Acyclovir Liposomes for Intranasal Systemic Delivery: Development and Pharmacokinetics Evaluation

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Intranasal route is one of the most attractive routes for distributing drugs to systemic circulation. Liposomes are used as bio-compatible carriers to improve delivery properties across nasal mucosa. The objective of the present study was to formulate acyclovir liposomes and partition into poly-N-vinyl-2-pyrrolidone. Entrapment efficiency showed that multilamellar and unilamellar liposomes were 43.2\% \pm 0.83 and 21\% \pm 1.01, respectively.

The bioavailability of acyclovir from nasal mucoadhesive gel was 60.72\% compared with intravenous route. The use of liposomes acyclovir and mucoadhesive gel not only promoted the prolonged contact between the drug and the absorptive sites in the nasal cavity, but also facilitated direct absorption through the nasal mucosa.

Keywords
Acyclovir Liposome, Mucoadhesion, Nasal Hydrogels, Pharmacokinetics

Liposomal drug delivery systems have received considerable attentions due to their immense advantages, including effective encapsulation of both small and large molecules with a wide range of hydrophobicity levels and pKa values (Cattel et al. 2003; Mura et al. 2007), prolonging and targeting release of therapeutic agents by modification of liposomal surface and minimizing clinical drug dose and reducing toxicity effects (Gabizon et al. 2003).

Liposomes delivery systems were found to enhance nasal penetration and absorption of calcitonin or insulin; this was attributed to increase penetration of peptides (Chen et al. 2007; Jain et al. 2007). Liposomes also have been reported as adjuvant for influenza vaccine formulations. Intranasal immunization with the liposome-supplemented vaccine conferred a better protection against an influenza infection (Ninomiya et al. 2002).

Liposomes can be obtained in different sizes and layers (single bilayer or multiple layers) depending on the preparation methods chosen. A common characteristic of the methods used for liposome preparation usually involves drying the lipids from organic solvents followed by dispersion in aqueous solution. When lipids are dispersed in an aqueous solution, multilamellar vesicle (MLV) structures form spontaneously. Usually MLVs are subjected to a sonication process (using bath-type or probe-type instrument) to reduce size and prepare small unilamellar vesicle (SUV). A problem associated with sonicator probe is its metal particle shedding. The bath sonicator has many advantages. For instance, the temperature of the product can be controlled during process by controlling the temperature of water bath and the product can be processed aseptically in a sealed container (Vemuri and Rhodes 1995).

The bioavailability of intranasally administered peptides and proteins including insulin may be low because of high molecular weight and hydrophilicity (Hinchcliffe and Illum 1999). The poor absorption of drugs across the nasal membrane is due to low permeability of the nose membrane to the high molecular weight polymers, mucociliary clearance, and enzymatic degradation (Illum 2003). To optimize nasal administration, bioadhesive hydrogels that increase residence time in nasal cavity, absorption enhancers, and enzymatic inhibitors that protect the enzymatic degradation of drugs have been used (Varshosaz et al. 2006).
Gels can be used for nasal drug delivery because they provide a high drug transport often governed by the longer residence time of the formulation at the site of absorption (Ugwoke et al. 1999). The residence time is only advantageous if the drug remains in formulation and is released throughout the complete period. The release can be controlled by having the drug suspended in the gel (Desai and Blanchard 2000) or by partitioning the drug to liposomes (Bochot et al. 1998).

Acyclovir is being used to treat herpes simplex virus (HSV) infection in both immunocompetent and nonimmunocompromised patients. Currently available dosage forms of acyclovir have a number of limitations. Absorption of acyclovir is slow, variable, and incomplete with low bioavailability of (15–20%) requiring a frequent dosing regimen (Thummel and Shen 2001). Its limited solubility in both water and lipid decreases the bioavailability (Shao et al. 1994) and makes its intramuscular administration rather difficult. Many researchers have tried to increase its solubility by modifying chemical structure or by prodrug approach (Chikhale et al. 1996), but none of the findings is very encouraging.

