol Oleique (8%) and water (50%). The *in vitro* intraduodenal diffusion and the *in vivo* study revealed a 12.8-fold increase of bioavailability after oral administration of the microemulsion as compared to the commercially available tablets.

3.5.2 *SMEDDS*

It is known that despite *in vitro* activity against herpes viruses and a favorable toxicity profile, many potential applications of acyclovir are limited by its poor absorption. Acyclovir is absorbed slowly and incompletely from the human gastrointestinal (GI) tract. To enhance the oral bioavailability of acyclovir, Patel and Sawant [106] have developed a self-microemulsifying drug delivery system (SMEDDS). SMEDDS is mixture of oils, surfactants and co-surfactants, which are emulsified in aqueous media under conditions of gentle agitation and digestive motility that would be encountered in the GI tract. Once dispersed, such

systems would be expected to behave *in vivo* in the same way as o/w emulsions. The spontaneous formulation of an emulsion upon drug release in the GI tract advantageously presents the drug in a solubilized form, and the small droplet size provides a large interfacial surface area for drug absorption. A SMEDDS formulation containing acyclovir (50 mg), Tween 60 (60%), glycerol (30%) and sunflower oil (9%) was compared with the pure drug solution by oral administration to male albino rats. The absorption of acyclovir from SMEDDS form resulted in a 3.5-fold increase in bioavailability compared with the pure drug solution.

3.5.3 *Transdermal systems*

Human skin serves a protective function by imposing physicochemical limitations to the type of permeant that can cross the barrier. A drug to be delivered via the skin needs to have a suitable lipophilicity and a molecular weight < 500 Da. The number of commercially available products based on transdermal or dermal delivery has been limited by these requirements. Recently, various passive and active strategies have emerged to optimize delivery [107,108]. The delivery of drugs of differing lipophilicity and molecular weight is improved by active methods such as iontophoresis, electroporation, mechanical perturbation and other energy-related techniques such as ultrasound and needleless injection.

For instance, iontophoresis (i.e., the application of a small electrical current to facilitate the transport of charged molecules into and across the skin) [109,110] has been considered as a means to increase cutaneous acyclovir bioavailability [111,112]. Abla *et al.* [110] investigated by *in vitro* experiments the iontophoretic delivery of both acyclovir and valacyclovir (positively charged) across the porcine skin. The prodrug approach would be expected to ameliorate treatment of cutaneous herpetic infections by targeting therapeutic levels of drug to this tissue layer without the undue systemic exposure associated with oral and parenteral delivery. It was demonstrated that the prodrug was more efficiently iontophoresed into the skin than acyclovir, but only the latter was detectable in the receptor chamber, suggesting that valacyclovir was enzymatically cleaved into the active metabolite during skin transit. Iontophoresis of valacyclovir was significantly more efficient than that of acyclovir, suggesting the potential of valacyclovir iontophoresis to improve the topical therapy of cutaneous herpes simplex infections.

In contrast, Brown *et al.* [113] described a practical example of an active skin abrasion device to demonstrate the success of such active methods. The *in vitro* permeation of acyclovir through human epidermal membrane, using a rotating brush abrasion device, was compared with acyclovir delivery using iontophoresis. It was found that application of the brush treatment (10 s at a pressure of 300 nm^{-2}) was comparable to 10 min of iontophoresis. The observed enhancement of permeability observed using the rotating brush was a result of disruption of the cells of the stratum corneum, causing a reduction of the barrier function of the skin and thus allowing the facilitated penetration of the hydrophilic acyclovir sodium.

3.5.4 *Doughnut-shaped mini-tablet (DSMT)*

In order to optimize the design of formulations for intraocular drug delivery, Choonara *et al.* [114] have produced a novel doughnut-shaped mini-tablet (DSMT) containing ganciclovir (an acyclovir-related molecule) and tried to evaluate the effect of two independent variables on the drug release. The two independent variables were the concentration of polymer (Resomer, % by weight) and the type of Resomer grade (RG502, RG503 and RG504). DSMT devices were prepared using a Manesty F3 tableting press fitted with a novel central rod, punch, and die set-up [114]. The response of the drug release rate was evaluated from a referenced marketed product. Dissolution data revealed biphasic drug release behavior, with 55 – 60% drug released over 120 days. Using the resultant statistical relationships with the release rate constant as a response, the optimum formulation predicted for devices formulated with ganciclovir was 92% wt/wt of Resomer RG503. The results of this study revealed that the full factorial design was a suitable tool to predict an optimized formulation for prolonged intraocular drug delivery.

4. Conclusion

The development of successful antiviral agents against HSV infections had been slow until the last decade; however progress in the production of delivery systems for acyclovir offers a promising alternative [115]. The studies reviewed in this article, summarized in **Table 1**, provide a foundation to suggest these systems for further therapeutic developments. This will undoubtedly result in an improved understanding of the physical factors, such as administration route and parameters of preparation. The advancement of both passive and active targeted formulations has been limited to a few successful studies in animal models. However, these basic studies identify a number of parameters that will effectively facilitate the development of therapeutically useful targeted delivery systems.

