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Nasal administration of Carbamazepine using chitosan microspheres: In vitro/in vivo studies

E. Gavini [a], A.B. Hegge [b], G. Rassu [a], V. Sanna [a], C. Testa [c], G. Pirisino [a], J. Karlsen [b], P. Giunchedi [a,*]

[a] *Dipartimento di Scienze del Farmaco, University of Sassari, via Muroni 23/a, 07100 Sassari, Italy*
[b] *Institute of Pharmacy, University of Oslo, Oslo, Norway*
[c] *Istituto Zooprofilattico Sperimentale della Sardegna, Sassari, Italy*

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Abstract

The nasal route is used both for local therapies and, more recently, for the systemic administration of drugs, as well as for the delivery of peptides and vaccines. In this study the nasal administration of Carbamazepine (CBZ) has been studied using microspheres constituted by chitosan hydrochloride (CH) or chitosan glutamate (CG). Blank microspheres were also prepared as a comparison. The microspheres were produced using a spray-drying technique and characterized in terms of morphology (scanning electron microscopy, SEM), drug content, particle size (laser diffraction method) and thermal behaviour (differential scanning calorimetry, DSC). In vitro drug release studies were performed in phosphate buffer (pH 7.0). In vivo tests were carried out in sheep using the microparticles containing chitosan glutamate, chosen on the basis of the results of in vitro studies. The results were compared to those obtained after the nasal administration of CBZ (raw material) alone. For the evaluation of in vivo data statistical analysis was carried out using the unpaired *t*-test.

Spray-drying was a good technique of preparation of CBZ-loaded microspheres. The loading of the drug into the polymeric network always led to an increase in the dissolution rate compared to CBZ raw material. The microspheres obtained using chitosan glutamate had the best behaviour both in vitro and in vivo. They increased the drug concentration in the serum when compared to the nasal administration of the pure drug (C_{max} 800 and 25 ng/ml for microspheres and pure drug, respectively). The results obtained indicate that the loading of CBZ in chitosan glutamate microspheres increases the amount of the drug absorbed through the nose.

Keywords: Carbamazepine; Chitosan salts; Spray-dried microspheres; Nasal route; In vivo study; Sheep

1. Introduction

Conventionally the nasal route has been used for the delivery of drugs in the treatment of local diseases, however the last decade has recognised the importance of the nasal cavity as potential route for drug delivery, particularly of small molecular weight polar drugs, peptides and proteins (Illum, 2003). There is an increasing number of research and review articles addressing topics on nasal drug delivery. This interest arises from the different possible advantages presented by the nasal cavity, such as: the epithelium very vascularized and with a relatively large surface area available for drug absorption, the porous endothelial basement membrane, the direct transport of absorbed drugs into the systemic circulation thereby avoiding the first-pass effect hepatic present in peroral administration, the lower enzymatic activity compared with the gastrointestinal tract and the liver (Ugwoke et al., 2001). For all these reasons the nasal route can be considered a useful alternative both to parenteral and oral routes (Edman and Bjork, 1992; Turker et al., 2004). A wide range of nasal products is in development, mostly in correlation with the rapid onset of action of nasal route, for example, for the treatment of pain (nasal morphine and ketamine) and for the treatment of erectile dysfunction (nasal apomorphine) (Illum, 2003).

A limitation of nasal drug delivery is the mucociliary clearance that determines a limited time available for adsorption within the nasal cavity (Soane et al., 1999). One strategy for increasing drug absorption is to prevent the rapid clearance of

* Corresponding author. Tel.: +39 079 228754; fax: +39 079 228733.
E-mail address: pgiunc@uniss.it (P. Giunchedi).

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the delivery system from the nasal mucosa and thereby prolong the contact between the nasal mucosa and the formulation. Mucoadhesive materials can increase the time available for drug absorption: starch microspheres were the first example of mucoadhesive microparticulate nasal delivery system (Illum et al., 1987); mucoadhesive microspheres were further investigated for the purpose of nasal delivery (Abd El-Hameed and Kellaway, 1997; Pereswetoff-Morath, 1998; Dyer et al., 2002; Fundueanu et al., 2004; Gavini et al., 2005).