When administered intravenously, most of acyclovir is excreted unchanged through urine since the drug molecules are excreted through kidneys by both glomerular filtration and tubular secretion. The intravenous administration of acyclovir sodium results in thrombophlebitis and has chances of drug crystal formation when excreted through kidneys during the systemic treatment of HSV infections in immunocompetent as well as immunocompromised patients (Brown et al. 2002). The terminal or beta-phase half-life of acyclovir was reported to be 2 to 3 hr for adults (Thummel and Shen 2001). Consequently, the dose of the drug is high and residence time of the drug in circulation is very less. Therefore, formulation of acyclovir as liposomal vesicles would help in maintaining the drug in the systemic circulation for a longer time and thus the dose-related toxicities may be reduced (Jain et al. 2005). Pavelič et al. (2005) prepared a liposomal delivery system for vaginal administration of acyclovir to improve its delivery characteristics.

Earlier studies attempted to improving the physicochemical characteristics of acyclovir was based on chemical modification using ester prodrugs and limited work has been reported on modification, which includes transbuccal delivery. An in vitro study using porcine tissue indicated that buccal transport of acyclovir occurs predominantly by a passive diffusion mechanism probably through paracellular route (Shojaei et al. 1998). Consequently, acyclovir may serve as a good model drug to study nasal absorption enhancement via liposomal formulation since no research has been carried out to formulate acyclovir as a liposomal nasal formulation. Acyclovir does not undergo degradation and it is not metabolized in the nasal cavity. The elimination route acyclovir is urinary excretion and metabolism of these drugs in the liver and nasal cavity might be negligible. Therefore, the objectives of this study were to evaluate the absorption promoting effect of nasal administration of acyclovir mucoadhesive liposomal gel and to study the pharmacokinetics parameter to assay the enhancement of bioavailability via nasal route.

MATERIALS AND METHODS

Acyclovir was kindly provided by Riyadh Pharma Pharmaceutical Company (Riyadh, Saudi Arabia). Cholesterol (CHOL) was purchased from Fisher Scientific Company (Fair Lawn, NJ, USA). L-α-Dipalmitoylphosphocholine (DPPC) was obtained from Avanti Polar Lipids (Birmingham, AL, USA). Chloroform and triton X-100 (iso-octylphenoxy polyethoxy ethanol) were purchased from BDH Laboratories (Poole, England, UK). One-decane sulphonate sodium salt was purchased from Fluka Chemie GmH (Buchs, Switzerland). Poly-N-vinyl-2-pyrrolidone (PVP) known as Luviskol® K-90 was purchased from Aktiengesellschaft (Ludwigshafen, Germany). Polyethylene glycol 600 (PEG) was purchased from Merck-Schuchardt (Hohenbrunn, Germany). Glycerin BP was purchased from Pacegrove Chemical and Biopharmaceutical Chemicals (Leicestershire, UK). Perchloric acid 70% PRS was purchased from Panreac Quimica, SA (Barcelona, Spain). Acetonitrile and HPLC grade triethanolamine reagent were purchased from Sigma-Aldrich, Chemie GmbH (Steinheim, Germany). All chemicals and organic solvents used were of HPLC grade and were used as received.

Preparation of Acyclovir Liposomes

Two methods were used to prepare the acyclovir-containing liposomes. The first method is a conventional drug-lipid film hydration method (Law and Hung 1998; Fersta et al. 1999). Lipid phase, which contains L-α-dipalmitoylphosphocholine (DPPC) and CHOL in a molar ratio of 1.6:1, respectively, was dissolved in chloroform. The solution was transferred to a 50 ml round-bottom flask. The flask was attached to a rotary evaporator Büchi ratavapor (Labortechnik AG, Flawil, Switzerland). The organic solvent were evaporated at 30°C under reduced pressure and a stream of nitrogen at 150 rpm until a thin film deposited on the wall of the flask. The flask was kept in vacuum desiccators overnight for complete removal of chloroform residual. The dried film was hydrated with aqueous phase containing 10 mg/ml acyclovir dissolved in phosphate buffer. The film was hydrated while the flask kept rotating at 200 rpm. The hydration was performed above the phase transition temperature (Tm) of the phospholipids (55°C) and was continued until the lipid film was dispersed in the aqueous phase. The most important parameter that must be considered in liposome preparation is the phase transition temperature (Tm) of the membrane lipid; the temperature must be kept above the transition temperature of the lipid. DPPC transition temperature is 41°C (Vemuri and Rhodes 1995).