5. Expert opinion

Herpes viruses (herpes simplex, varicella zoster, cytomegalovirus) are the main cause agents of a wide variety of human infections. Although infections are often subclinical, HSV can cause mild to severe diseases, especially in immunocompromised patients. Lesions in immunocompetent patients may be benign; in contrast in immunocompromised patients lesions can be life threatening, with high mortality and morbidity. Herpes simplex viruses establish latency in the nuclei of neuronal cells and may reactivate, with or without symptoms, throughout the host's lifetime. There are few drugs licensed for the treatment of HSV infections and indeed acyclovir remains the reference treatment some

Table 1. Summary of the principal delivery systems described in the present review.

Delivery system	Composition	Administration/application site	Ref.
Prodrug and polymer conjugates	Amino acid esters	i.v., s.c.	[25-29]
	Non-peptidyl peptides (PTAP)	Cell	[30]
	Thiolated chitosan	Oral	[36-38]
	Ethylmethacrylate (EMA) Hexyl methacrylate (HMA)	Injection	[39,40]
	Ethylene vinyl acetate copolymer (EVA)	Oral	[41]
SADDS	Cholesterol derivatives	Macrophages	[43]
Vesicles	Liposomes MVL	Intradermal	[58]
	Liposomes	Corneal	[62]
	Liposomes PC	Vaginal	[64,65]
	Elastic liposomes	Transdermal	[70]
	PEGylated liposomes	Gene therapy	[71]
	Niosomes	Oral	[81]
	Niosomes	i.v.	[82]
	Ethosomes Soy-PC	Lips (herpes labialis)	[87]
Microparticles	PLGA–PEG–PLGA	Intravitreal	[88-89]
	Eudragit RL, RS, NE	Ophthalmic	[90]
	Chitosan	Ophthalmic	[91]
	IPN acrylamide dextran chitosan	Oral	[92]
Nanoparticles	PLA (PEG-coated)	Ophthalmic	[85]
	SLN	Topical	[86]
Cyclodextrins	Polamidoamine (PAA)	Injection	[87]
Microemulsions	Labrafac–labrasol–plurol oleique	Oral	[105]
SMEDDS	Tween 60–glycerol–sunflower oil	Oral	[106]
DSMT	Resomer	Intraocular	[114]

30 years after its discovery. However, progress in organ transplant, cancer therapy and the upsurge of human immunodeficiency virus (HIV) have stimulated the development of newer compounds for the treatment of individuals with herpes infection. In addition, it is well known that the method by which a drug is delivered influences its efficacy. Some drugs have an optimum concentration range within which maximum benefit is derived, and concentrations above or below this range can be toxic or produce no therapeutic benefit at all. In this view, new ideas on controlling the pharmacokinetics, pharmacodynamics, immunogenicity, biorecognition and efficacy of drugs have to be considered. These strategies, called drug delivery systems, are based on interdisciplinary approaches combining polymer science, pharmaceutics, bioconjugate chemistry and molecular biology. Among drug carriers, soluble polymers, microparticles (made of insoluble or biodegradable natural and synthetic polymers), microcapsules, cells, cell ghosts, lipoproteins, liposomes and micelles can be mentioned.

As previously stated, the currently available dosage regimens of acyclovir are characterized by a number of limitations including: i) variable bioavailability by oral administration; ii) poor percutaneous absorption; and iii) thrombophlebitis on IV bolus injection [2,18]. Most of the earlier studies to improve the physicochemical characteristics of acyclovir were based on chemical modification, such as amino acid ester [30,116,117] or highly water soluble alkylamines and benzylcarbamate prodrugs [31-33], aliphatic [118,119] or 6-deoxyacyclovir prodrug to enhance intradermal delivery [120] and 1-valyl ester prodrug to increase oral bioavailability. Limited work has been reported on dosage form modification for transbuccal [114,115,121] and ocular delivery [122,123].

Although identifying novel antiviral agents remains a priority, the development of drug delivery systems for currently used agents may represent a cost-effective and promising alternative. The above-reported investigations indicate that all the considered formulations are able to improve the bioavailability of acyclovir, usually known as a poorly adsorbed drug. Their major advantages, such as improvement of drug bioavailability and reduction of the dosing frequency, may create a better management of the disease, making the treatment more practical and affordable. In particular,

prodrugs and polymer conjugates showed in animals an increase in acyclovir absorption [123,124] and a controlled release after oral administration [125].

