International Journal of Pharmaceutics 400 (2010) 32–36

Contents lists available at ScienceDirect

International Journal of Pharmaceutics

journal homepage: www.elsevier.com/locate/ijpharm

Poloxamer 407 microspheres for orotransmucosal drug delivery. Part II: In vitro/in vivo evaluation

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ARTICLE INFO

Article history:
Received 10 May 2010
Received in revised form 12 August 2010
Accepted 13 August 2010
Available online 20 August 2010

Keywords:
Atenolol
Microspheres
Transbuccal permeation
Pig mucosa
Rabbit

ABSTRACT

Aim of this research was to evaluate novel microspheres based on poloxamer 407, alone or in mixture with Gelucire® 50/13, as possible buccal delivery system for atenolol (AT). The microspheres have been prepared by spray congealing and investigated to assess AT in vitro delivery through cellulose membranes and ex vivo permeation using porcine buccal mucosa. The microparticles were tested as such or directly compacted to obtain tablets. For comparison the physical mixtures, tablets of the physical mixtures and an AT solution were examined. Finally, the microparticles were sublingually administered in rabbits to evaluate AT pharmacokinetics compared to a market oral tablet (reference). The AT release from microspheres through the synthetic membrane was delayed with respect to the drug solution, more markedly when microparticles contained poloxamer as unique adjuvant; this formulation enhanced AT transmucosal permeation. The enhancement effect of poloxamer was confirmed by the permeation experiments on the corresponding physical mixture. Tabletting hindered both release through cellulose membranes and transmucosal permeation of drug. In vivo studies revealed that the absolute bioavailability of microsphere formulations was higher than that of reference in spite of a lower dosage of drug, suggesting a possible dose reduction by AT microparticles orotransmucosal administration.

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1. Introduction

Over the last decades, systemic drug delivery through oral mucosa has received a great deal of attention. Actually buccal mucosa is an attractive route due to its easy accessibility, rapid efficacy, smooth and relatively immobile surface, high patient compliance, avoidance of gastro-intestinal or hepatic first-pass metabolism and suitability for the placement of controlled-release systems (De Vries et al., 1991; Junginger et al., 1999; Madhav et al., 2009). However, once the drugs are applied on buccal mucosa, the therapeutic efficacy mainly depends on the ability of molecules to permeate through the tissue, providing the required plasma concentrations. The limitations in buccal drug delivery are related to the low permeability of the epithelium, relatively small surface area available for absorption and short residence time of formulations due to involuntary swallowing and constant salivary scavenging within the oral cavity (Senel and Hincal, 2001; Madhav et al., 2009). The use of penetration enhancers is a logical approach to increase the drug permeation across the epithelium. The effect of many classes of penetration enhancers such as surfactants and bile salts, fatty acids, ethanol (Nicolazzo et al., 2005; Burgalassi et al., 2006), cyclodextrin derivatives (Figueiras et al., 2009) or chitosan derivatives (Sandri et al., 2004a) has been studied. To provide a better retention of the dosage form in the site of application, bioadhesive polymers have been used extensively in buccal drug delivery systems (Burgalassi et al., 1996; Shojaei et al., 2000; Kockisch et al., 2003; Sandri et al., 2004b), hence the mucoadhesion/enhancer combination can lengthen the residence time and improve the drug bioavailability.

Poloxamers, a class of non-ionic surfactants, polyoxyethylene–polyoxypropylene block-type copolymers, exhibited mucoadhesive properties and the ability to promote the permeation of drugs through the mucosae of different parts of the body (Shin and Kim, 2000; Morishita et al., 2001; Bromberg and Alakhov, 2003; Chun et al., 2003; Brüsewitz et al., 2007; Lin et al., 2007).

The main goal of this research was to evaluate novel microparticles based on poloxamer 407, alone or in mixture with Gelucire® 50/13 as possible orotransmucosal delivery systems. To this aim, atenolol (AT), a cardio selective β-blocker, was selected as drug due to its low permeability and high solubility (Class III drug according to the Biopharmaceutics Classification System) (Vogelpoel et al., 2004).

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doi:10.1016/j.ijpharm.2010.08.018

Table 1
Formulation under study.