After hydration, the dispersion was allowed to stand for 1 hr for vesicle formation, then kept at 4°C for 24 hr. Hydration was carried out under a stream of nitrogen and in reduced pressure to avoid oxidation of the DPPC. According to the sonication method, MLV suspensions (obtained as described above) were vesicles submitted to a bath-sonicator for 1 hr to form a reduced
vesicle size. Acyclovir-containing liposomes was separated from unentrapped acyclovir by centrifugation at 5000 rpm at 4°C for 4 min (Law and Hung 1998) and was used in further study.

The second method to prepare the liposomes was a partial modification of the drug-lipid hydration method (Chetoni et al. 2004; Law and Hung 1998). DPPC:CHOL in a molar ratio of 1:6.1, respectively, was used. The total lipid was 163 mg; the quantity of lipid phase was 124 mg of DPPC and 39 mg of CH dissolved in chloroform and 7 mg of acyclovir dissolved in 10 ml methanol were mixed and dried to form a thin film as described in the first method. Then it was hydrated with 10 ml of phosphate buffer saline containing of acyclovir. This method yielded a unilamellar liposomes.

Particle Size and Morphology Determination
The particle size of freshly prepared liposomes was measured by a laser particle analysis technique using Brookhaven Instruments (New York, NY, USA).

Morphology of the vesicles was performed with the help of a scanning electron microscope, JSM-6060 JSM, Jeol Ltd. (Tokyo, Japan).

Entrapment Efficiency
The liposomes entrapping acyclovir were estimated after removing unentrapped one. Various attempts were made to destroy the liposomes, one of these approaches was destroying the liposomes using triton X-100. A sample of the liposome suspension with dialyze 10% triton X-100 solution (in a ratio of 1:1) to annihilate the liposomes and centrifuged for 6000 rpm for 10 min (Jain et al. 2005). A clear solution was removed for analysis. Briefly, the analysis was preformed by high performance liquid chromatography (HPLC) system at 254 nm with µ-Bondapak C18 column maintained at 25°C (column oven). The mobile phase consisted of 0.02 M potassium dihydrogen phosphate pH 3.5 (99%) and acetonitrile (1%) and injection volume was 20 μl (Jain et al. 2005). Entrapment efficiency was calculated according using the following equation:

\[
\text{Encapsulation efficiency} = \frac{E_0 - E_1}{E_0} \times 100
\]

where \(E_0\) = amount of acyclovir added initially, \(E_1\) = amount of acyclovir determined, and \((E_0 - E_1)\) represents the amount of acyclovir trapped in the liposome formulation.

Preparation of Poly-N-Vinyl-2-Pyrrolidone Gel
Preparation of poly-N-vinyl-2-pyrrolidone (PVP) gel was prepared according to the method described by Lugão et al. (2002) with some modifications. PVP (3% w/v) with 1% PEG solution was prepared in 100 ml of distilled water and stirred by magnetic stirrer (25 rpm for 3 min), then mixtures were filled in glass bottles. The irradiation of PVP hydrogel was carried out using gamma irradiation (Gamma Irradiation Facility, King Faisal Specialist Hospital and Research Center, Riyadh, Saudi Arabia) with 60°C source at room temperature at doses of 20 kGy (preliminary study indicated these doses are appropriate doses for PVP cross-linking). The irradiation time was calculated based on the radiation source dosing rate (51.71 Gy/min).