Concerning vesicles, liposomes, ethosomes and niosomes were considered. In particular their use was addressed to increase adsorption and effectiveness through different routes of administration, such as ocular, corneal, subcutaneous, transdermal and vaginal routes. However, for niosomes oral administration was additionally proposed. *In vitro* and *in vivo* results are encouraging. Microemulsions [103-106] showed an absolute bioavailability almost 13-fold higher than a commercially available tablet (Aquivir®, FDC, Mumbai, India) [105]. In contrast complexes based on the use of cyclodextrins showed high potentiality as systems to obtain both an increase of solubility and antiviral activity [102].

Concerning micro and nanoparticles, a feasibility of the versatile routes of drug administration was shown and their high stability suggests a long shelf life [88-97,126,127].

It can be expected that future research will concentrate on the development of the vectorized delivery systems, combining advantages of the colloidal carriers, such as the large payloads of a drug, with active targeting to the infection sites. Moreover, development of innovative formulation technologies suggests that nanoparticles can be incorporated into various solid dosage forms (i.e., granules or tablets) that can release the nanoparticles at the site of action [128-130]. These approaches would further improve efficacy and practicability of the nanoparticle-based formulations.

In conclusion, it has to be underlined that all the drug delivery systems proposed here for the release of acyclovir have potential. The success of these technologies will probably depend on toxicologic issues associated with understanding the fate of nanocarriers and their polymeric constituents in the body, as well as elimination of the risk of the residual organic solvents. However, the potential of these new ways to deliver acyclovir is interesting. However, it is also easy to understand how the therapeutic use of these systems, especially for immunocompromised patients, is still a long way from the reality. In fact, many studies have still to be performed: first of all, after designing new delivery systems, it is necessary to select those with optimal *in vitro* and *in vivo* characteristics for obtaining maximum therapeutic efficacy; secondly clinical trials have to be recruited; and last (but not least) an industry interested in the production of at least one of these new systems has to be discovered. Luckily many researchers and pharmaceutical industries throughout the world are interested in new findings. Currently the delivery system with most chance could be the liposome-based system; some pharmaceutical industries have launched their liposome-based products/formulations, obtaining very good outlines. For example antimycotic drugs, such as econazol (Pevaryl® Lipogel, Janssen Pharmaceutica, NJ, USA), amphotericin (Ambisome®, NeXtar Pharmaceutical, CO, USA and Abelcet®, Elan Pharmaceuticals, Inc., NJ, USA), nistatin (Nyotran®, Aronex Pharmaceuticals Inc., TX, USA) amikacin (Mikasome®, Gilead, CO, USA) and also antitumor drugs, such as doxorubicin (Doxil®, Alza Pharmaceuticals, CA, USA), daunorubicin (DaunoXome®, NeXtar Pharmaceutical, CO, USA), cisplatin (Aroplatin™, Aronex Pharmaceuticals Inc., TX, USA) or ATRA (Atragen®, Aronex Pharmaceuticals Inc., TX, USA) have been commercialized or are under clinical trials. Moreover, a number of liposome-based diagnostic, therapeutic and veterinary products are facing the test of the marketplace. Meanwhile, promising research results indicate that highly focused research and development programs could lead to a new generation of anticancer and, perhaps, antiviral agents (i.e., acyclovir or its derivates) based upon these new systems' ability to stimulate cellular processes.

Acknowledgements

The authors acknowledge Regione Emilia Romagna (Spinner Project) and PRIN-2006 for financial support.

Bibliography

Papers of special note have been highlighted as either of interest (•) or of considerable interest (••) to readers.

1. Richards DM, Carmine AA, Brogden RN, et al. Acyclovir. A review of its pharmacodynamic properties and therapeutic efficacy. Drugs 1983;26:378-438

• **Good review on the properties of acyclovir. Mainly pharmacological content.**

2. Wagstaff AJ, Faulds D, Goa KL. Aciclovir. A reappraisal of its antiviral activity, pharmacokinetic properties and therapeutic efficacy. Drugs 1994;47:153-205

3. Fletcher C, Bean B. Evaluation of oral acyclovir therapy. Drug Intell Clin Pharm 1985;19:518-24

4. Blum MR, Liao SHT, Miranad PD. Overview of acyclovir pharmacokinetic disposition in adults and children. Am J Med 1982;73(Suppl):186-92

5. Douglas JM, Davis LG, Remington ML, et al. A double-blind, placebo-controlled trial of the effect of chronically administered acyclovir on sperm production in men with frequently recurring genital herpes. J Infect Dis 1988;157:588-90

6. Stahlmann R, Klug S, Lewandowski C, et al. Teratogenicity of acyclovir in rats. Infection 1987;15:261-2

7. Tucker WE. Pre-clinical toxicology profile of acyclovir: an overview. Am J Med 1982;73:27-30

8. Ga De Jalon E, Blanco-Priieto MJ, Ygartua P, et al. Increased efficacy of acyclovir-loaded microparticles against herpes simplex virus type 1 in cell culture. Eur J Pharm Biopharm 2003;56:183-7