Formulation	AT	Vehicle
AT-sol	1.7% (w/v)	Buffer solution
M5	15.51% (w/w)	PF127
M6	14.73% (w/w)	PF127/GLC (3:1)
PM-M5	15.51% (w/w)	PF127
PM-M6	14.73% (w/w)	PF127/GLC (3:1)
TAB-M5	50 mg	PF127
TAB-M6	50 mg	PF127/GLC (3:1)
TAB-PM-M5	50 mg	PF127
TAB-PM-M6	50 mg	PF127/GLC (3:1)

The microspheres used in this research have been previously prepared and characterized as regards morphology, particle size, drug loading and solubility, bioadhesion to buccal tissue, physicochemical properties and stability; the results are reported in the Part I of the study (Albertini et al., 2010). In the present paper, the best formulations were further investigated to assess AT in vitro delivery through cellulose membranes and ex vivo permeation using porcine buccal mucosa. Finally pharmacokinetic experiments were performed on rabbits.

2. Materials and methods

2.1. Materials

Atenolol (AT) was purchased from Sigma–Aldrich (lot n. 075K1888) (Italy); poloxamer 407 (PF127) was a gift of BASF (Germany) while stearoyl polyoxylglycerides (GLC, Gelucire® 50/13) was kindly supplied by Gattefossè (France). All other reagents were analytical grade.

2.2. Formulations

The formulations investigated in this part of the study are summarized in Table 1. M5 and M6 microspheres with drug loading of 15.51 and 14.73% (w/w), respectively, were produced by the spray congealing process using a wide pneumatic nozzle (Albertini et al., 2008, 2009; Passerini et al., 2010) and contained as adjuvant PF127 or a PF127:GLC mixture. The preparation of the microspheres has been described in detail in the Part I of this research (Albertini et al., 2010). The microspheres were directly compacted to obtain round flat tablets of 11 mm in diameter and 2.48 mm in thickness containing 50 mg of AT (TAB-M5 and TAB-M6). For comparison the physical mixtures (PM-5 and PM-6), tablets made up of the physical mixtures (TAB-PM-M5 and TAB-PM-M6) and AT in isotonic, pH 6.8 phosphate buffer solution (AT-sol) were prepared.

2.3. Analytical method

AT concentration in the samples was assayed by HPLC (liquid chromatograph with LC-6A pump and 20-μl Rheodyne injector, SPD-10A detector and computer integrating system, Shimadzu Corp.). The column (Gemini 250 mm × 4.6 mm, Phenomenex, CA, U.S.A.) was packed with C18 phase (size 5 μm). The mobile phase was acetonitrile/water (10:90, v/v) containing 20 mM NaH_2PO_4 adjusted to pH 3.8 with H_3PO_4; the flow rate was 1.0 ml/min. The determination was performed at 274 nm. The sensitivity of the assay was greater than 80 ng/ml. The amount of drug in the samples was determined by comparison with appropriate standard curves. To determine AT concentration in plasma, the standard curve was obtained by adding increasing amounts of drug to pools of blank plasma.

2.4. AT delivery/permeation experiments

2.4.1. In vitro through cellulose acetate membranes

Release in vitro of AT from the formulations through cellulose acetate membranes (SpectraPore3, MWCO3500, Spectrum®, NL) was investigated using vertical Gummer cells (Gummer et al., 1987) with an effective diffusion area of 1.23 cm^2. 5 ml of isotonic, 66.7 mM, pH 7.4 Sorensen phosphate buffer solution (PBS), kept at 37 °C and stirred at 600 rpm, were used as the receptor medium. The formulations (donor phase: M5, TAB-M5; M6, TAB-M6) were put in contact with the membrane and moistened with 700 μl of isotonic pH 6.8 PBS. AT-sol was used as reference. The donor section was hermetically sealed to avoid evaporation. At predetermined time intervals, samples of the receiving phase were withdrawn for analysis and replaced with an equal volume of fresh buffer to maintain the sink conditions. The amount of AT released was determined by HPLC. Each release test was replicated at least three times.

2.4.2. Ex vivo across porcine buccal mucosa

The porcine buccal mucosa were taken from Clinical Physiology Institute of CNR (National Research Council) and excised immediately after animals' death. The mucosa was transported to the laboratory in PBS and used within 2 h of animal sacrifice. The majority of the underlying connective tissue was removed with the help of a scalpel blade and then the remaining buccal mucosa was carefully trimmed with a pair of surgical scissors to uniform thicknesses of 500–600 μm. The thickness of the tissues was measured using a micrometer.

The permeation tests were carried out using the same apparatus and conditions described in Section 2.4.1. The formulations tested (M5, PM-M5, TAB-M5, TAB-PM-M5, M6, PM-M6, TAB-M6, TAB-PM-M6, AT-sol), placed in contact with the mucosal epithelial side, were moistened with an appropriate amount of isotonic, pH 6.8 PBS. The donor section was then hermetically sealed to avoid evaporation phenomena and the consequent desiccation of the mucosa. The receiving phase (5.0 ml), consisting of isotonic pH 7.4 PBS containing 0.003% (w/v) sodium azide to prevent bacterial growth, was stirred at 600 rpm. At predetermined time intervals, samples of the receiving phase were withdrawn for analysis, and replaced with an equal volume of fresh solution. The samples were analyzed by HPLC. Each permeation test was performed at least in quadruplicate.

