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Clarithromycin targeting to lung: characterization, size distribution and in vivo evaluation of the human serum albumin microspheres☆

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Abstract

Microspheres of clarithromycin have been prepared from human serum albumin using the emulsion polymerization technique. Albumin microspheres containing the active substance were injected into the tail vein of mice. Mice were sacrificed at intervals and microspheres collected from lungs and livers. The clarithromycin amount in microspheres was determined by reversed phase high performance liquid chromatographic (HPLC) method from the mice organs. Morphological and histopathological observations were also reported. The microsphere accumulation began at 10 min, and increased gradually until 6 h, then a decrease was observed. The microspheres were still present after 24 h. In the liver sample, no microsphere accumulation was observed at any time.

Keywords: Clarithromycin; Drug targeting; Human serum albumin microsphere; Morphology

1. Introduction

Clarithromycin acts like erythromycin and has a similar spectrum of antibacterial activity, i.e. mainly against Gram-positive organisms. It is rapidly and completely absorbed from the gastrointestinal tract, 60% of the dose is inactivated by metabolism, which is saturable and the remainder is eliminated in the urine [1]. Clarithromycin is used for respiratory tract infections including atypical pneumonias and soft tissue infections. It causes some undesirable side effects to the gastrointestinal tract, such as diarrhoea, vomiting, nausea, headache and abdominal pain. The incidence of these adverse effects is lower (7%) than for erythromycin [1]. There is a great interest in the development of alternative dosage forms to overcome these side effects problems.

Targeting of an active substance is an important issue with regard to pharmaceutical, biopharmaceutical, clinical and commercial aspects. Recently, a great deal of attention has been focused on microsphere preparations for albumin delivery. Several formulations based on these albumins have been investigated for injectable microspheres [2–4]. Their purpose is to change the body distribution of carried drugs, targeting them to specific organs or cells, thereby protecting normal tissue from toxic side effects.

It has been demonstrated that for the targeting of drugs with microspheres, the size plays a great role in controlling drug delivery to the target organs and the subsequent uptake of drugs in tissue [5,6]. It is also critical to estimate the degradation process of microspheres in vivo for the drug release studies.

The present study is an extension of previous work on the design of targeted microspheres using two differ-

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ent albumins. The preparation, size optimization, drug loading and in vitro release characteristics of albumin microspheres containing clarithromycin were evaluated in our previous work [7]. The physical characteristics of two different serum albumins (human serum albumine and bovine serum albumine) and the effects of their physical characteristics on the particle size of microspheres, percentage of drug loading, release of the drug from microspheres were investigated [7]. Based on the above mentioned results, we prepared the appropriate particle size in the range of 7–15 μm to ensure that these particle could target passively the lungs. It was found that the optimum particle size distribution desired for in vivo conditions was obtained by using a stirring rate at 2500 rpm. Among the matrix materials used in the formulations in the group having 7–15 μm of particle size distribution, the best compliance according to the total particle ratio was obtained in HSA (%94). HSA microsphere formulation releasing rate was much faster than BSA microsphere formulations. Based on the in vitro studies, HSA microsphere was selected for the optimum formulation for the in vivo studies.

The aim of this study is to target these microspheres to the lungs, to control the in vivo distribution of microspheres by examining the optical micrograph of mouse lungs and liver at different times after administration and evaluate this distribution histologically.

2. Experimental

2.1. Materials

Clarithromycin was kindly provided by Fako Products Inc. (Istanbul, Turkey). Human serum albumin (HSA) and Bovine serum albumin (BSA) were obtained from Sigma Chemicals. (St. Louis, MO, USA). Cotton seed oil was supplied by Karam Inc. (Turkey). All other chemicals used were of analytical reagent-grades.

2.2. Preparation of albumin microspheres

Microspheres were prepared as reported by Özkan et al. [7].

2.3. Morphological studies

An optical microscope supplied with a camera was used to observe the microspheres localized in lungs and liver.

2.4. Determination of drug content of microspheres

A reversed-phase high performance liquid chromatographic method was used for the determination of clarithromycin as a pure drug, in microspheres and in tissue. Details of the chromatographic conditions were described previously [7].