9. Modiano P, Salloum E, Gillet-Terver M, et al. Acyclovir-resistant chronic cutaneous herpes simplex in Wiskott–Aldrich syndrom. Br J Dermatol 1995;133:475-8

10. Reichman RC, Badger GJ, Mertz GJ, et al. Treatment of recurrent genital herpes simplex infections with oral acyclovir: a controlled trial. J Am Med Ass 1984;251:2103-7

11. Miranda PD, Blum MR. Pharmacokinetics of acyclovir after intravenous and oral administration. Antimicrob Chemother 1983;12:29-37
12. Bryson YJ, Dillon M, Lovett M, et al. Treatment of first episodes of genital herpes simplex virus infection with oral acyclovir. A randomized double-blind controlled trial in normal subjects. N Engl J Med 1983;308:916-21
13. Snoeck R, De Clercq E. New treatments for genital herpes. Curr Opin Infect Dis 2002;15:49-55
14. Goldberg LH, Kaufman R, Kurtz TO, et al. Long-term suppression of recurrent genital herpes with acyclovir. Arch Dermatol 1993;129:582-7
15. Wade JC, Newton B, McLaren C, et al. Intravenous acyclovir to treat mucocutaneous herpes simplex virus infection after marrow transplantation: a double blind trial. Ann Intern Med 1982;96:265-9
16. Spruance SL, Nett R, Marbury T, et al. Acyclovir cream for treatment of herpes simplex labialis: results of two randomized, double-blind, vehicle controlled, multicenter clinical trials. Antimicrob Agents Chemother 2002;46:2238-43
17. Raborn GW, Grace MGA. Recurrent herpes simplex labialis: selected therapeutic options. J Can Dent Assoc 2003;69:498-503
18. Spruance SL, Crumpacker CS. Topical 5 percent acyclovir in polyethylene glycol for herpes simplex labialis. Antiviral effect without clinical benefit. Am J Med 1982;73:315-9
19. Spruance SL, Crumpacker CS, Schnipper LE, et al. Early, patient-initiated treatment of herpes labialis with topical 10% acyclovir. Antimicrob Agents Chemother 1984;25:553-5
20. Freeman DJ, Sheth NV, Spruance SL. Failure of topical acyclovir in ointment to penetrate human skin. Antimicrob Agents Chemother 1986;29:730-2
21. Parry GE, Dunn P, Shah VP, et al. Acyclovir bioavailability in human skin. J Invest Dermatol 1992;98:856-63
22. Piret J, Desormeaux A, Gourde P, et al. Efficacies of topical formulations of foscarnet and acyclovir and of 5-percent acyclovir ointment (Zovirax) in a murine model of cutaneous herpes simplex virus type 1 infection. Antimicrob Agents Chemother 2000;44:30-8
23. Albert A. Chemical aspects of selective toxicity. Nature 1958;182:421-3
24. Krenitsky TA, Hall WW, De Miranda PD, et al. 6-Deoxyacyclovir: a xanthine oxidase activated prodrug of acyclovir. Proc Natl Acad Sci USA 1984;81:3209-13
25. De Clercq H, Field HJ. Antiviral prodrugs – the development of successful prodrug strategies for antiviral chemotherapy. Br J Pharmacol 2006;147:1-11

•• **Excellent review on antiviral prodrug.**

26. Granero GE, Amidon GL. Stability of valacyclovir: implications for its oral bioavailability. Int J Pharm 2006;317:14-8
27. Perry CM, Faulds D. Valaciclovir. A review of its antiviral activity, pharmacokinetic properties and therapeutic efficacy in herpes virus infections. Drugs 1996;52:754-72