Chitosan, a linear polysaccharide produced by a process of deacetylation from chitin, is one such material that has been shown to be mucoadhesive (Soane et al., 1999). The free amino groups resultant from the deacetylation process of chitin enable the formation of positively charged chitosan salts with organic and inorganic acids. Chitosan may be a good option in nasal delivery as it binds to the nasal mucosal membrane with an increased retention time and it is a good absorption enhancer (Ravi Kumar et al., 2004; Illum, 2003). Furthermore chitosan is an excipient able to enhance the dissolution rate of low water-soluble drugs (Giunchedi et al., 2002; Maestrelli et al., 2004).

Aim of this work is the preparation of a microparticulate delivery system (spray-dried microspheres) for the nasal administration of Carbamazepine (CBZ) based on two chitosan salts: chitosan hydrochloride (CH) and chitosan glutamate (CG).

Carbamazepine is a drug widely used as antiepileptic agent, in the therapy of psychomotor seizures and trigeminal neuralgia; it is traditionally given by oral administration but due to its poor water solubility (about 170 mg/l at 24 °C) it is characterised by slow and irregular gastrointestinal absorption (Levy et al., 1989; Jackson et al., 1986). Many reports were focused on improving its dissolution characteristics (Moneghini et al., 2002). Furthermore this drug is characterised by a considerable hepatic first-pass effect owing to the enzymatic auto-induction of its metabolism (Larkin et al., 1989). This latter characteristic besides the need of a therapeutic prompt action make CBZ a possible candidate for the development of a nasal formulation.

The microspheres were prepared by a spray-drying method, with two different drug to polymer weight ratios: 1–2 and 1–3. Blank microspheres were prepared as comparison.

The microspheres were characterised in terms of encapsulation efficiency, morphology and particle size; the in vitro release behaviour of the drug from the microparticles was studied and compared to the dissolution profile of the drug alone. In vivo tests were performed on sheep which are one of the preferred animal model for pharmacokinetic and formulation studies in nasal drug delivery (Lindhardt et al., 2002). As a comparison, the nasal administration of a powder constituted by CBZ alone (raw material) was carried out. For the evaluation of in vivo behaviour, data statistical analysis was carried out using the unpaired *t*-test.

2. Materials and methods

2.1. Materials

Carbamazepine USP-grade was supplied by Sigma–Aldrich (Milano, Italy); chitosan hydrochloride Protasan UP CL 113 batch FP-110-02 (Mw: 160000; degree of acetylation: 86%) and chitosan glutamate batch 904-360-01 were purchased from FMC BioPolymer AS, (Drammen, Norway); 1-butanol 99.4% A.C.S. reagent, acetone, analytic reagent were supplied by Sigma–Aldrich (Milano, Italy); silicon oil was supplied by Cruciani (Roma, Italy); ethanol, 95% (v/v) and acetic acid glacial were furnished from Carlo Erba Reagenti (Milano, Italy); acetonitrile and methanol (HPLC-grade) were supplied by Thomas Baker Chemicals Pvt. Ltd., (Mumbai, India). Monopowder P® insufflators were kindly purchased by Valois Dispray, (Mezzovico, Switzerland). All other solvents and chemicals were of analytical grade.

Table 1
Theoretical composition of spray-dried microspheres

Microspheres	Polymer	Polymer to drug weight ratio
CH-CBZ 1	Chitosan hydrochloride	2:1
CH-CBZ 2	Chitosan hydrochloride	3:1
CG-CBZ 1	Chitosan glutamate	2:1
CG-CBZ 2	Chitosan glutamate	3:1
CH	Chitosan hydrochloride	1:0
CG	Chitosan glutamate	1:0

2.2. Preparation of microspheres

Drug-loaded microspheres were prepared using chitosan hydrochloride or chitosan glutamate in two different drug to polymer weight ratios. Their compositions are reported in Table 1. Blank microspheres were also prepared as comparison.