2.5. Optical microscope images of the microspheres having optimum characteristics

The image that the microsphere formulation, which was determined as having the suitable characteristics for in vivo studies from the results of the in vitro controls of the prepared microsphere formulations, was controlled. For this purpose, the image of the sample of the microsphere formulation that has optimum properties, prepared by the 'Cell Blockade Technique' was microscopically examined. The cell blockage technique is one of the more accurate techniques of preparing material for microscopic analysis. Its greatest advantage is the ability of examining a lot of parts of the same material routinely using dyes, such as haematoxylene-eosin, or other specific staining methods. The microspheres were taken into 10% formol at first, for the cell block technique. The mixture was centrifuged at 2000 rpm for 10 min and left standing for 2 h. The upper part of the liquid was removed, the precipitate at the bottom was taken by a spatula. The precipitate was divided in two parts at the middle of it and the cut surface was settled on liquid agar. It was coated by liquid agar completely and left to harden for 2 min. The hardened sample was settled in the tissue casette and taken again in the cover containing 10% formol after being fixed, it was settled in tissue pursuit device. The sample was then embedded in clean paraffin, and portions of 4–6 μm were taken. They were stained by haematoxylene–eosin and the images were examined [8].

2.6. Administration of the optimum formulation to the laboratory animals

To examine the appropriate properties of the formulations in vivo, thirty two Swiss Webster mice weighing 30–35 g were divided into 8 groups to test the drug release at the following periods (0, 10, 30 min, 1, 2, 4, 6 and 24 h) after administration. Considering the LD_{50} values (850–1030 mg/kg) of clarithromycin [9] and to facilitate its quantitative determination in the target organ, the microspheres containing 1 mg/ml active substance were suspended in isotonic sodium chloride containing 0.1% Tween 80. The mixture (0.4 ml) was injected into the tail veins. Four mice from each group were killed by cervical dislocation at the previously determined periods. The lungs, which were selected as target organs and the liver, for control were removed. One of the separated organs were taken into 0.1% formol suspension for histopathological examination. The other three mouse organs were weighed and stored at -20°C until the extraction procedure.

These samples were extracted for clarithromycin quantitative analysis by HPLC. The organs were homogenized, by adding saline at the ratio of 0.1 mg/ml. Biphenyl was added as internal standard at the ratio of 300 ng/ml. The proteins in the mixture were precipitated by addition of 2 M perchloric acid (100 μl). The mixture was vortexed for 5 s, centrifuged at 5000 cycle/min. One millilitre was taken from the upper clear phase and 200 μl 0.1 M sodium carbonate solution was added. This mixture was extracted with 3 ml 1:1 ethyl acetate–hexane (v/v). The organic phase was evaporated until dryness. The residue was dissolved in methanol (250 μl) and injected into HPLC device for analysis [10]. After the active substance was determined, the periods which showed the higher accumulation were selected. An ethanol solution of clarithromycin (1 mg/ml) was administered to a group of three mice. Quantitative determination of the active substance in the lungs and livers was performed as described before. The results of targeting with microspheres were compared to results using only the active substance. Lung samples weighing 0.217 ± 0.013 g and liver samples weighing 1.693 ± 0.091 g were used in quantitative determinations.

2.7. Histopathological investigation of the optimum formulation

The lungs and livers obtained after the administration of the formulations at 0, 10, 30 min, 1, 2, 4, 6, 24 h were kept in 10% formol solution. Samples were investigated by the tissue device (Autotechnicon, Shandon-Lipshaw, Germany). Tissues were embedded in paraffin and cut by microthom at 5 μm thickness and deparaffinized in stove at 60°C. These sections were stained by haematoxylene–eosin and examined histopathologically.

3. Results and discussion

The physico-chemical characteristics of clarithromycin loaded serum albumin microspheres used in this investigation were described previously [7]. Spherical, discrete and free flowing clarithromycin microspheres were prepared using the emulsion polymerization technique.

Quantitative determination of clarithromycin was performed by reversed phase HPLC method. Calibration parameters obtained at a concentration range of 1–100 μg/ml of clarithromycin using 300 ng/ml biphenyl as an internal standard are shown in Table 1.