• **Interesting review on the properties of valacyclovir.**

28. Acosta EP, Fletcher CV. Valacyclovir. Ann Pharmacother 1997;31:185-91
29. Anand BS, Katragadda S, Nashed YE, et al. Amino acid prodrugs of acyclovir as possible antiviral agents against ocular HSV-1 infections: interactions with the neutral and cationic amino acid transporter on the corneal epithelium. Curr Eye Res 2004;29:153-66
30. Amidon GL, Leesman GD, Elliott RL. Improving intestinal absorption of water-insoluble compounds: a membrane metabolism strategy. J Pharm Sci 1980;69:1363-8
31. Han HK, Oh DM, Amidon GL. Cellular uptake mechanism of amino acid ester prodrugs in Caco-2/hPEPT1 cells overexpressing a human peptide transporter. Pharm Res 1998;15:1382-6
32. Bai JP, Hu M, Subramanian P, et al. Utilization of peptide carrier system to improve intestinal absorption: targeting prolidase as a prodrug-converting enzyme. J Pharm Sci 1992;81:113-6
33. Balimane PV, Tamai I, Guo A, et al. Direct evidence for peptide transporter (PepT1)-mediated uptake of a nonpeptide prodrug, valacyclovir. Biochem Biophys Res Commun 1998;250:246-51
34. Sinko PJ, Balimane PV. Carrier-mediated intestinal absorption of valacyclovir, the L-valyl ester prodrug of acyclovir: 1. Interactions with peptides, organic anions and organic cations in rats. Biopharm Drug Dispos 1998;19:209-17
35. Palmberger TF, Hombach J, Bernkop-Schnürch A. Thiolated chitosan: development and in vitro evaluation of an oral delivery system for acyclovir. Int J Pharm 2008;348:54-60
36. Cascorbi I. Role of pharmacogenetics of ATP-binding cassette transporters in the pharmacokinetics of drugs. Pharmacol Ther 2006;112:457-73
37. Salama NN, Scott KR, Eddington ND. DM27, an enaminone, modifies the in vitro transport of antiviral therapeutic agents. Biopharm Drug Dispos 2004;25:227-36
38. Yang ZG, Meng H, Zhang X, et al. Effect of quercetin on the acyclovir intestinal absorption. Beijing Da Xue Xue Bao 2004;36:309-12
39. Tallury P, Airrabeelli R, Li J, et al. Release of antimicrobial and antiviral drugs from methacrylate copolymer system: effect of copolymer molecular weight and drug loading on drug release. Dent Mater 2008;24:274-80
40. Tallury P, Randall MK, Thaw KL, et al. Effects of solubilizing surfactants and loading of antiviral, antimicrobial, and antifungal drugs on their release rates from ethylene vinyl acetate copolymer. Dent Mater 2007;23:977-82
41. Kalachandra S, Takamata T, Lin DM, et al. Stability and release of antiviral drugs from ethylene vinyl acetate (EVA) copolymer. J Mater Sci Mater Med 2006;17:1227-36

• **Description of polymer-based drug delivery systems.**

42. Jin Y, Tong L, Ai P, et al. Self-assembled drug delivery systems. 1. Properties and in vitro/in vivo behavior of acyclovir self-assembled nanoparticles (SAN). Int J Pharm 2006;309:199-207

• **Description of SADDS as drug delivery systems.**

43. Jin Y, Xin R, Ai P, Chen D. Self-assembled drug delivery systems 2. Cholesteryl derivatives of antiviral nucleoside analogues: synthesis, properties and the vesicle formation. Int J Pharm 2008;350:330-7
44. Aquaro S, Calio R, Balzarini J, et al. Macrophages and HIV infection: therapeutical approaches toward this strategic virus reservoir. Antiviral Res 2002;55:209-25
45. Mogensen S. Role of macrophages in hepatitis induced by Herpes simplex virus types 1 and 2 in mice. Infect Immun 1977;15:686-91

46. Mott K, Brick DJ, Van Rooijen N, et al. Macrophages are important determinants of acute ocular HSV-1 infection in immunized mice. Invest Ophthalmol Vis Sci 2007;48:5605-15

47. Ogihara-Umeda I, Sasaki T, Toyama H, et al. Rapid diagnostic imaging of cancer using radiolabeled liposomes. Cancer Detect Prev 1997;21:490-6

48. Park JW, Hong K, Kirpotin DB, et al. Immunoliposomes for cancer treatment. Adv Pharmacol 1997;40:399-435

49. Kao GY, Change LJ, Allen TM. Use of targeted cationic liposomes in enhanced DNA delivery to cancer cells. Cancer Gene Ther 1996;3:250-6

50. Lasic DD. Novel applications of liposomes. TIBTECH 1998;16:307-21

51. Ran Y, Yalkowsky SH. Halothane, a novel solvent for the preparation of liposomes containing 2-40-amino-30-methylphenul benzothiazole (AMPB), an anticancer drug: a technical note. AAPS PharmSciTech 2003;4:E20

52. Mumper RJ, Hoffman AS. The stabilization and release of hirudin from liposomes or lipid-assemblies coated with hydrophobically modified dextran. AAPS PharmSciTech 2000;1:E3

53. Riaz M, Martin F, Weiner H. Liposomes as a drug delivery system. Drug Dev Ind Pharm 1989;15:1523-4

54. Lasic DD, Papadjopoulos D. Liposomes revisited. Science 1995;267:1275-6

55. Meyer J, Whitcomb L, Collins D. Efficient encapsulation of proteins within liposomes for slow release in vivo. Biochim Biophys Res Commun 1994;199:433-8

56. Kim T, Murdane S, Gruber A, Kim S. Sustained-release morphine for epidural analgesia in rats. Anesthesiology 1996;85:331-8

57. Khatibi S, Howell Sb, McCully C. Prolongation of action in CSF by encapsulation into multivesicular liposomes. Am Soc Clin Oncol 1991;10:282-6

58. Jain SK, Jain RK, Chourasia MK, et al. Design and development of multivesicular liposomal depot delivery system for controlled systemic delivery of acyclovir sodium. AAPS PharmSciTech 2005;6:E35-41