CBZ was dissolved in one part of acetone; the chitosan salt was separately dissolved in one part of distilled water. CBZ solution and polymer solution were mixed and then homogenized (Ultra-Turrax, T25, IKA, Germany) at 8000 rpm for about 30 s. A total solid final concentration of 1% (w/v) (drug and chitosan salt) in feed-solutions was always obtained.

CBZ-loaded microspheres were obtained by spraying the feed-solution with a spray-dryer (Mini Büchi B-191, Büchi Laboratorius-Technik AG, Flawil, Switzerland) using a standard 0.5 mm nozzle. The solution was fed to the nozzle with a peristaltic pump, atomized by the force of compressed air and blown together with heated air to the chamber where the solvent in the droplets was evaporated. The dried microparticles were harvested from the apparatus collector and kept under vacuum for 48 h.

The process conditions of the spray-drying process were: inlet temperature 100–110 °C; outlet temperature 60–70 °C; spray-pressure about 2 atm and spray-rate of feed 3–4 ml/min. The total volume of feed-solution sprayed for the preparation of each batch of microspheres was always 200 ml.

Blank microspheres were prepared using the same conditions of drug-loaded particles.

2.3. Characterization of microspheres

2.3.1. Yields of production

The yields of production were calculated as the weight percentage of the final product after drying, with respect to the

initial total amount of CBZ and chitosan salts used for the preparations.

2.3.2. Drug content

Samples (10 mg) of drug-loaded microspheres were transferred to a 200 ml volumetric flask. A total volume of 200 ml of dichloromethane was added and the dispersion obtained was sonicated for 30 s to dissolve CBZ. Samples were withdrawn from the undiluted solution using a syringe, then filtered (PTFE 0.45 μm) before detection. The concentration of CBZ in dichloromethane was determined using a UV-spectrophotometer (Hitachi spectrophotometer U-2001, Hitachi Instruments, Tokyo, Japan) at the wavelength of 240 nm. Triplicate measurements were performed (relative standard deviation, R.S.D., within 2%). Real drug content was calculated as the detected amount of CBZ (UV-spectrophotometric determination) with respect to the real amount of total solid in the sprayed solution (polymer and CBZ). The real drug content was expressed in percent (R.S.D. within 1.7%).

2.3.3. Particle size analysis

CBZ (raw material) and the spray-dried microspheres prepared were characterized in terms of dimensions. Particle size distributions were determined by laser diffractometry using Coulter LS 100Q laser size (Beckman Coulter Particle Characterization, Miami, Florida, USA). CBZ (raw material) was dispersed in silicon oil, while the microspheres were dispersed in 1-butanol, always by sonication (about 15–20 s).

The average particle size was expressed as the volume-surface diameter, d_{vs} (μm) (Edmundson, 1967).

The particle size distribution was also expressed in terms of SPAN factor determined as:

$$\text{SPAN} : \frac{d_{90} - d_{10}}{d_{50}}$$

where d_{10}, d_{50} and d_{90} are the diameter sizes and the given percentage value is the percentage of particles smaller than that size. A high SPAN value indicates a wide size distribution (Dubey and Parikh, 2004).

2.3.4. Scanning electron microscopy (SEM)

Shape and surface characteristics of spray-dried microspheres were studied by scanning electron microscopy (Zeiss, Germany). The samples were placed on double-sided tape that had previously been secured on aluminium stubs and then analysed at 20 kV acceleration voltage after gold sputtering, under argon atmosphere.

2.3.5. Differential scanning calorimetry (DSC)

The thermal behaviour of blank and drug-loaded microparticles and of pure drug were determined using differential scanning calorimeter (DSC) (METTLER STAR^e system equipped with a DSC821^e module, PChemLabs, Albuqerque, USA). Samples of 3–5 mg (Mettler M3 Microbalance) were scanned in crimped sealed aluminium pans, under static air atmosphere. An empty pan was used as reference. The heating rate was 10 °C/min, and the temperature interval used was 30.0–300.0 °C.