Incorporation of Liposome into Hydrogel
Pervious study by Pavelić et al. (2001) showed that liposomes are compatible with viscosity-increasing agents, e.g., methylcellulose and carbopol. To provide suitable vehicles for nasal application, liposomes can be distributed uniformly by incorporation into hydrogel and can preserve its structure. Liposomes (10% (w/w)) containing acyclovir (previously separated from the unencapsulated drug) were mixed into the hydrogels with continuous stirring using spatula. Control hydrogels (drug suspended in gels) were prepared using the same conditions.

In Vivo Studies
The protocol of animal studies adhered to the principle guide of Laboratory Animal Research Committee, King Saud University (Riyadh, Saudi Arabia). New Zealand white rabbits with mean weight of 2.5 ± 0.3 kg were obtained from Laboratory Animal Facility, Central Laboratory for Drug and Food Analysis (Ministry of Health, Saudi Arabia). The rabbits were neither sedated nor anesthetized during the experiment. For this reason, rabbits were accommodated to the dosing for 1 month before the study to prevent withdrawal and defense reaction that may lead to inaccurate dosing.

The rabbits were kept in a single cage and fasted for 12 hr before the study with free access of water during the experiments. A cannula was inserted into marginal ear vein for blood sampling and flushed with heparinazed normal saline solution.

Study Design and Plasma Sampling
New Zealand white rabbit was selected as an animal model because it provides a well-controlled animal model for screening the nasal absorption potential of nasal formulations (Dondeti et al. 1995). The blood volume of the rabbit is sufficiently large to permit frequent blood samples and allow full characterization of pharmacokinetics profile of the drug (Gizurarson 1990). In addition, the rabbit was chosen as it offered certain advantages such as ease of handling and low cost of maintaining (Dondeti et al. 1995).

Fifteen rabbits were used in this study. The rabbits were divided into three groups; the first group received acyclovir liposomes as nasal gel. The second group received the free drug as nasal gel (control), the need for the control group was required to confirm our hypothesis that a liposome formulation should demonstrate a better permeation of acyclovir across nasal membrane. The third group received intravenous injection of acyclovir solution. Intranasal preparations were administered by
wide nasal droppers with a wide orifice inserted about 5 mm into nostril to the rabbit while in a supine position. The rabbit was kept in this position for 1 min after administration. A total dose of 20 mg/nostril (10 mg) of acyclovir was administered in each side of the rabbit. The intravenous 10 mg/kg (Zovirax®) was administered via the previously placed cannula into marginal ear vein over 1 hr (slow intravenous injection of acyclovir is preferred since rapid intravenous administration will affect kidney functions) to reduce renal tubular damage (GlaxoSmithKline 2006; Sweetman 2007).

Multiple blood samples (1 ml) were collected from cannulated marginal vein before administration of the dose to obtain a reference level (time point zero) and after administration at different time intervals at 0.5, 1, 2, 4, 6, and 8 hr in heparinized vacuum glass tubes. The blood volume withdrawal during the study was 6 ml for each rabbit; the cannula was flushed with heparin in normal saline solution after each sample was taken. The plasma was separated by centrifugation at 13000 rpm for 5 min and stored at −20°C until the time of analysis. Acyclovir plasma sample was found to be stable over 4 weeks when stored at −20°C (Bangaru et al. 2000).

The extraction for acyclovir in plasma was similar to the method described by (Bangaru et al. 2000; Teshima et al. 2003). The extraction was achieved by adding 35 µl of 7% (v/v) of freshly prepared perchloric acid into evacuated tube containing 200 µl of spiked plasma and vortexed for 5 min; the precipitated plasma proteins were separated out by centrifugation for 30 min at 1500 rpm. A clear supernatant layer was obtained, snapped and filtered through Milipore into a HPLC vial directly. The vial was arranged in the autosampler and programmed to inject 100 µl of the sample into chromatographic system. The blank plasma samples were injected after applying the same extraction procedure and tested for interference to ensure the selectivity of the method.