Fig. 1 shows the chromatogram of the standard mixture containing the active substance at a concentration of 50 μg/ml and the internal standard concentration of 300 ng/ml. The determination limit was found to be 1 μg/ml. Human serum albumin (HSA) microspheres were selected since optimum values for particle size distribution (7 μm), amount of delivery and amount of substance in microspheres were obtained with this species [7].

Fig. 2 shows optical microscope images of these microspheres.

Quantitative determination as described in the experimental section was done in the lung and liver samples belonging to mice that were intravenously administered with the microsphere formulations. It is of interest to mention that no clarithromycin was found in the liver samples. Table 2 shows the results of quantitative determination obtained from the lungs. Mice administered with the clarithromycin solution containing only the active substance, showed maximum accumulation after 4 and 6 h (18 ± 4.68 and 34 ± 6.55 μg/ml, $P < 0.01$, respectively).

Accuracy and recovery values were 96 and 92%, respectively, which were the mean of six parallel experiments.

One group of samples was separated for quantitative determination, another one was examined histopathologically. The optical microscope images (20×10 enlargements) of the lung and liver samples obtained from the mice which were not administered active substance for control are shown in Fig. 3a and b.

The lung and liver images obtained 4 h after administration, where maximum accumulation of clarithromycin occurs, are shown in Fig. 4a and b. These samples revealed that the lung samples, in addition to congestion, especially in perialveolar localizations, microspheric accumulations were present as indicated by the arrows in Fig. 4a. It was noted that these microsphere accumulation began from the tenth min, increased gradually in the 4 and 6-h samples and then decreased, however, they were still present in the 24-h samples.

Table 1
Characteristics of clarithromycin calibration curve

Mobile phase	Concentration range (μg/ml)	Slope	*y*-intercept	Correlation coefficient	SE of slope [a]	SE of intercept
Methanol: 0.067 M KH_2PO_4 (650:350) Adjusted to pH 4.0 with H_3PO_4	1-100	0.1904	0.483	0.998	4.06×10^{-3}	0.218

[a] SE = Standard Error.

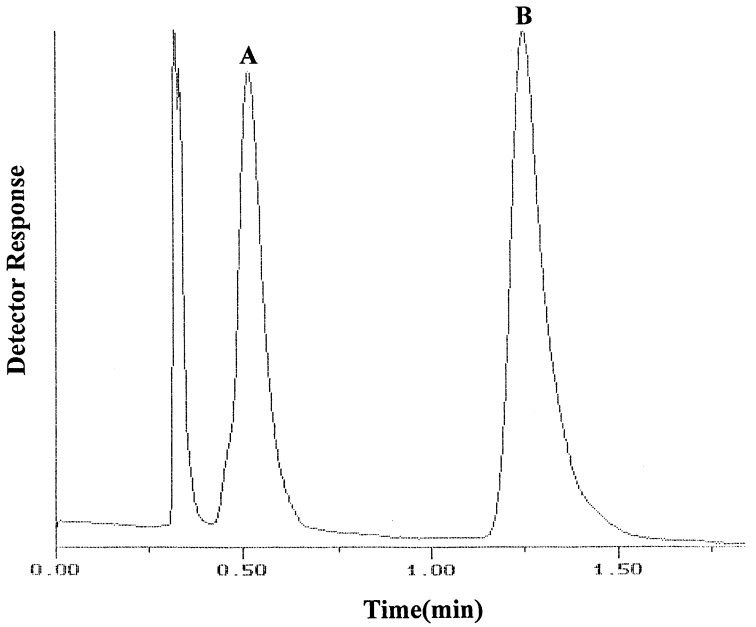

Fig. 1. The typical chromatogram of standard mixture containing clarithromycin (A) at a concentration of 50 μg/ml (internal standard is biphenyl (B) at a concentration of 300 ng/ml).

In liver sample portions, no microspheric accumulation was seen at any time periods.

The accumulation of particles in lungs is a function of particle size. The uptake of microspheres by the lung probably takes place due to mechanical filtration caused by capillary blockade before distribution to the whole body.

4. Conclusions

The accumulation of particles in lungs is a function of particle size. It is generally accepted that, following intravenous injection, microspheres of 7 μm or more in diameter are rapidly entrapped in the lungs by mechanical filtration whereas microspheres with a diameter of 5 μm or less are mainly taken up by cells of the reticuloendothelial system predominantly in the liver [11]. Therefore the clarithromycin microspheres of 7–15 μm in diameter were injected.