2.3.6. In vitro release studies

In vitro drug release tests were performed with a USP dissolution equipment, Apparatus n.1, (Erweka, Heusenstamm, Germany) using a modified form of the rotating basket method. Samples of microspheres containing 10 mg of CBZ were tested in 1 l of phosphate buffer (pH 7.0), to assure *sink* conditions. The rotational speed was set at 30 rpm and the temperature for the dissolution medium was set at 37 °C. Samples (1 ml) were withdrawn at predetermined time points (3, 6, 10, 15, 25, 35, 50, 70, 90, 120 and 180 min), and for each withdrawal the corresponding volume was replaced with fresh medium of the same temperature. Samples were filtered (PTFE 0.45 μm) and assayed spectrophotometrically for CBZ (Hitachi spectrophotometer U-2001, Hitachi Instruments, Tokyo, Japan) at 285 nm. The in vitro dissolution tests carried out on CBZ (raw material) were made in the same conditions above described. All the in vitro tests were carried out in triplicate (R.S.D. within 2%).

2.4. In vivo experiments in sheep

The in vivo experiments in sheep were performed at the University of Sassari, Facoltà di Veterinaria, Italy. All animal studies were performed according to "Principles of laboratory animal care" (NAH publication no. 85-23, revised 1985) and approved by the Ministero della Sanità, Generale Servizi Veterinari Divisione VIII, Roma, Italy.

2.4.1. Preparation of microspheres and CBZ pure drug for administration to sheep

Microspheres (CG-CBZ 1) containing chitosan glutamate and CBZ in a 2:1 ratio were chosen for this experiment on the basis of the results of in vitro studies. Before use, microspheres were sieved (0.125 mesh) to avoid aggregation, and filled into the monopowder insufflators kindly furnished by Valois (Valois Dispray, Mezzovico, Switzerland). About 70 mg of microspheres were used.

The CBZ powder (drug raw material alone) was directly filled in dispenser. About 20 mg of CBZ was administered.

The exact amount of powder given was calculated from the difference between the weight of the insufflator before and after administration. Possible mucus from the sheep's nose was gently removed from the tip with a paper tissue before weighing and calculating the exact amount administered.

2.4.2. Administration of CG-CBZ 1 and CBZ to sheep

Ten Sardinian female sheep (5–6 years) of 35–40 kg were divided into two groups. Group 1 ($n = 4$) received CBZ (about 20 mg) and group 2 ($n = 6$) received CG-CBZ 1 (about 70 mg). The spray-dried microspheres were administrated by gently placing the tip of the pre-filled monopowder insufflator (Valois Dispray) into the left nostril of the sheep and spraying. Blood samples (2 ml) from the jugular vein were withdrawn before drug administration and at different times after administration (10, 20, 30, 60, 180 and 240 min). Blood samples were immediately stored in sterile tubes (8.5 ml) containing a coagulation-promoting substance to separate the serum from the whole blood. The animals were conscious during the whole experiment and

Table 2
Drug contents, encapsulation efficiencies and d_{vs} of spray-dried microspheres

Microspheres	Theoretical drug content (%)	Actual drug content (%) ± S.D.	Encapsulation efficiency (%)	d_{vs} (μm) ± S.D.
CH–CBZ 1	33	29.3 ± 1.1	88.6	2.0 ± 0.2
CH–CBZ 2	25	23.1 ± 1.7	92.6	1.8 ± 0.1
CG–CBZ 1	33	31.9 ± 0.7	95.1	1.7 ± 0.1
CG–CBZ 2	25	23.7 ± 0.1	94.9	1.9 ± 0.0
CH	0	–	–	2.4 ± 0.1
CG	0	–	–	3.1 ± 0.1

between each blood sampling they were allowed to move freely within an enclosed area.

2.4.3. Determination of CBZ in blood samples from sheep

The blood samples from sheep were stored in tubes and frozen before analysis.