The method used to assay acyclovir concentration in plasma was a modification of the methods described previously (Bangaru et al. 2000; European Pharmacopoeia 2001). The mobile phase consists of 6.0 g of sodium dihydrogen phosphate and 1.0 g of sodium decaene sulphonate in 900 ml of water and pH adjusted to pH 3 ± 0.1 with phosphoric acid. Then 40 ml of acetonitrile was added and diluted to 1 liter with distilled water, filtered through 0.45 µm Milipore, and degassed. The effluent from the reverse phase column (Nucleosil chrompak C18) was monitored at 254 nm. The flow rate was maintained at 2 ml/min and the operating temperature was 40°C.

Pharmacokinetic Studies

Various pharmacokinetic parameters such as T_{max} (time required to achieved maximum plasma concentration), C_{max} (maximum plasma concentration) and AUC (area under the plasma concentration-time curve) were calculated from rabbit plasma and represented as ± SD. Intranasal bioavailability was calculated relatively to the intravenous administration, where intravenous bioavailability was considered 100% as shown in following equation:

\[
\%F = \frac{\text{AUC}_{\text{IV, Dose}_\text{IV}}}{\text{AUC}_{\text{IV, Dose}_\text{IN}}} \times 100
\]

RESULTS

Acyclovir Liposomes

Liposomes were prepared by two methods both of them based on drug-lipid film hydration method. However, in the first method the drug was dissolved in aqueous phase whereas in the second method the drug was dissolved in both phases. MLVs were achieved by the first method whereas the second method yielded unilamellar vesicles.

L-α-dipalmitoylphosphocholine was used as a membrane since this phospholipid is able to mimic many aspects of biological membranes, being one of their most abundant constituent. Fersta et al. (1999) studied the interaction between positively, negatively, and neutral charged membrane via electrostatic or hydrogen bound. They found that no interaction was observed with neutral membrane made up of DPPC. Therefore, in this study neutral liposomes were prepared. However, no literature reported the toxicity of anionic liposome (anionic charged membrane), while cationic liposome (positively charged membrane) were proven to be toxic in vitro and in vivo (Dokka et al. 2000; Hui et al. 2004), especially those containing stearylamine (Aramaki et al. 2000).

Particle Size Determination

The size of liposomal prepared by the first method was 1048.1 ± 101.3 nm and 627.4 ± 36.9 nm for the second method. The liposomal size ranges falls within an acceptable range (3.9–2088 µm) as reported previously by many researchers (Law et al. 2001; Shahiwala and Misra 2006; Jain et al. 2007). This large size range may be attributed to the preparation conditions such as the hydration time, the degree of shaking and the sonication time, which may have a role in determining the vesicle size and size range.

Liposome Shape and Morphology

Figures 1 and 2 show photomicrographs of the freshly prepared acyclovir liposomes as recorded by scanning electron microscope. The figures show large unilamellar vesicles with a spherical shape.

Entrapment Efficiency

The amount of acyclovir entrapped in liposomes was determined as a difference between the amount of drug added during liposome preparation and the amount of the entrapped drug present in the supernatant. The encapsulation efficiency was expressed as percentage of the total amount of acyclovir that became entrapped.
FIG. 1. Scanning electron micrograph of liposomes prepared by conventional drug-lipid film hydration method.

The calculated average percent entrapment of the liposomes prepared by the second method was $43.2\% \pm 0.83$ ($n = 3$). The results showed an enhancement of the entrapment efficiency compared with those reported for other acyclovir liposomal preparations (Law and Hung 1998; Law et al. 2000). The entrapment efficiency of the first method was $21\% \pm 1.01$ ($n = 3$).