To verify tissue integrity, at the end of permeation experiments 1 ml of methylene blue solution was added to the donor phase and the incidental colour change of receiving phase after 5 min was detected.

Linear regression analysis of pseudo steady-state diffusion data allowed calculation of J, the steady-state flux (given by $Q/A{\cdot}t$, where Q is the amount of permeant diffusing across the area A in time t). AT apparent permeability coefficients (Papp) were obtained from the relationship: $Papp = J/C_d$ where C_d is the initial drug concentration in the donor phase. The permeation lag times (indicating the time taken by the drug to saturate the skin and to reach the receiving compartment) were calculated from the x-axis intercept values of the regression lines. Enhancement factors (EF) were calculated from the Papp ratio of formulations under study and AT-sol.

2.4.3. In vivo in rabbits

Female, New Zealand albino rabbits, weighing 4.0–4.5 kg (Pampaloni Rabbitry, Italy) were used and treated as prescribed in the publication "Guide for the care and use of laboratory animals" (NIH Publication No. 92-93, revised 1985). All experiments conformed to the ARVO Resolution on the Use of Animals in Research; they were carried out under veterinary supervision and the protocols were approved by the ethical-scientific committee of the University of Pisa.

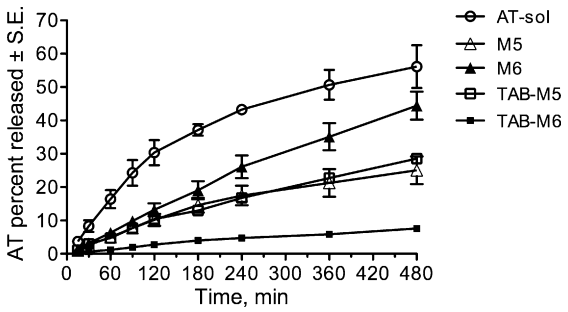

Fig. 1. Profiles of AT diffusion through cellulose membranes from the formulations under study.

The animals were housed singly in standard cages in a room with controlled lighting, at 19 ± 1 °C and 50 ± 5% R.H., with no restriction of food or water.

A market tablet (50 mg AT, reference formulation) per os and 250 mg of M5 or M6 placed in the sublingual area, in intimate contact with the ventral surface of tongue, were administered to rabbits anaesthetised by i.m. administration of 8.73 mg kg^{-1} tiletamine hydrochloride and 8.63 mg kg^{-1} zolazepam hydrochloride (Zoletil 100®, Laboratories Virdac, France). To evaluate absolute bioavailability, 2.5 mg of drug were administered by i.v. injection. Blood samples (400–500 μl) were collected from the ear marginal vein 10, 20 (only for i.v. administration), 30, 60, 90 min and 2, 3, 4, 6, 8, 12, 16, 20 and 24 h after administration of the formulations. After centrifugation (10 min at 10,000 rpm, Microcentrifugette 4214, ALC International Srl, Italy), the plasma samples were separated and stored at −20 °C before analysis. Finally the plasma samples were deproteinized by mixing with an equal volume of methanol containing 6.0% (v/v) perchloric acid; after centrifugation (10 min at 12,000 rpm), 20 μl of the supernatant were submitted to HPLC analysis. Each test was performed at least four times.

3. Results and discussion

3.1. Assessment of AT delivery/permeation

Fig. 1 illustrates the profiles of AT diffusion through cellulose membranes (percent drug released vs time) from the formulations under study. It is noteworthy that AT-sol showed the highest release percentage (56.13 ± 6.55% after 480 min); the encapsulation of drug into microspheres produced a decrease of AT release more significant for M5, containing poloxamer 407 as unique adjuvant, than for M6, containing also Gelucire® 50/13 (25.05 ± 3.86 and 44.44 ± 4.20%, respectively). This effect might be attributed to micellar complexation of the drug, combined with the absence of promoting effect due to the non-biological nature of the membrane.

PF127, as surfactant, causes AT micellization with subsequent increase of drug solubility (Albertini et al., 2010) but also the reduction of the diffusion since the membranes (non-biological and biological) are hardly permeable to the drug-micelle species (Ganem-Quintanar et al., 1997). The presence of GLC would seem to reduce PF127 micellization ability, increasing free AT amount able to cross the cellulose membrane. Such an antagonistic effect between these two products on inhibition of poorly water-soluble drug precipitation had already been observed by Dai et al. (2007).