59. Langston MV, Rampresad MP, Karali TT, et al. Modulation of the sustained delivery of myelopoietin (Leridistim) encapsulated in multivesicular liposomes (DepoFoam). J Control Rel 2003;89:87-99

60. Xiao CJ, Qi XR, Aini W, et al. Preparation of cisplatin multivesicular liposomes and release of cisplatin from the liposomes in vitro. Yao Xue Xue Bao 2003;38:133-7

61. Kim S, Howell S. Multivesicular liposomes having a biologically active substance encapsulated therein in the presence of a hydrochloride. US5807572; 1998

62. Law SL, Huang KJ, Chiang CH. Acyclovir-containing liposomes for potential ocular delivery. Corneal penetration and absorption. J Control Rel 2000;63:135-40

63. Lasch J, Bouwstra J. Interaction of external lipid (lipid vesicles) with the skin. J Liposome Res 1995;5:543-69

64. Pavelic Z, Skalko-Basnet N, Schubert R. Liposomal gels for vaginal drug delivery. Int J Pharm 2001;219:139-49

65. Pavelic Z, Skalko-Basnet N, Filipovic-Grcic JE, et al. Development and in vitro evaluation of a liposomal vaginal delivery system for acyclovir. J Control Rel 2005;106:34-43

66. Jain SK, Singh R, Jain VV. Mucoadhesive liposomes bearing metronidazole for controlled and localized vaginal delivery. Proc Int Symp Control Release Bioact Mater 1996;23:701-2

67. Foldvari M, Moreland A. Clinical observations with topical liposome-encapsulated interferon alpha for the treatment of genital papilloma virus infections. J Liposome Res 1997;7:115-26

68. Jain SK, Singh R, Sahu B. Development of a liposome based contraceptive system for intravaginal administration of progesterone. Drug Dev Ind Pharm 1997;23:827-30

69. Kikuchi H, Yamauchi H, Hirota S. A polyol dilution method for mass production of liposomes. J Liposome Res 1994;4:71-91

70. Jain SK, Gupta Y, Jain A, et al. Enhanced transdermal delivery of acyclovir sodium via elastic liposomes. Drug Deliv 2008;15:141-7

71. Kajiwara E, Kawano K, Hattori Y, et al. Long-circulating liposome-encapsulated ganciclovir enhances the efficacy of HSV-TK suicide gene therapy. J Control Release 2007;120:104-10

72. Gregoriadis G. Liposome technology. 3rd edition. volume1. CRC Press, Florida; 2007

73. Monnard PA, Oberholzer T, Luisi P. Entrapment of nucleic acids in liposomes. Biochim Biophys Acta 1997;1329:39-50

74. Biju SS, Talegaonkar S, Mishra PR, et al. Vesicular systems: an overview. Indian J Pharm Sci 2006;68:141-53

• **Simple but complete review on vesicular systems.**

75. Sahin NO. Niosomes as nanocarrier systems. In: Mozafari MR, editor, Nanomaterials and nanosystems for biomedical applications. Springer, Dordrecht. The Netherlands; 2007. p. 67-82

•• **This book is complete, simple and clear.**

76. Baillie AJ, Florence AT, Hume LR, et al. The preparation and properties of niosome-non ionic surfactant vesicles. J Pharm Pharmacol 1985;37:863-8

77. Echoyen LE, Hernandez JC, Kaifer AE, et al. Aggregates of steroidal lariat ethers: the first example of non-ionic liposomes (niosomes) formed from neutral crown ethers compounds. J Chem Soc Chem Comm 1988;8:836-7

78. Hofland HEJ, Bouwstra JA, Ponec M, et al. Interactions of non-ionic surfactant vesicles with cultured keratinocytes and human skin in vitro: a survey of toxicological aspects and ultrastructural changes in stratum corneum. J Control Rel 1991;16:155-68

79. Hofland HEJ, Bouwstra JA, Verhoef JC, et al. Safety aspects of non-ionic surfactant vesicles – a toxicity study related to the physicochemical characteristics of non-ionic surfactants. J Pharm Pharmacol 1992;44:287-94

80. Parthasarathi G, Udupa N, Umadevi P, et al. Niosome encapsulated of vincristine sulfateimproved anticancer activity with reduced toxicity in mice. J Drug Target 1994;2:173-82

81. Attia IA, El-Gizawy SA, Fouda MAA, et al. Influence of a niosomal formulation on the oral bioavailability of acyclovir in rabbits. AAPS PharmSciTech 2007;8:E106

82. Mukherjee B, Patra B, Layek B, et al. Sustained release of acyclovir from nano-liposomes and nano-niosomes: an in vitro study. Int J Nanomed 2007;2:213-25