Preparation of PVP Gel

Ionizing radiation possesses a unique ability to initiate polymerization and/or cross-linking reactions without the need to add toxic chemicals. Therefore radiation processing is emerging as an excellent tool to produce hydrogels for a variety of medical applications (Lugão et al. 2002). PVP hydrogel itself is of limited adhesiveness; consequently blending PVP with other polymers has a significant role in a series of PVP hydrogels as biomedical materials. PEG is an additive typically used in PVP hydrogels for biomedical applications because of its nontoxic property. PEG also allows changes in the rheological characteristics of hydrogels of PVP. The presence of PEG usually increases the elasticity, adhesion, and tacky properties of the hydrogels because of its plasticizing effect. The PEG chains settle among those of PVP, avoid cross-linking, and decrease the physical interactions between PVP chains (Sen and Avcı 2005).

In Vivo Studies

In the present study the rabbits remained conscious through the experiment, thereby providing functional mucociliary transport function. Usual anesthetizing agents could affect the in vivo absorption of drug (Mayor and Illum 1997), therefore we avoided using anesthesia. The pharmacokinetics parameters were calculated and expressed as the mean ± SD. $C_{\text{max}}$ and $T_{\text{max}}$ were calculated from the pharmacokinetics profiles generated by plotting the drug plasma concentrations expressed as $\mu g/ml$ at each sampling time point and were plotted against time. The results are represented in Table 1 and Figure 3. The area under the plasma level curve concentration-time curve (AUC) was calculated from the beginning ($t_0$) to the end of observation time ($t_\infty$) using trapezoidal rule.

Intravenous acyclovir used as a reference comparison has a 100% bioavailability while acyclovir suspended in gel was used as control to examine the effect of liposomes preparation in acyclovir penetration across nasal membrane. Our results showed that in vivo administration of acyclovir mucoadhesive liposomal gel remarkably increased the acyclovir concentration found in serum when compared with the concentration found after administration of acyclovir suspended in gel. The AUC values of acyclovir mucoadhesive liposomal gel and acyclovir suspended in gel were 1.91175 and 21.90264 ($\mu g \cdot hr \cdot ml$), respectively. This variation in the AUC values is due to the differences in the drug profile of acyclovir suspended in gel.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Intravenous acyclovir</th>
<th>Intranasal free drug in gel</th>
<th>Intranasal liposomal gel</th>
</tr>
</thead>
<tbody>
<tr>
<td>$C_{\text{max}}$ ($\mu g/ml$)</td>
<td>21.03</td>
<td>0.097</td>
<td>6.98</td>
</tr>
<tr>
<td>$T_{\text{max}}$ (hr)</td>
<td>0.5</td>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td>AUC$_{0 \rightarrow t}$ ($\mu g \cdot hr \cdot ml$)</td>
<td>45.125</td>
<td>1.91175</td>
<td>21.90264</td>
</tr>
<tr>
<td>Bioavailability (%)</td>
<td>—</td>
<td>5.3</td>
<td>60.72</td>
</tr>
</tbody>
</table>

$C_{\text{max}}$ = Maximum plasma concentration; $T_{\text{max}}$ = time required to reach maximum plasma concentration; AUC = area under the plasma concentration-time curve.
DISCUSSION

Systemic immunization studies using liposomes loaded with tetanus toxoid suggested that intranasal administration of liposomes encapsulated with vaccines is an effective way for inducing mucosal immune responses. Lack of membrane toxicity and local irritation of neutral liposomes in human nose was studied by a standard hemolysis test and local irritations were indicated. This finding also demonstrates one safety aspect of these liposomes in practical use (Tafaghodi et al. 2006).

