International Journal of Pharmaceutics 469 (2014) 23–30

Contents lists available at ScienceDirect

International Journal of Pharmaceutics

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

Injectable long-term control-released *in situ* gels of hydrochloric thiothixene for the treatment of schizophrenia: Preparation, *in vitro* and *in vivo* evaluation

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ARTICLE INFO

Article history:
Received 27 January 2014
Received in revised form 5 April 2014
Accepted 17 April 2014
Available online 18 April 2014

Keywords:
Schizophrenia
Hydrochloric thiothixene
Injectable
Biodegradable
PLA
Control-released
In situ gel

ABSTRACT

Hydrochloric thiothixene (HT) is an antipsychotic drug used in the treatment of various psychoses including schizophrenia, mania, polar disorder, and in behavior disturbances. However, because the psychotics often could not control their behaviors, the independent administration of antipsychotic drug based on medical order was difficult. The omissions of the administration often brought an unsatisfactory therapeutic efficacy. A novel injectable long-term control-released *in situ* gel of HT for the treatment of schizophrenia was developed based on biodegradable material polylactic acid (PLA). The optimum formulation of the injectable PLA-based HT *in situ* gel containing 15% (w/w) HT and 45% (w/w) PLA with benzyl benzoate was used as a gelling solvent. The results of the *in vitro* and *in vivo* studies showed that this *in situ* gel had a long-term period of drug release for several weeks and a good histocompatibility without any remarkable inflammatory reactions.

1. Introduction

Schizophrenia is a major psychosis with unknown etiopathogenisis. Hydrochloric thiothixene (HT), a commonly used drug for schizophrenia, is widely used in clinics due to its seldom side reaction of extracorticospinal tract. HT is an antipsychotic drug used in the treatment of various psychoses including schizophrenia, mania, polar disorder, and in behavior disturbances. HT is usually given orally as doses of 5–15 mg or intramuscularly injected as small doses of 4–8 mg every 8–12 h. Because the psychotics often could not control their behaviors, the independent administration of antipsychotic drug based on medical order was difficult. The omissions of the administration often brought an unsatisfactory therapeutic efficacy. Therefore, a long-term control-released preparation that could last the drug release for several weeks after one administration was desired by doctors in clinics (Dong et al., 2011; Wang et al., 2012).

At present, *in situ* gel is widely used in control-released and sustained-released drug delivery system (Packhaeuser et al., 2004; Kempe and Mäder, 2012; Kapoor et al., 2012). It was in a solution state before its administration. Whereas, when it administered into the body, the phase variation occurred. It often transformed from solution state into solid state or semisolid state (Fogueri and Singh, 2009; Chen and Singh, 2005). Injectable *in situ* gel was a new *in situ* preparation developed in the last decade (Mao et al., 2012; Hate and Amsden, 2002; Agarwal and Rupenthal, 2013; Kranz and Bodmeier, 2007). Injectable *in situ* gel not only had the general features of the gels, but also had good syringeability, biodegradable ability and histocompatibility (Liu et al., 2010; Madhu et al., 2009).

PLA was a widely used biodegradable material in pharmaceutics (Wischke et al., 2010; Brodbeck et al., 1999; Eliaz et al., 2000; Athanasiou et al., 1996). It had a good histocompatibility and was prepared by lactic acid condensation polymerization (Fredenberg et al., 2011). PLA could be hydrolyzed slowly in the body when it met with the body fluid (Camargo et al., 2013). The production of the hydrolization was lactic acid. Because the lactic acid was a common metabolic product in our body, the hydrolization of PLA was very safe (Faisant et al., 2002; Jain et al., 2000). The lactic acid hydrolyzed from PLA could be taken away by the blood, and completely metabolized into water and CO_2 in other organs, such as liver, heart muscle and skeletal muscle. Thus, PLA was a highly safe biomaterial with high histocompatibility and sufficient biodegradable ability (Gad et al., 2008; Qin et al., 2012).

The purpose of this study was to develop a novel injectable long-term control-released *in situ* gel of HT for the treatment of

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http://dx.doi.org/10.1016/j.ijpharm.2014.04.044
0378-5173/

schizophrenia based on biodegradable material PLA, and evaluate its *in vitro* and *in vivo* properties.

2. Materials and methods

2.1. Materials

Polylactic acid (PLA) (MW 5500) was used as an *in situ* gelling-agent and was supplied by Shenghe Chemical Co., Ltd. (Hubei, China). *N*-Methylpyrrolidone (NMP), triacetin and benzyl benzoate were used as *in situ* gelling solvent, respectively, and was supplied by Sinopharm Chemical Reagent Co., Ltd. (Beijing, China). Hydrochloric thiothixene (HT) was used as a model drug and was also purchased from Sinopharm Chemical Reagent Co., Ltd. (Beijing, China). Acetonitrile and methanol used in HPLC analysis were of chromatographic purity and were supplied by Fisher Scientific (Fair Lawn, New Jersey, USA). Natrium biphosphoricum, dipotassium phosphate and phosphoric acid, to prepare phosphate buffer saline (PBS, pH 7.4), were purchased from Pengli Chemical Reagent Co., Ltd. (Beijing, China). The other chemicals used in the present study were all of analytical grade.

