Microparticles on the Basis of Segmented Polyurethanes for the Treatment of Tuberculosis

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ABSTRACT

The objective of the research is to develop a new drug delivery system based on polyurethane (PU) microparticles for the treatment of tuberculosis by pulmonary administration to the lungs. The synthesis and characterization of polyurethane microcapsules are studied making use three molecular weight polyethylene glycol (PEG) and tolylene-2,4-diisocyanate (TDI). Antitubercular drugs such as isoniazid was incorporated into the polyurethane microcapsules. The effects of nature and concentration of drug and diols were studied by electronic microscope and size distribution by dynamic light scattering analysis. It was shown that the size of microcapsules at maximum distribution band increases also with the PEG length. The size of polymeric microcapsules and size distribution are important factors for their application for pulmonary administration of drug by respiratory way. The size of microparticles is limited to 1–10 μm. Particles of such size are mainly to be placed in the periphery of the lung and must be phagocytized by alveolar macrophages, the primary site of tuberculosis infection.

Keywords: polyurethane, microparticles, tuberculosis treatment, drug delivery, isoniazid.

1 INTRODUCTION

Inhalable aerosol particles containing tuberculostatic agents are useful for the treatment of tuberculosis, which is recognized as a global public health emergency by the declare of World Health Organization [1]. Carrier systems of antitubercular drug currently in use include liposomes and polymers such as poly (DL-lactide-co-glycolide) [2,3].

Biodegradable microsphere drug delivery systems have shown application for oral and parenteral administration [4]. Administration of microparticles to the lungs (alveolar region) may provide the opportunity for the prolonged delivery active agent to tuberculosis infected macrophages. Microspheres can be produced to meet certain morphological requirements such as size, shape and porosity by varying the process parameters. However, the morphology of the lung is such that to achieve effective drug deposition it is necessary to control the particle size of microparticles [5].

Polyurethanes are an attractive class of polymers that have found many applications as biomaterials due to their excellent physical properties, superior blood compatibility and biodegradation [6,7]. Basically, polyurethane may be produced by two chemical processes: by reaction between a diol and a diisocyanate. Polyurethane microspheres can be prepared by interfacial poly-condensation in emulsions [8].

The objective of the research is to develop a new drug delivery system based on polyurethane microparticles for the treatment of tuberculosis by pulmonary administration to the lungs.

2 MATERIALS AND METHODS

2.1. Materials

Poly(ethylene glycol)s with various molecular weight (M_W 400, 600, 1000, 1450) were used as received, 2,4-toluene (tolylene) diisocyanate (TDI) were obtained from Aldrich, USA. TDI was distilled under a vacuum before use. Emulsion was stabilized with Tween 40 (SIGMA, USA). Toluene used as the oil phase.

2.2. Preparation of microparticles

Polycondensation was carried out in a 1 L double-neck flask fitted with a stirrer. Three solutions were prepared separately. Solution A: 10 mg Tween 40 was dissolved in 100 ml of toluene; solution B: *x* mmol diol and isoniazid were added in *y* ml of water; amount of isoniazid was 10, 20, and 30 mol % from PEG; solution C: 2.5 *x* mmol TDI was dissolved in 10 ml of solution A. Water/oil ratio was 1:10 vol.%

Solution B was poured into the reaction flask, contaning 90 ml of solution A under the stirring at 1000 rpm during 15 minutes. After microemultion formation solution C dropwise was added. After 180 min the polymerization was stopped. Microparticles were filtered, carefully washed with distillated water and dried at ambient conditions. Yield of polymers was estimated from the total amount of introduced monomers compared to the weight of polycondensation products.

2.3. Microparticles characterization

IR spectra were obtained by a Nicolet 5700 FT-IR (USA) infrared spectrophotometer in KBr.

Microparticle size and shape were characterized visually by scanning electron microscopy (SEM) using electron

probe microanalyzer Superprobe733 (JEOL, Japan) with energy dispersive spectrometer INCA ENERGY. Dried microparticles were spread on the conductive type and coated with gold in FINE COAT

2.4. Release measurements

The release behavior of microparticles loaded with isoniazid was studied with UV-spectroscopy. For calibration, physiological solutions of isoniazid with concentration ranging from 0.004 to 0.05 mg/ml were prepared and their absorption was measured at 263.5 nm with Jasco UV/VIS 7850 spectrophotometer (Japan). The calibration equation was $C=(D-0.01)/31.5$, where C is the concentration of isoniazid in physiological solutions (mg/ml), D is the value of absorbance.