The entrapment efficiencies of the first method were lower than the second method. We suggest that for the second method of the preparation, the acyclovir in the organic solvent is present in neutral form and interacts with phospholipids in the organic phase before the film formation. This interaction may be significant when both acyclovir and phospholipids are in dry mixture. As aqueous phase is added, interaction becomes more effectively hydrophobic between the acyclovir and phospholipids bilayers. Therefore, more drug molecules are encapsulated in the liposomes. For the first method of acyclovir preparation, the acyclovir was dissolved in the aqueous phase in hydrophilic state, which may interact in less manner with the hydrophobic phospholipids bilayers leading to low drug entrapment. The large size of the prepared unilamellar liposomes may be responsible for the high drug entrapment. For watersoluble compounds, large- or intermediate-sized unilamellar vesicles are the most appropriate types to achieve as high a value as possible for entrapped volume-to-lipid ratio. This is due to the large core available for entrapping large volume of the drug solution. It is widely recognized that entrapment efficiency decreases with decreasing liposome size (Mayer et al. 1993).

Berger et al. (2001) found that liposomes with size ranging from 50 to 400 nm led to an entrapment efficiency varying from ∼10% to 40%. The high entrapment efficiency was obtained by the second method. Therefore, further studies were conducted with liposomes prepared by second method of preparation.

Since acyclovir is freely soluble in aqueous environment, the plasma protein and other nucleic acid were precipitated at pH less than 2.5. Some organic solvents such as dichloromethane and acetonitrile were tried for extraction. Different HPLC methods have been reported to extract acyclovir from plasma. The first method of extracting acyclovir from plasma used 1 ml of plasma and 2 ml of acetonitrile that was vortexed and centrifuged to collect the organic phase. Then the mixture was evaporated under nitrogen gas and reconstituted with water (Chavanpatil and Vavia 2004). In another method of extraction based on dichloromethane and isopropyl alcohol (1:1), the organic phase was removed, evaporated under stream of nitrogen, and reconstituted with water (Bahrami et al. 2005). When we used these methods that involve evaporation of plasma and reconstitution, it resulted in a bad resolution and did not permit adequate purification of the extract.

Jain et al. (2005) reported an extraction method based on using 35% perchloric acid and provided direct injection of supernatant into chromatographic column. By applying this method, we obtained a good sample separation. However, the column was deteriorated after three injections, thus we excluded this method. Consequently, we attempted to use a low concentration of perchloric acid. By applying this method a good peak separation was obtained while at the same time maintaining the column integrity (Teshima et al. 2003).

Perchloric acid solution was added to precipitate plasma protein, which could allow direct injection of clear solution containing no particulate matter into the separating column. Figure 4 illustrates the chromatograms obtained of plasma spiked with acyclovir. The acyclovir peak has a retention time of 6.192 min. Good peak retention resolved well with no interference from the endogenous component in rabbit plasma. The assay was completed in ~12 min. The standard calibration curve of five triplicates was linear over the concentration range used (0.05–2 µg/ml); the calibration curve revealed a good linear fit with a correlation coefficient of 0.9991. Limit of detection was defined as the concentration of drug giving a signal-to-noise ratio of 3:1 and was found to be 0.025 µg/ml and the limit of quantification was 0.05 µg/ml. The linear regression of calibration curve was used to calculate acyclovir concentration in plasma samples of rabbits. Mascher et al. (1992) reported that a strong acidic mobile phase could further improve the detection limit (10 ng/ml). The composition of mobile phase used had a measured pH value of ∼1.5. However, this is not recommended as it may lead to rapid deterioration of the column. At pH 3.5 used in present study, the detection limit of 0.05 µg/ml obtained was deemed satisfactory for the method used in pharmacokinetics studies of acyclovir.

The AUC values of acyclovir mucoadhesive liposomal gel and acyclovir suspended in gel were 1.91175 and 21.90264 (µg·hr·ml), respectively. This variation in the AUC values is due to the differences in the drug profile of acyclovir suspended in