83. Godin B, Touitou E. Ethosomes: new prospects in transdermal delivery. Crit Rev Ther Drug Carrier Syst 2003;20:63-102

84. Touitou E, Godin B. Dermal drug delivery with ethosomes: therapeutic potential. Therapy 2007;4:465-72

•• **Excellent review on the potential dermal use of ethosomes.**

85. Touitou E. Drug delivery across the skin. Expert Opin Biol Ther 2002;2:723-33

86. Huff JC, Kreuger GC, Overall JC, et al. The histopathologic evolution of recurrent herpes simplex labialis. J Am Acad Dermatol 1981;5:550-7

87. Horwitz E, Pisanty S, Czerninski R, et al. A clinical evaluation of a novel liposomal carrier for acyclovir in the topical treatment of recurrent herpes labialis. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 1999;88:700-5

88. Duvvuri S, Janoria Kg, Pal D, et al. Controlled delivery of ganciclovir to the retina with drug-loaded Poly(d,L-lactide-co-glycolide) (PLGA) microspheres dispersed in PLGA-PEG-PLGA Gel: a novel intravitreal delivery system for the treatment of cytomegalovirus retinitis. J Ocul Pharmacol Ther 2007;23:264-74

89. Duvvuri S, Gaurav Janoria K, Mitra AK. Effect of polymer blending on the release of ganciclovir from PLGA microspheres. Pharm Res 2006;23:215-23

90. Cortesi R, Ajanji SC, Sivieri E, et al. Eudragit microparticles as a possible tool for ophthalmic administration of acyclovir. J Microencapsul 2007;24:445-56

91. Genta I, Conti B, Perugini P, et al. Bioadhesive microspheres for ophthalmic administration of acyclovir. J Pharm Pharmacol 1997;49:737-42

92. Rokhade AP, Patil SA, Aminabhavi TM. Synthesis and characterization of semi-interpenetrating polymer network microspheres of acrylamide grafted dextran and chitosan for controlled release of acyclovir. Carbohydr Polym 2007;67:605-13

93. Kim SC, Sperling LH. IPNs around the world: Science and Engineering, New York: Wiley; 1997

94. Diez-Sales O, Dolz M, Hernandez MJ, et al. Acyclovir delivery matrices based on poly(ethylene glycol)/chitosan semi-interpenetrating networks. J Pharm Sci 2007;96:1653-7

95. Kubik T, Bogunia-Kubik K, Sugisaka M. Nanotechnology on duty in medical applications. Curr Pharm Biotechnol 2005;6:17-33

•• **Very good review on the relationship between nanotechnology and medical fields.**

96. Giannavola C, Bucolo C, Maltese A, et al. Influence of preparation conditions on acyclovir-loaded poly-d,l-lactic acid nanospheres and effect of PEG coating on ocular drug bioavailability. Pharm Res 2003;20:584-90

97. Cortesi R, Drechsler M, Esposito E. SLN as a tool to administrate acyclovir. (Manuscript in preparation)

98. Duchêne D, Vaultion C, Glomot F. Cyclodextrins, their value in pharmaceutical technology. Drug Dev Ind Pharm 1986;12:2193-215

99. Loftsson T, Brewste ME. Pharmaceutical applications of cyclodextrins. 1. Drug solubilization and stabilization. J Pharm Sci 1996;85:1017-25

100. Loftsson T, Duchêne D. Cyclodextrins and their pharmaceutical applications. Int J Pharm 2007;329:1-11

101. Loftsson T, Hreinsdóttir D, Másson M. Evaluation of cyclodextrin solubilization of drugs. Int J Pharm 2005;302:18-28

102. Bencini M, Ranucci E, Ferruti P, et al. Preparation and in vitro evaluation of the antiviral activity of the Acyclovir complex of a beta-cyclodextrin/poly(amidoamine) copolymer. J Control Rel 2008;126:17-25

103. Constantinides P, Scalart J, Lancaster C, et al. Formulation and intestinal absorption enhancement evaluation of water-in-oil microemulsions incorporating medium-chain glycerides. Pharm Res 1994;11:1385-90

104. Charman S, Charman W, Rogge M, et al. Self-emulsifying drug delivery systems: formulation and biopharmaceutic evaluation of an investigational lipophilic compound. Pharm Res 1992;9:87-93

105. Ghosh PK, Majithiya RJ, Umrethia ML, et al. Design and development of microemulsion drug delivery system of acyclovir for improvement of oral bioavailability. AAPS PharmSciTech 2006;7:77

• **Description of the characteristics of microemulsions as a delivery system.**

106. Patel D, Sawant KK. Oral bioavailability enhancement of acyclovir by self-microemulsifying drug delivery systems (SMEDDS). Drug Dev Ind Pharm 2007;33:1318-26

• **Description of SMEDDS for drug delivery.**

107. Abla N, Naik A, Guy RH, et al. Topical iontophoresis of valaciclovir hydrochloride improves cutaneous aciclovir delivery. Pharm Res 2006;23:1842-9

108. Ita KB, Du Preez J, Lane ME, et al. Dermal delivery of selected hydrophilic drugs from elastic liposomes: effect of phospholipid formulation and surfactants. J Pharm Pharmacol 2007;59:1215-22