An extraction procedure was performed before analysis: a tube containing 400 μl of acetonitrile was added to 200 μl of serum (Kappelhoff et al., 2003). Then samples were vortexed for 2 s and mixed 15 min on a shaking device. The tubes were centrifuged for 10 min at 3000 rpm, and 200 μl of the clear supernatant was transferred to another tube. The supernatant was then mixed with 200 μl of HPLC mobile phase, filtered and submitted to HPLC analysis.

Determination of CBZ was performed by a liquid chromatography system equipped with DAD detector 1100 at 285 nm (Agilent Technologies, Milano, Italy). A Luna® phenyl hexyl 5 μm (Phenomenex, Torrance, USA) 250 mm × 4.6 mm column with cartridge precolumn was used for separation at a flow rate of 0.60 ml/min (Van Rooyen et al., 2002). The mobile phase consisted of acetonitrile, methanol and formic acid (0.1%) 10:70:20 (v/v) and the injection volume was 100 μl (Bugamelli et al., 2002).

The amount of CBZ in serum was expressed as ng/ml serum.

2.4.4. Pharmacokinetics analysis

The AUC values (areas under the concentration versus time curves) for CBZ in serum were calculated from the beginning (t_0) to the end of the observation time (t last), from appropriate graphs using the linear trapezoidal rule (KaleidaGraph 3.6, Synergy Software).

2.4.5. Statistical data analysis

Results of in vivo experiments are reported as mean ± S.E. of mean (n = 4 or 6). Statistical tests of significance were performed using KaleidaGraph 3.6 (Synergy Software); differences were considered to be statistically significant when $P < 0.05$ using a two-tailed unpaired t-test.

3. Results and Discussion

3.1. Preparation and characterization of microspheres

The spray-drying method here described appeared to be a suitable and simple technique to prepare chitosan microspheres loaded with CBZ. Other techniques such as emulsification/solvent evaporation involve different steps and the use of surfactants to stabilize the emulsion. Spray-drying was rapid and simply involved the preparation of a feed-solution containing drug and polymer. Yields of production ranged between 30 and 50%, the microspheres prepared using chitosan glutamate showed the highest values. These relatively low values can be explained both by the small volumes of feed-solution sprayed for the preparation of each batch of microspheres (200 ml) and by the structure of the spray-dryer apparatus, which is not equipped with a trap to recover the smaller and lighter particles which are exhausted by the aspirator, as already pointed out (Giunchedi et al., 2000).

The encapsulation efficiencies obtained from the drug content analysis for the batches based on chitosan salts were ranging from about 89 to 95% (Table 2).

Size analyses (Table 2) showed that drug-loaded microspheres were characterised by a d_{vs} always around 2 μm, independently on polymer salt and drug to polymer ratios used, while blank microspheres had a d_{vs} of about 2.5–3 μm. These results indicate that the loading of CBZ in the microparticles substantially did not influence their sizes. All microspheres showed SPAN factors ranging between 2 and 3.7, indicating a narrow size distribution. The microspheres based on chitosan hydrochloride were characterised by a particle size distribution dependent upon drug loading as SPAN factors showed the following rank order: CH < CH-CBZ 2 < CH-CBZ 1 (SPAN = 2.36, 2.96 and 3.69, respectively). No remarkable differences were found in particle size distributions of microspheres based on chitosan glutamate. Size analysis was also carried out on CBZ (raw material) and it showed a size of about 12 μm.

SEM photomicrographs of CBZ and CG-CBZ 1 microspheres (chosen as an example) are reported in Fig. 1(a and b). The drug was characterised by particles of irregular shape and heterogeneous particle size (Fig. 1a); drug-loaded microspheres (based on chitosan glutamate) showed regular shape and smooth surface and no free drug was present (Fig. 1b); CG-CBZ 2 showed a similar morphology. Drug-loaded microspheres made with chitosan hydrochloride showed a regular morphology, but also few needle-like structures were present (pictures not reported) possibly due to CBZ not completely loaded into the polymeric network. The presence of free drug could explain the increase of SPAN factors found increasing the drug to polymer ratios. All blank microparticles were characterised by a morphology analogous to that of microspheres based on chitosan glutamate.