FIG. 3. Mean acyclovir plasma concentrations versus time profile for intravenous (♦), acyclovir suspended in gel (□), and acyclovir mucoadhesive liposomal gel administered by nasal route (■).
gel. This behavior could be due to its limited permeability across nasal mucosa and hydrophilicity. The effect of PEG present in PVP gel formulation may be responsible of acyclovir penetration (even low) across nasal membrane. Nasal absorption of nifedipine from gel preparations, PEG 400, aqueous carbopol gel, and carbopol-PEG has been studied in rats. Nasal administration of nifedipine in PEG resulted in rapid absorption and high C max value (i.e., 138); however, the elimination of nifedipine from plasma was very rapid. The plasma concentration of nifedipine after nasal administration in aqueous carbopol gel formulation was very low. The use of PEG 400 in high concentration in humans should be considered carefully because PEG 400 is known to cause nasal irritation in concentrations higher than 10% (Morimoto et al. 1987).

The enhancement of bioavailability of the prepared liposomal formulations in comparison to free drug suspended in gel could be attributed to both encapsulation and incorporation of acyclovir in nasal mucoadhesive gel. Nasal bioavailability of acyclovir was 60.72% calculated relative to the serum acyclovir levels over a period of 8 hr after intravenous injection of acyclovir. Liposomes have been demonstrated to have good permeability characteristics to enhance nasal penetration of many drugs. Our results were found to be in agreement with Ding et al. (2007) who studied nasal administration of liposomes formulation of levonorgestrel and evaluated their pharmacokinetic properties. They found that administration of levonorgestrel liposomes via nasal route increased its bioavailability when compared with levonorgestrel suspension by oral route. The liposomes greatly facilitated levonorgestrel nasal absorption and may provide a rapid onset of action of levonorgestrel for emergency contraception.

The permeability of liposome-entrapping insulin through the nasal mucosa of rabbit has been studied and compared with the permeability of insulin solution with or without pretreatment by sodium glycocholate (SGC). A comparison of the insulin solution and liposome suspension showed that the liposome had permeated more effectively. The result demonstrated that liposomes could be considered as a useful carrier of insulin administered intranasally (Murraymatsu et al. 1999). Jain et al. (2007) studied the usefulness of mucoadhesive multivesicular liposomes as a mucoadhesive to prolong the release of insulin via nasal and ocular route. They concluded that the multivesicular liposomes carriers were shown to be marginally effective after nasal administration compared with ocular route, although better therapeutic profile as the hypoglycemic effects were prolonged until 72 hr.

Nasal administration of a liposomal leuprorelin acetate formulation with chitosan produced contraception in male and female rat when compared with subcutaneous delivery. For leuprorelin acetate administered by nasal route a low dose of drug was needed and hence it would be expected to increase the therapeutic index and reduce the adverse effect (Shahiwala and Misra 2006). Liposomes are phospholipid vesicles composed of lipid bilayer enclosing one or more aqueous compartments. Liposomes provide an efficient drug delivery system because they can alter the pharmacokinetics and pharmacodynamics of the entrapped drugs. Liposomes are interesting drug carriers that have been widely used to deliver a wide variety of medications via the nasal and lung route (Illum 1997).

Nasal delivery of tetanus toxoid entrapped in liposomes improved the immune response compared with delivery of free antigen. Furthermore, if the liposomes were taken up intact, the superficial layer of nose is highly vascularized. Therefore a quantity of liposomes will pass directly into systemic circulation resulting in a systemic immune response and some liposomes probably would be taken up and delivered to underlying lymphoid cells of nasal associated lymphoid tissue (Alpar et al. 1992). Liposomes also provided protection of entrapped drugs from enzymatic degradation (Murraymatsu et al. 1999) and disrupted the mucosal membrane to increase absorption (Lee et al. 1991).
CONCLUSION

Nasal liposomal delivery system combined with mucoadhesive gel system provides therapeutically significant plasma levels of low molecular weight hydrophilic drug such as acyclovir. The enhanced bioavailability of the prepared liposomal formulations in comparison to free drug suspended in gel could be attributed to both encapsulation and incorporation of acyclovir in nasal mucoadhesive gel. Liposomes have been demonstrated to have good permeability characteristics to enhance nasal penetration of acyclovir.

REFERENCES