10 mg of isoniazid-loaded microparticles were dispersed in 10 ml of physiological solution under light stirring at constant temperature 37 ^{0}C. After fixed time interval 2 ml of solution was taken out by the squirt equipped with the special filter. The efficiency of capsulation was calculated as ratio of introduced INH to solution B compared with amount of delivered INH into water during 3 weeks. Isoniazid loading was weight of INH (mg) contain in 1 g of microparticles.

2.5. In vivo studies of PU microparticles

PU microparticles were administrated subcutaneously to mice BL/6. Histologic analyses of the underskin tissue was carried out at a different period of microparticles administration in the mice by using electron microscope LEO F360, equipped with X-ray analyzer EDS Oxford ISI 300.

3. RESULTS

3.1. Preparation and characterization of drug - loaded PU microparticles

Isoniazid is hydrophilic water-soluble compound, and it is insoluble in toluene. Thus INH could be capsulated by interfacial polycondensation technique using water-in-toluene emulsion, which prevents transferring of INH to the external phase. And drug encapsulation is possible during the process of the polymer wall formation.

In our previous study [9] we synthesized microparticles on the basis of segmented polyurethanes using polycondensation process in inverse emulsion (water-in-oil systems, w/o). It was shown that using this technique, it is possible to obtain microparticles in the range 6 - 10 μm with good mechanical properties. And such particles are very suitable as a drug carrier system.

In this work, INH-loaded segmented polyurethane microcapsules were synthesized in water-in-toluene emulsion. Isocyanate groups of TDI react with hydroxyl groups of PEG to form polyurethane chains according to the Scheme 1.

TDI can also reacts with molecules of water at the border of reaction to form unstable NH-COOH group, which dissociates into amine and carbon dioxide. Chains with amine end-group reacts with the isocyanate groups of growing polymer with urea segments formation.

The completion of polycondensation process was estimated from decreasing of the adsorption band at 2270-2320 cm$^-$1, which correspond to -N=C=O isocianate group. In the IR-spectra of microparticles the N-H stretching vibration were observed at 3450–3300 cm^{-1}, absorption bands at 1740–1700 cm^{-1} for the C=O stretching of urethane and at 1690–1650 cm^{-1} for urethane-urea formation presence also. Band at 1100 cm^{-1} of C-O-C ether group and band at 2850 -2950 cm^{-1} of C-H presence in the IR-spectra and also demonstrated polyurethane formation.

In FT-IR spectra of microparticles containing INH, the new stretching vibrations appeared at 1350 cm^{-1}, 1000 см$^{-1}$ and 690 cm^{-1}, wich also presence in FT-IR spectra of pure isoniazid, that indicate the prevail of the physical mechanism of INH capsulation.

In the process of interfacial polycondensation, two PU products of reaction were detected: the main product - PU microparticles, and the secondary product - linear precipitated polyurethane. The increase of PEG content in water phase result in increase amount of the secondary product, and as the PEG content in water phase reach 60 vol.%, maximum of the secondary product was observed (about 40%).

Decreasing PEG concentration in water phase leads to increase yield of polyurethane microparticles. Maximum of yield was reached at PEG concentration 22 - 27 vol.% and in that conditions whole olygomer reacted at surface of emulsion drops with microparticles formation. Reduction of microparticle yield after the maximum is due mainly to increasing contribution of the hydrolysis process of isocianate groups.

Appearance of the secondary product and increase of it's yield, probably, can be attributed to increase of PEG concentration and result in PEG partially transfer from the water phase to the internal phase of toluene and process of the polycondensation between PEG and TDI take place with linear polyurethane formation.

At the end of reaction rate of PEG diffusion to surface, namely at the reaction region, seems to be a limit stage of the process. Reducing of PEG concentration causes to decrease of system viscosity and enhance of mobility of PEG molecules.

Fig.1 shows SEM photos of interfacial polycondensation products prepared by reaction between TDI and PEG 400 at water/PEG ratio 11,8 : 88,2 vol.% in water phase. According to Figs. 3a and 3b two products of polycondensation with different structure were formed. PU microparticles have spherical shape and size about 5 - 10 μm (Fig. 1a). The secondary product has fibril structure with diameter less then 500 nm (Fig 1b). The effect of

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water/PEG ratio on morphology of microparticle walls is shown in Figs. 1c and 1d. PU microparticles prepared at water/PEG ratio 82,4 :17,6 (Fig. 1c) have rough surface. On the contrary the surface of PU microparticles prepared at water/PEG ratio 11,8 : 88,2 (Fig 1d) were dense and smooth.