Fig. 2 reports the thermal profiles of CBZ as pure drug (curve a) and of drug-loaded microspheres CG-CBZ 1, cho-

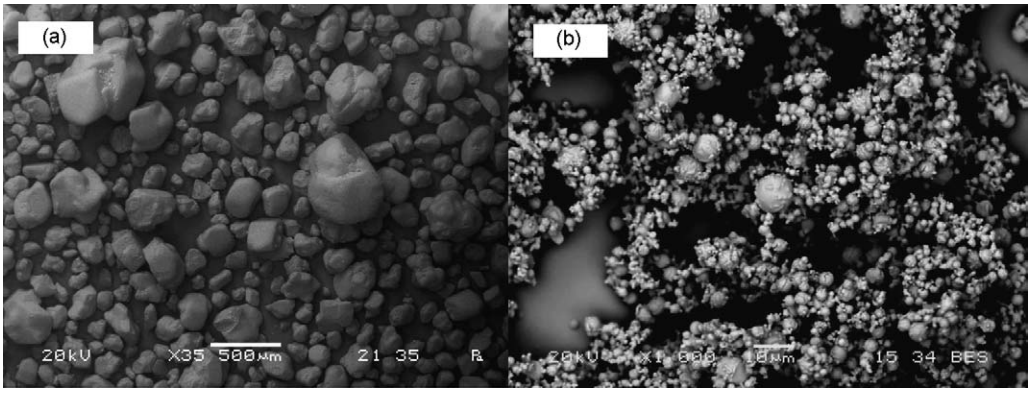

Fig. 1. SEM photographs of CBZ raw material (a) and of microspheres CG-CBZ 1 (b). Magnification: 35× (a) and 1000× (b).

sen as an example (curve b). DSC analysis of CBZ showed two endotherms of fusion and one exothermic peak. The first endothermic peak corresponded to the melting of β-polymorph (about 178 °C), followed by exothermic recrystallization as the α-polymorph (180 °C) which subsequently melted at 197 °C. This DSC profile confirmed previously studies on β-polymorph of CBZ (Ochoa Machiste et al., 1995; Katzhendler et al., 1998; Rustichelli et al., 2000). The DSC analysis of the drug-loaded microspheres made with glutamate showed only a little endothermic peak at 178 °C, indicating that the loading of CBZ into the polymeric network partially maintained the drug as β-polymorph, but mainly led to its amorphization. No exothermic or endothermic peak corresponding to the α-polymorph was present. Analogous results were obtained from the other spray-dried particles (data not reported).

Fig. 3 shows the in vitro release profiles obtained from the drug-loaded microparticles, compared to the dissolution profile of the drug alone. The rate of dissolution of CBZ powder was significantly slow (approximately less than 60% of the drug dissolved in 3 h). The loading of CBZ into chitosan microspheres led to an improvement of its dissolution/release rate. The increase in the rate of dissolution was always remarkable, independently on the kind of chitosan used (hydrochloride or glutamate) and on the drug to polymer ratio. In fact, about 90–100% of released drug was achieved in less than 2 h from the spray-dried microparticles.

Chitosan is a polymeric material which is already known for its properties of dissolution rate enhancer of drugs poorly soluble in water. For example, prednisolone when ground with chitosan achieved an improvement of its dissolution behaviour (Sawanagi et al., 1983); dexamethasone improved its dissolution rate upon loading into spray-dried chitosan microparticles (Genta et al., 1995). The improvement of the dissolution rate of the drug from the microparticles can be also due to their small size and to the spraying process that leads to the uniform dispersion of the drug into the polymeric network and thus to its (partial) amorphization, as shown by DSC analyses.

The improvement of the dissolution behaviour due to the loading into the polymeric chitosan network of the microspheres can be a useful characteristic for the nasal delivery of a drug of low water solubility such as CBZ.