109. Kalia YN, Naik A, Garrison J, et al. Iontophoretic drug delivery. Adv Drug Deliv Rev 2004;56:619-58

110. Abla N, Naik A, Guy RH, et al. Iontophoresis: clinical applications and future challenges. In: Smith EW, Maibach HI, editors, Percutaneous penetration enhancers. CRC, Boca Raton, Florida; 2005. p. 177-219

111. Gangarosa S, Hill JM. Modern iontophoresis for local drug delivery. Int J Pharm 1995;123:159-71

112. Stagni G, Ali ME, Weng D. Pharmacokinetics of acyclovir in rabbit skin after IV-bolus, ointment, and iontophoretic administrations. Int J Pharm 2004;274:201-11

113. Brown MB, Traynor MJ, Martin GP, et al. Transdermal drug delivery systems: skin perturbation devices. Methods Mol Biol 2008;437:119-39

114. Choonara YE, Pillay V, Carmichael T, et al. Studies on a novel doughnut-shaped minitablet for intraocular drug delivery. AAPS PharmSciTech 2007;8:E118

115. Kaparissides C, Alexandridou S, Kotti K et al. Recent advances in novel drug delivery systems. AZojono – Journal of Nanotechnology Online, 2007. Available from: http://www.azonano.com/nanotechnology

116. Han HK, Oh DM, Amidon GL. Cellular uptake mechanism of amino acid ester prodrugs in Caco-2/hPEPT1 cells overexpressing a human peptide transporter. Pharm Res 1998;15:1382-6

117. Han H, De Vrueh RL, Rhie JK, et al. 5′-Amino acid esters of antiviral nucleosides, acyclovir, and AZT are absorbed by the intestinal PEPT1 peptide transporter. Pharm Res 1998;15:1154-9

118. Bundgaard H, Jensen E, Falch E. Water-soluble, solution-stable, and biolabile N-substituted (aminomethyl) benzoate ester prodrugs of acyclovir. Pharm Res 1991;8:1087-93

119. Shao Z, Park G, Krishnamoorthy R, et al. The physicochemical properties, plasma enzymatic hydrolysis, and nasal absorption of acyclovir and its 2′-ester prodrugs. Pharm Res 1994;11:237-42

120. Chikhale P, Bodor N. Improved delivery of acyclovir to the skin using a dihydrotrigonelline in equilibrium with trigonelline redox carrier. J Pharm Sci 1991;80:402-3

121. Shojaei AH, Zhou S, Xiaoling L. Transbuccal delivery of acyclovir (II): feasibility, system design, and in vitro permeation studies. J Pharm Pharm Sci 1998;1:66-73

122. Greco A, Diaz JJ, Thouvenot D, et al. Novel targets for the development of anti-herpes compounds. Infect Disord Drug Targets 2007;7:11-8

•• **Interesting review on the development of molecules with antiherpetic activity.**

123. Yasukawa T, Ogura Y, Sakurai E, et al. Intraocular sustained drug delivery using implantable polymeric devices. Adv Drug Deliv Rev 2005;57:2033-46

124. Anand BS, Dey S, Mitra AK. Current prodrug strategies via membrane transporters/receptors. Expert Opin Biol Ther 2002;2:607-20

•• **Review on the use of prodrugs and their effect.**

125. Neves SS, Sarmento-Ribeiro AB, Simões SP, et al. Transfection of oral cancer cells mediated by transferrin-associated lipoplexes: mechanisms of cell death induced by herpes simplex virus thymidine kinase/ganciclovir therapy. Biochim Biophys Acta 2006;1758:1703-12

126. Magenheim B, Benita S. Nanoparticle characterization: a comprehensive physicochemical approach. STP Pharm Sci 1991;1:221-41

127. Iwasaki Y, Ueda M, Yamada T, et al. Gene therapy of liver tumors with human liver-specific nanoparticles. Cancer Gene Ther 2007;14:74-81

128. Sham JO, Zhang Y, Finlay WH, et al. Formulation and characterization of spray-dried powders containing nanoparticles for aerosol delivery to the lung. Int J Pharm 2004;269:457-67

129. Tsapis N, Bennett D, Jackson B, et al. Trojan particles: large porous carriers of nanoparticles for drug delivery. Proc Natl Acad Sci USA 2002;99:12001-05

130. Schmidt C, Bodmeier R. Incorporation of polymeric nanoparticles into solid dosage forms. J Control Rel 1999;57:115-25

Affiliation

Rita Cortesi† PhD & Elisabetta Esposito PhD
†Author for correspondence
University of Ferrara,
Department of Pharmaceutical Sciences,
CoReS Techno Group,
Via Fossato di Mortara,
19-44100 Ferrara, Italy
Tel: +39 0532 455259; Fax: +39 0532 455953;
E-mail: crt@unife.it