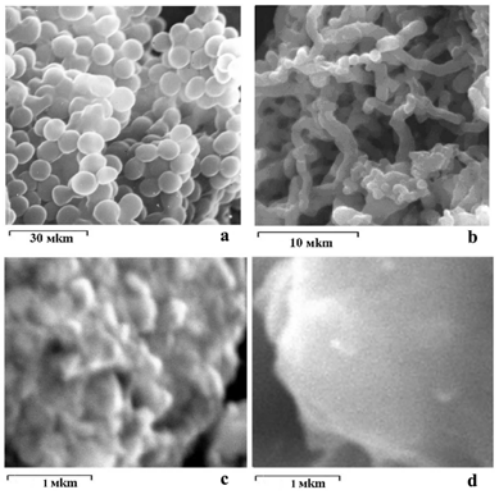

Figure 1 SEM photographs of products of interfacial polycondensation. between TDI and PEG 400 at 60^0C. PU microparticles (a) and PU secondary product synthesized at water/PEG ratio 11.8 : 88.2 in water phase. Surface of PU microparticles prepared at water/PEG ratio 82.4 :17.6 (c) and 11.8 : 88.2 (d) in water phase.

Fig. 2 shows that composition of the water phase influences upon effectiveness of capsulation. Increase of PEG concentration results in decreasing effectiveness of capsulation and decrease of INH loading correspondingly. The high PEG concentration promotes miscibility of INH in the internal oil phase - toluene.

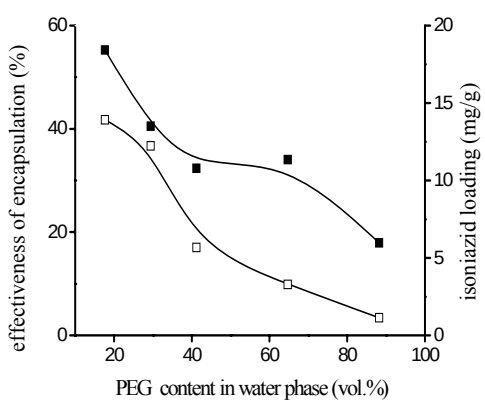

Figure 2. The effect of compound water phase on containing isoniazid in PU microparticles and effectiveness of encapsulation. Effectiveness of encapsulation (open); Isoniazid loading in PU microparticles (closed).

3.2. In vitro release of isoniazid from PU microparticles

The release behavior of isoniazid from PU microparticles was carried out and different conditions of synthesis such as water/PEG ratio, molecular weight of PEG and isoniazid concentration was investigated.

. Microparticles prepared with less concentration of PEG in the water phase demonstrated faster diffusion of INH through walls of microparticles. The increase PEG content in water phase of reaction, results in decreasing INH diffusion, due to formation of PU microparticles with denser polymer wall.

Microparticles prepared with PEG concentration 17.6, 29.4 и 41.2 vol.% showed the release 58 - 66 % of isoniazid during 3 h. However, due to denser wall of microparticles prepared with PEG 64.7 и 88.2 vol. % demonstrated the release no more then 35% of the drug within the same time.

The effect of molecular weight of PEG on release of isoniazid from PU microparticles was investigated (Fig 3) Microparticles were prepared by using PEG with molecular weight 400, 600, 1000 and 1450. Increasing molecular weight of soft segments (PEG) results in the increase of diffusion rate of isoniazid into solution. This phenomenon can be attributed to increasing molecular weight of PEG which leads to accelerating diffusion of water-soluble isoniazid through hydrophilic PEG chains.