Under the experimental conditions used, tabletting did not affect the AT release from TAB-M5 formulation (28.56 ± 1.16%) while it produced a remarkable reduction of AT percentage released when the co-carrier was introduced (TAB-M6, 7.64 ± 0.59%), showing a constant and slowed down release of drug, already seen in the dissolution studies (Albertini et al., 2010).

Table 2
Permeation parameters of AT through porcine buccal mucosa from formulations under study.

Formulation	Papp × 10^3 (cm/h)	Lag time (h)	EF
AT-sol	1.18 ± 0.29	6.65 ± 0.34	–
PM-M5	2.20 ± 0.34	3.46 ± 0.03	1.88
M5	4.22 ± 0.65	3.47 ± 0.49	3.57
TAB-PM-M5	0.11 ± 0.04	9.11 ± 0.58	0.10
TAB-M5	0.85 ± 0.39	9.27 ± 0.34	0.72
PM-M6	0.91 ± 0.18	4.36 ± 0.21	0.77
M6	1.64 ± 0.48	4.61 ± 0.57	1.39
TAB-PM-M6	0.36 ± 0.09	9.98 ± 0.59	0.31
TAB-M6	0.13 ± 0.046	6.71 ± 0.54	0.11

AT permeation parameters through porcine buccal mucosa from each tested formulation (apparent permeability coefficient, lag time, enhancement factor over AT-sol) are listed in Table 2.

The apparent permeability coefficient of AT at steady state from AT-sol was 1.18 × 10^{-3} cm/h. In spite of that observed in release studies, microspheres based on poloxamer 407 rose 3.6-fold (EF = 3.57) the transmucosal permeation of the drug (Papp = 4.22 ± 0.65 × 10^{-3} cm/h), suggesting that this vehicle appeared useful to carry AT through mucosal structure. Moreover, the replacement of a part of PF127 with GLC in the preparation of microparticles proved a Papp value of 1.64 ± 0.48 × 10^{-3} cm/h very close to AT-sol (EF = 1.38) demonstrating that this co-carrier did not give a significant contribution as promoter.

These results stand in apparent contrast with those observed with the cellulose membranes. An explanation for this discrepancy can derive from the consideration that drug permeation through biological membranes is known to be promoted by surfactants. They, in fact, can interact with mucosae by protein denaturation or extraction of lipid components, thus altering their barrier properties and allowing absorption of many drugs by passive diffusion through intercellular pathway, the most generally accepted route for drug buccal absorption (Squier, 1973; Squier and Lesch, 1988).

The enhancement effect of PF127 surfactant was confirmed in the permeation experiments when the corresponding physical mixture (PM-M5) was tested, which an appreciable increase of the mucosal permeation of AT (EF = 1.88) was revealed in. Instead adding GLC (PM-M6) appeared to reduce the solubilization effect of PF127 making the drug less available for permeation of the tissue (Papp: 0.91 × 10^{-3} cm/h). This phenomenon has already been observed from Fukushima et al. (2007) on oral absorption of atazanavir (ATV): the addition of GLC to ATV solid dispersion consisted of sodium lauryl sulfate, hydrophilic surfactant (HLB = 40.0), did not improve the bioavailability of the vehicle.

It should be remembered that enhancement effect of surfactants does not occur towards non-biological membranes, so that, in the presence of these barriers, only their complexing, diffusion-reducing effect is evident.

PF127 demonstrated a positive effect on lag time that dropped from 6.6 h (AT-sol) to about 3.5 h for both M5 and physical mixture, highlighting further a modification of tissue structure while the introduction of GLC (as already observed above) antagonized this effect: lag time rose up to 4.6 h.

As already noted in the diffusion tests, tabletting hindered the transmucosal permeation of drug probably owing to the need of additional time for the hydration/gelation phase and then to the achievement of the steady state: Papp ranged from 0.1 to 0.8 × 10^{-3} cm/h.

3.2. In vivo AT permeation studies

The results of the delivery/permeation studies demonstrated the good performance of the microspheres as such for orotransmu-

Table 3
Pharmacokinetic parameters of AT after administration to rabbits.