2.2. Preparation of in situ gels

The *in situ* gels were prepared by dissolving a predetermined amount of PLA in the *in situ* gelling solvents NMP, benzyl benzoate and triacetin, respectively. In detail, PLA was added into the solvent with a continuous agitation at 25 °C for at least 20 min until PLA was dissolved completely. Then, a fixed amount of drug HT (15%, w/v) was added into the PLA-based *in situ* gels that have been prepared in the previous step. Afterward, the *in situ* gels were agitated by a magnetic stirrer until HT was either completely dissolved or suspended in the *in situ* gels. The prepared *in situ* gels were stored at 4 °C before a further experiment.

In order to avoid the additions of the bacterial inhibitors in the gels, in this study, all the preparations of the gels were be performed under an aseptic condition. Therefore, all the preparations were performed in a bio-safety cabinet (Haier®, Haier Biological Medical Co., Ltd., Qingdao, China). Additionally, all the experimental glassware in this study was also previously sterilized using an autoclave (Lead-Tech (Shanghai) Scientific Instrument Co., Ltd., Shanghai, China).

2.3. Syringeability

Because the successful administration by a syringe with an appropriate needle of a preparation is essential for the injectable gels, the syringeabilities of the formulations were tested in this study. Syringeability was defined as a capacity of the injection of a preparation at a determined injection rate through a needle (Burckbuchler et al., 2010). The syringeabilities of the formulations were tested by a Stable Micro Systems TA-XT Plus Texture Analyser (Stable Micro Systems, Godalming, UK). The pre-test speed was set at 1.0 mm/s; the test speed was set at 1.0 mm/s; the post-test speed was set at 10.0 mm/s; the distance covered by the plunger was 20 mm; the trigger force was 0.05 N. The study was performed using a 5 ml glass syring with a 21 gauge needle.

2.4. Dissolution study

In order to investigate the releasing property of HT from the PLA-based *in situ* gels, an *in vitro* drug release study was carried out by using a RX-6 dissolution test apparatus (Tianjin Optical Instrument Factory, Tianjin, China) (Kranz et al., 2008; Kang and Singh, 2005). 10 ml samples were added into every dissolution cup. Every formulation was tested in triplicate. The release medium was a 900 ml phosphate buffer solution (pH 7.40). Moreover, because the period of the dissolution test was long, sodium azide (0.02%, w/v) was added into the phosphate buffer solution in order to prevent bacterial contamination. The test temperature was set at 37 °C. In the dissolution test, the paddle method was used. In detail, the rotating rate of the paddle was set at 50 rpm. At the interval time of 1, 3, 6, 9, 12 days, and so on, or until the gel was completely dissolved, 1.0 ml samples were withdrawn and replaced with 1.0 ml fresh medium. Afterwards, the samples were filtered by a centrifugal filter (Shanghai Chaoyan Biological Technology Co., Ltd., Shanghai, China) before they were analyzed by HPLC.

2.5. Analysis of HT by HPLC

The quantification of HT was performed by a high performance liquid chromatograph (HLPC). The HPLC consisted of a Model 501 pump (Waters Associates, Milford, MA, USA), Rheodyne Model 7125 loop injector (Cotati, CA, USA), Model 484 variable-wavelength absorbance detector (Waters Associates) and Model 3396A integrating recorder (Hewlett-Packard, Palo Alto, CA, USA), together with a C_{18} guard column (Direct Connect; Alltech Associates, Australia) and a C_{18} column 10 μm particle size, 25 cm × 4.6 mm, (Waters Associates). The mobile phase was methanol–water–cholamine (350:50:0.05) at a flow rate of 1.0 ml/min. The injection volume was 20 μl, and the detecting wavelength was set at 229 nm.

2.6. Stability

Stability study of the selected *in situ* gels formulation was performed to investigate the influence of the storage temperature on the physical–chemical properties of the gels (Asmus et al., 2013). The *in situ* gels were filled into 50 ml screw-capped glass vials. Then, the vials filled with the gels were placed in a stability test chamber (Shanghai Linpin Instrument Stock Co., Ltd., Shanghai, China). The test temperatures were set at 4, 25 and 40 °C, respectively. The test was carried out without illumination. Every month, drug content, color, limpidity, syringeability and pH of the tested gels were evaluated. In this study, in order to presume the store condition of the selected *in situ* gel formulation, a stability investigation was also performed at 4 °C for at least 12 months. The content of HT in the tested gels was measured by HPLC. In detail, 0.1 ml gel was taken from the glass vial. Then, the gel that eas taken out was added into 1-ml acetonitrile (HPLC grade). The mixture was vortex-mixed for 5 min, and then centrifuged at 3000 rpm. Finally, the supernatant liquid was withdrawn and filtered by 0.45 μm micropore film before analysis by HPLC. The pH of the tested gels was tested by a Leici PHS-3C pH meter (Leici Instrument Factory, Shanghai, China). The pH of the gels was tested by directly immersing the electrode in the samples.

2.7. In vivo evaluation

In this study, twenty four male Wistar rats whose weight should be in the range of 190–250 g were used as test animals. Two of them were put in one mouse cage and were accessing freely to the mouse diet and water throughout the study. The rats were divided into two groups. The first group (Group I) was injected with HT *in situ* gel; the second group (Group II) was injected with HT solution. The HT *in situ* gel and the HT solution were both injected intramuscularly into the right musculus rectus of the rats (Patel et al., 2010). The HT *in situ* gel or the HT solution equivalent to 15 mg of HT was intramuscularly injected into the animals, respectively. The blood was taken from the tail vein of the tested rats. Briefly, the tested animal was put in a body restrainer, and the tail of the rat was warmed by an incandescent lamp in order to