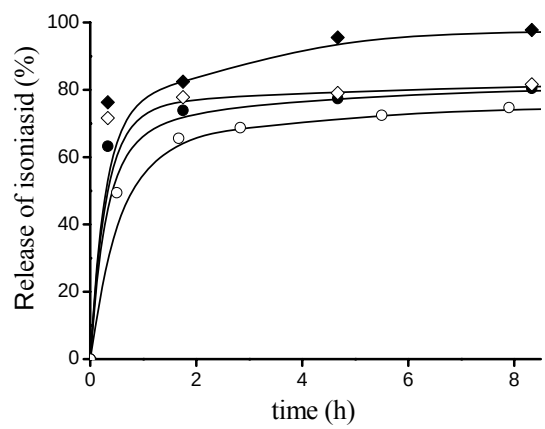

Figure 3. Release of isoniazid from polyurethane microparticles synthesized at various molecular weigh of PEG. M_W = 400 (○), 600 (●), 1000 (◇) and 1450 (◆).

Microparticles with different isoniazid loading, namely 18.4, 35.3 and 65.6 mg/g were produced. It was shown that mroparticles with higher isoniazid loading demonstrate faster release rate of the drug due to increase gradient of concentrations between the external solution and core of microparticles.

3.3. In vivo studies of PU microparticles

The histologic analyses of tissue under skin shows that within 5 days of microparticles deposition the thickening of the surrounding tissue due to primary macrophage reaction and fibrillar tissue formation were detected as shown in Fig. 8b. Up to 21 day some enzymatic lysis of polyurethane – C(O) – NH – group took probably place (Fig. 8c) and its partial biodegradation of PU microparticles were observed. For all experimental animals no casting-off or necrosis of tissue were observed.

4. DISCUSSION

There are no any reports about the using polyurethane microparticles as antitubercular drug carrier. M. Dutt and G.K. Khuller reported that the percentage entrapment of isoniazid in liposomes and PEG-PLG found be 8 - 10 and 7 % respectively and in PLG 10-16 %. Effectiveness of isoniazid capsulation in PU microparticles ranged from 3.4 to 41.7 % and significantly depended on water/PEG ratio in the water phase of emulsion [3].

Morphology of the surface of microparticles is very important factor, which affect release behavior of active agent. The wall structure depends on conditions of interfacial polycondensation process, such as molecular weigh and chemical structure of diol, the concentration of the monomers etc.

E. Jabbari, M. Khakpour prepared PU microparticles with 4,4'-methylene bisphenyl isocyanate, PEG 400 and 1,4-butanediol as the, they showed effect of the ratio of hard to soft segments of the PU chains on the morphology oh PU microparticles. As the amount of the chain extending agent increases the number of pores decreased [8].

The effect of water/PEG ratio in aqueous phase on morphology of microparticle was investigated. Microparticles prepared from PEG solutions of higher concentration have dense surface so that INH diffused mach slower. At the high concentration of PEG reaction between PEG and TDI is significantly limited on the interface of the drops. Furthermore excessive PEG transfers to the external surface of microparticles and react with TDI and less penetrable wall was formed.

Hong K. and Park S. [10] showed that morphology of polyurethane microcapsules became smoother when molecular weigh of PEG increases and demonstrated release behavior of 1,4-diamino antraquione (DAA). Microparticles from a low molecular weight of PEG (400, 600) is more permeable for hydrophobic DAA, whereas microcapsules with PEG 2000 was not permeable for DAA in that concentration. In our case increase of PEG molecular weight result in increase the release rate of isoniazid through PU walls of microparticles. Increase the length of hydrophilic PEG chain result in increase the permeability of water-soluble isoniazid through walls of PU microparticles in contrary of hydrophobic compounds.

Shukla P.G. et al. investigated microcapsulation of water-soluble pesticide monocrotophos in PU microcapsules. Microcapsules with higher per cent loading showed slower release [11]. On the contrary increase of isoniazid loading result in the increase release rate of the drug from PU microparticles.

5. CONCLUSION

Polyurethane microparticles containing isoniazid were prepared by interfacial reaction between PEG and TDI in water in toluene emulsion. Two products of polycondensation were detected: the main product is spherical microparticles with size about 5-10 μm and the second product is fibrils of linear polyurethane, which precipitates in toluene. The effect of water/PEG ratio on morphology of microparticles and release behavior was shown. The low PEG content in aqueous phase result in formation microparticles with rough surface, which demonstrate faster diffusion of INH in comparison with polyurethane microparticles produced from more concentrated PEG solutions, they have smooth surface and less penetrable walls for INH.

Increase molecular weight of PEG and isoniazid loading leads to increase diffusion rate of isoniazid from polyurethane microparticles. Biodegradation of PU microparticles administrated in mice BL/6 subcutaneously was observed due to enzymatic lysis of polyurethane group.

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