Formulation	$AUC_{0-24\,h}$ (μg/ml h)	C_{max} (μg/ml)	T_{max} (h)	$C_{24\,h}$ (μg/ml)	A.B. (%)
Market tablet	21.91 ± 6.89	2.77 ± 0.67	3.00	0.20 ± 0.10	17.42
M5	28.65 ± 3.41	2.69 ± 0.45	6.00	0.56 ± 0.15	29.38
M6	30.51 ± 3.09	2.63 ± 0.05	6.00	0.75 ± 0.10*	33.07

* Significantly different from market tablet ($p < 0.05$, unpaired two tailed t-test).

cosal administration of AT; so M5 and M6 were further examined after administration in rabbits.

The pharmacokinetic parameters following single dose oral (market tablet) and sublingual (M5, M6) AT administration are summarized in Table 3, where are reported: AUC_{0-24} (areas under the plasma concentration–time curves up to 24 h), C_{max}, T_{max} and $C_{24\,h}$ (AT concentration in plasma after 24 h). The absolute bioavailability (A.B., %) of AT from the formulations under study was calculated using the classical formula:

$$\text{A.B.} = \frac{AUC_x \times dose_{iv}}{AUC_{iv} \times dose_x} \times 100$$

where AUC_x and AUC_{iv} are the AUCs calculated after buccal or oral and intravenous administration, respectively; $dose_x$ and $dose_{iv}$ are the corresponding AT doses.

Pharmacokinetic data of the market tablet showed a peak of AT concentration (2.77 ± 0.67 μg/ml) 3 h after administration and a residual AT concentration at 24 h of 0.20 ± 0.10 μg/ml with AUC value of 21.91 ± 6.89 μg/ml h. The scientific literature reports opposing data about AT pharmacokinetic on rabbits. Gupta and Jain (2006) obtained C_{max} and AUC values much lower than those of the present research in spite of the same T_{max} value (3.00 h). In a comparative study among different formulations containing AT (oral, intravenous and transdermal matrices), Shin and Choi (2003) found AUC and C_{max} values similar to our data after oral administration although the peak time was 1.6 h and absolute bioavailability very higher (77.4% vs 17.42%).

Administration of microspheres (M5, M6) determined a shift of T_{max} from 3.00 to 6.00 h compared to reference formulation with the same values of C_{max} (2.69 and 2.63 μg/ml vs 2.77 μg/ml). This behavior could be due to numerous phenomena which the formulations underwent in the site of application: slow hydration in the modest volume of salivary fluid, gelation of PF127, AT diffusion through the gel, and finally AT diffusion through the oral membrane to reach systemic circulation.

After administration of M5 and M6 formulations, AT amount remained higher than the reference tablet during the entire elimination phase with significant statistically difference for M6, showing a sustained release profile; the concentrations at 24 h were 0.56 ± 0.15 and 0.75 ± 0.1 μg/ml vs 0.2 ± 0.1 μg/ml for M5, M6 and market tablet, respectively. These data seem to confirm the well known depot function of buccal mucosa (Le Brun et al., 1989).

Moreover, the best performance of microparticle systems was also reflected by area under curve measurements which were higher than those of orally administered conventional tablets; in particular M6 formulation showed the highest AUC values (30.51 ± 3.09 μg/ml h).

The high amount of AT permeated from microspheres through the buccal tissue is due to the enhancing activity of PF127, already discussed above. The slight difference between M5 and M6 formulations can be ascribed to the different times of hydration process: M5 formulation formed a uniform gel with a firm texture already after 10 min, whereas M6, containing a less hydrophilic additive (GLC), evidenced a lower gel formation at the same time (Albertini et al., 2010). When the formulations are in gel state, they undergo an easier wash-out by saliva, consequently M5 is more easily removed from the site of absorption. Moreover, the addition of a sparsely hydrophilic excipient to PF127 (M6) appeared cause an increase of drug permeated, even if the pharmacokinetics parameters of M5 and M6 were not significantly different.

It had already been shown that the addition of lipophilic adjuvants to PF127 gels increased the bioavailability of insulin after buccal administration in rats (Morishita et al., 2001).

Finally it is important to note that the amount of drug administered was 50 mg for oral tablets and about 40 mg for microspheres; in spite of lower dosage of drug the microparticles produced absolute bioavailability up to 2-fold compared to reference. Hence a dose reduction could be possible by administering AT microspheres via buccal route.

4. Conclusions

Although the study demonstrates that the incorporation into poloxamer/Gelucire microspheres may be an interesting potential platform for transbuccal administration of AT, it does not allow to strictly correlate in vitro data with in vivo performance. However, the use of the microspheres produced a higher bioavailability compared to oral market product in spite of a lower drug dose. So the microparticulate system could overcome consistently incomplete oral absorption and decrease the side-effects due to the high dosage of drug following oral administration.

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