The HSA-clarithromycin microspheres prepared with the appropriate characteristics showed favorable targeting to the lungs than using clarithromycin alone.

The results of using these microspheres for in vivo studies were comparable with in vitro studies. The microspheres could be a useful dosage form for pulmonary disease therapy thus avoiding the undesirable gastrointestinal and other side-effects reported for clarithromycin.

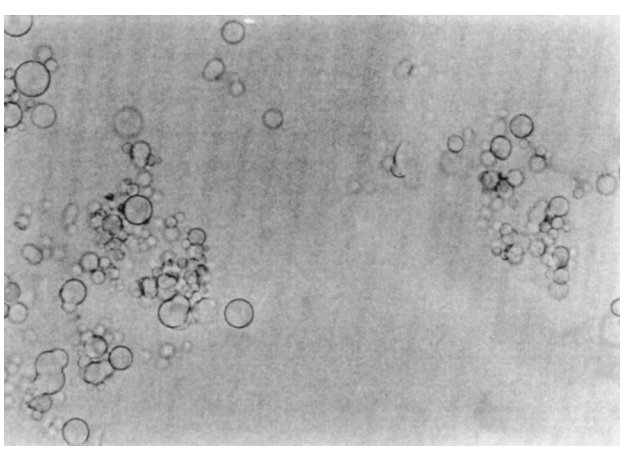

Fig. 2. Optical microscope image of HSA–clarithromycin formulation (× 200).

Table 2
Clarithromycin found in the lungs versus time

Time (min)	Clarithromycin [a] (μg/ml)
10	12 ± 3.45
30	18 ± 5.13
60	26 ± 3.90
120	54 ± 7.63
240	163 ± 13.54
360	176 ± 16.65
1440	23 ± 4.36

[a] Each value is the mean of three experiments.

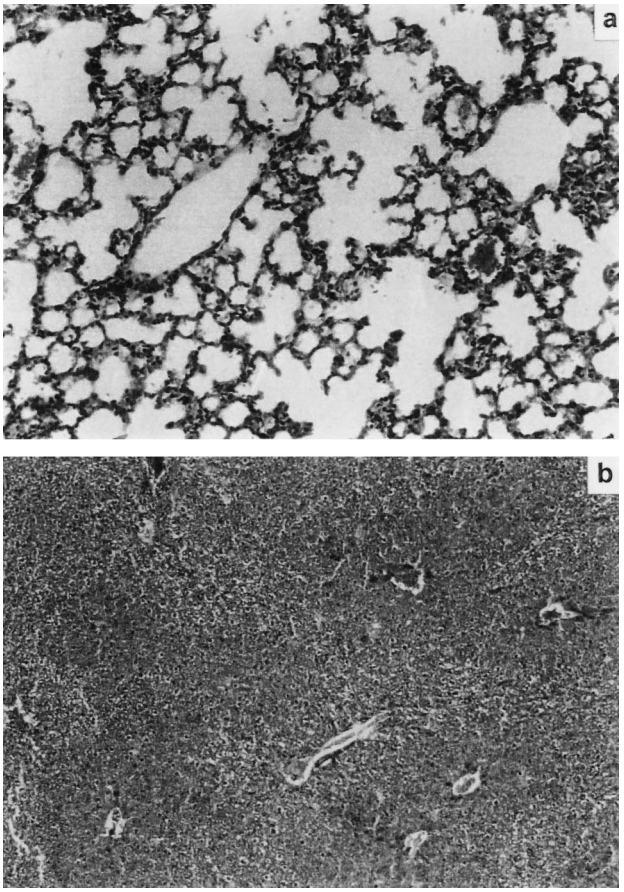

Fig. 3. Optical microscope image of the lung (a) and liver (b) samples obtained from the mice which did not receive the active substance (× 200).