3.2. Nasal administration of CG-CBZ 1 microspheres and of CBZ as pure drug to sheep

For in vivo testing the microspheres based on chitosan glutamate were chosen instead of the microparticles based on chitosan hydrochloride because these latter showed free drug crystals. CG-CBZ 1 batch was finally chosen because characterised by a

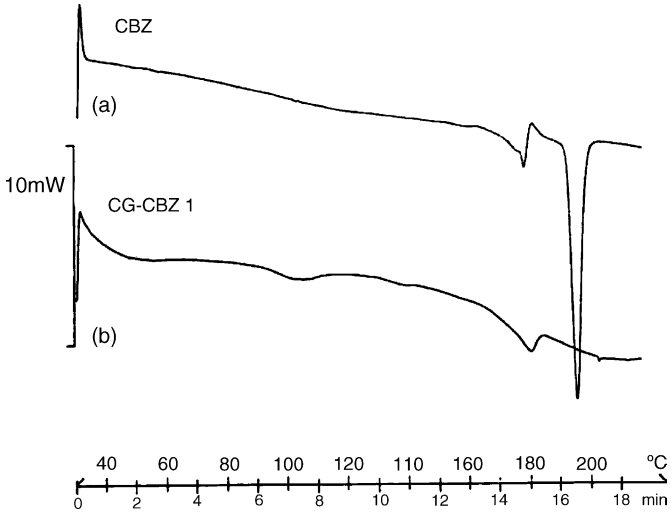

Fig. 2. DSC profiles of (a) CBZ (as pure drug) and (b) CG-CBZ 1 microspheres, chosen as an example.

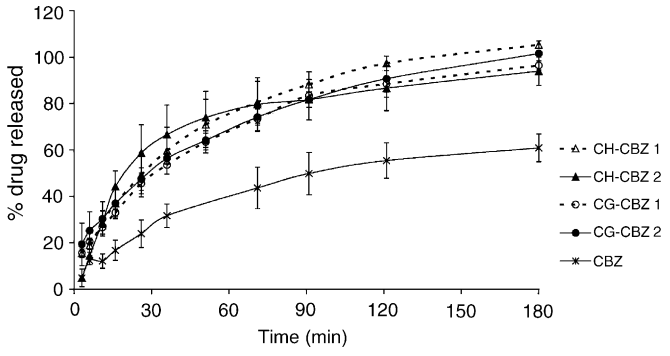

Fig. 3. In vitro release profiles of CBZ from spray-dried chitosan microspheres in comparison with the dissolution rate of CBZ as pure drug.

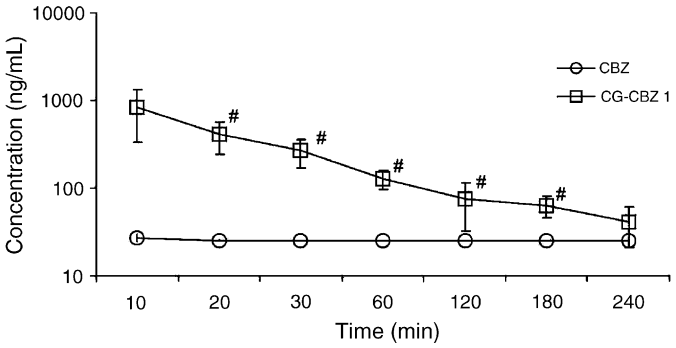

Fig. 4. The concentration of CBZ (ng/ml) in the sheep serum after nasal administration of CG-CBZ 1 ($n = 6 \pm$ S.E.M.) or CBZ pure drug ($n = 4 \pm$ S.E.M.). Unpaired *t*-test: (#) significantly different from CBZ pure drug, $P < 0.05$.

similar release profile with respect to CB-CBZ 2, but by a lower polymer content.

The results obtained from the in vivo tests in sheep are shown in Fig. 4 and expressed as CBZ levels (ng/ml) in the serum taken from sheep after nasal administration of CG-CBZ 1 microspheres and pure drug used as reference.

Results showed that the in vivo administration of drug-loaded glutamate microspheres (CG-CBZ 1) remarkably increased the CBZ concentration found in the serum when compared to the concentrations found after administration of CBZ powder (pure drug).

The two-tailed unpaired *t*-test showed statistically significant differences ($P < 0.05$) between the microparticulate delivery system and the CBZ pure drug at each time point except at 240 min.

The AUC values of CG-CBZ 1 and CBZ pure drug are remarkably different being 33.08 and 5.90 min μg/ml, respectively: when CBZ is formulated in microspheres based on chitosan glutamate and it is administered by nasal route, it has a higher bioavailability (AUC relative = 5.6) compared to the administration of the drug alone (as powder).

The drug profile of CBZ as powder had two aspects: it was low and "flat". This behaviour could be due to the low water solubility of the drug that determines both a low dissolution rate and probably the formation of a saturated drug solution on the surface of mucosa, responsible of a constant (even if low) drug absorption.

A considerably high CBZ concentration in the serum was reached 10 min after administration of the microspheres CG-CBZ 1 (C_{max} about 800 ng/ml: T_{max} 10 min) with respect to the pure drug as reference ($C_{10\,min}$ about 25 ng/ml for CBZ powder). CBZ concentration always remained significantly higher ($P < 0.05$) with respect to the reference during 180 min. T_{max} at 10 min after the administration of microspheres clearly indicated a very rapid absorption from the nose which did not occur in the case of the direct administration of the pure drug as powder or when CBZ is administered by oral route: in fact, the peak concentration after oral administration ranges from 4 to 8 h or longer due to the very low water solubility of this drug and its dissolution rate-limited absorption (Levy et al., 1992; Koester et al., 2004).

Both encapsulation of CBZ in microspheres and the nasal route determine the enhancement of the drug bioavailability: the high CBZ absorption through nasal mucosa occurs because the drug is administered loaded into microspheres based on chitosan glutamate, which adhere on the mucosal surface and at the same time improve the dissolution of the drug in the aqueous environment of the mucosa.

It has been demonstrated that the bioadhesive microparticles have a significant effect on the mucosal uptake of drugs (Pereswetoff-Morath, 1998; Ugwoke et al., 2001; Alpar et al., 2005).

Illum et al. (2001) describe the mechanism of action of the bioadhesive starch microsphere for nasal administration which are retained in the nasal cavity for an extended time period because of their bioadhesive nature and from which, after gelling, a local high drug concentration in close contact with the absorption site is reached. This could affect the passage of the drug through the paracellular tight junctions.

Many authors report that enhancing agents greatly enhanced bioavailability of the drug after nasal administration. These enhancer systems work by a variety of mechanisms but generally change the permeability of the epithelial cell layer by modifying the phospholipid bilayer, leaching out protein from the membrane or even stripping off the outer layer of the mucosa. Some of these enhancers also have an effect on the tight junctions and/or work as enzymatic inhibitors (Illum, 2003).

It is known that chitosan is characterised by absorption enhancing effects, as it improves the paracellular transport by opening the tight junctions, as reported in previous studies (Bjork et al., 1995; Illum, 2003; Gavini et al., 2005; Alpar et al., 2005; Davis, in press).

This could be important also for lipophilic drugs such as CBZ, for which the transcellular transport might dominate; in fact, in this case the drug is characterised by a high dissolution rate, even if poorly soluble, due to the presence of the chitosan. Therefore, both the higher local drug concentration and the increased paracellular transport are likely to play an important role in absorption.

Carbamazepine loaded microspheres based on chitosan hydrochloride and glutamate can be easily obtained by the spray-drying technique. The loading of the drug in the microparticulate polymeric network led to a significant increase in its in vitro dissolution rate.

The results of in vivo studies (carried out in sheep) showed that the microparticulate system based on chitosan glutamate was able to promote a rapid drug absorption through the nasal mucosa and to remarkably improve the bioavailability of the drug.

These preliminary results support the development of microparticulate nasal delivery systems based on chitosan glutamate and loaded with drugs of poor water solubility such as carbamazepine.

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