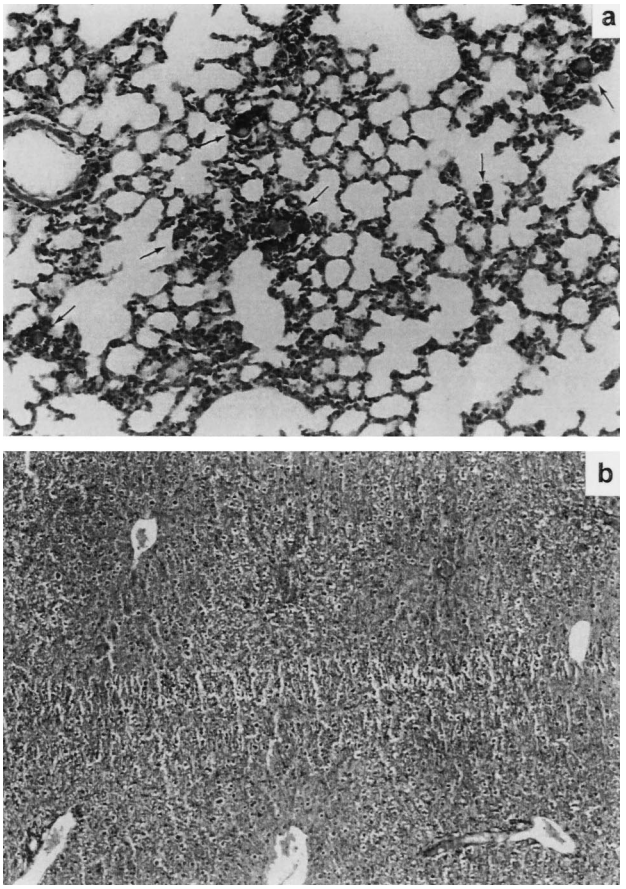

Fig. 4. Optical microscope image of the lung (a) and liver (b) samples at 4 h after administration (× 200).

References

[1] D.R. Laurence, P.N. Bennett, M.J. Brown, Clinical Pharmacology, eigth ed., Churchill Livingstone Medical Division of Pearson Professional Lim., New York, 1997, p. 209.
[2] M.A. Onur, I. Vural, H.S. Kaş, A.A. Hıncal, T. Coşkun, G. Kanraş, A. Tümer, Decrease in the placental transfer of chloramphenicol when administered in albumin microspheres into rats, J. Microencapsulation 10 (1993) 367–374.
[3] K. Çiftçi, A.A. Hincal, H.S. Kaş, M.T. Ercan, Ş. Ruacan, Microspheres of 5-fluorouracil using poly (DL-lactic acid): in vitro release properties and distribution in mice after in vitro administration, Eur. J. Pharm. Sci. 1 (1994) 249–258.
[4] D.J. Burgess, S.S. Davis, Potential use of albumin microspheres as a drug delivery system II. In vivo deposition and release of steroids, Int. J. Pharm. 46 (1988) 69–76.
[5] J.J. Burger, E. Tomlinson, E.M.A. Mulder, J.G. McVie, Albumin microspheres for intra-arterial tumor targeting I. Pharmaceutical aspects, Int. J. Pharm. 23 (1985) 333–344.
[6] M. Kanke, G.H. Simmons, D.L. Weiss, B.A. Brack, P.P. DeLuca, Clearance of ^{141}Ce-labeled microspheres from blood and distribution in specific organs following intravenous and intra-arterial administration in beagle dogs, J. Pharm. Sci. 69 (1980) 755–762.
[7] Y. Özkan, N. Dikmen, A. Işımer, Clarithromycin targeting to lung: optimization of the size, morphology and release characteristics of albumin microspheres. Eur. J. Pharm. Sci., Submitted for publication.
[8] L.G. Koss, Diagnostic cytology and its histopathologic bases. fourth ed., Vol II, J.B. Lippincott Comp., Philadelphia, PA, 1992, pp. 1472–1474.
[9] S. Budavari, The Merck Index, twelfth ed., Merck and Co., Whitehouse Station, NJ, 1996, p. 2400.
[10] D.N. Fish, M.H. Gotfried, L.H. Danziger, K.A. Rodvold, Penetration of clarithromycin into lung tissues from patients undergoing lung resection, Antimicrobial agents and chemotherapy, April (1994) 876–878.
[11] L. Illum, S.S. Davis, C.G. Wilson, N.W. Thomas, M. Frier, J.G. Hardy, Blood clearance and organ deposition of intravenously administered colloidal particles. The effects of particle size, nature and shape, Int. J. Pharm. 12 (1982) 135–146.