INFLUENCE OF SURFACE AND STRUCTURAL PROPERTIES ON THE INITIAL RELEASE OF RISPERIDONE FROM POLYMERIC DRUG CARRIERS

Artur Turek[1]*, Janusz Kasperczyk[1,2], Katarzyna Jelonek[2], Piotr Dobrzyński[2], Joanna Walichiewicz[1], Katarzyna Krzemińska[1], Anna Smola[2], Monika Musiał–Kulik[2], Andrzej Marcinkowski[2], Marcin Libera[2], Katarzyna Gębarowska[2]

[1]Medical University of Silesia, Department of Biopharmacy, 1 Narcyzów Str., 41-200 Sosnowiec, Poland
[2] Polish Academy of Sciences, Centre of Polymer and Carbon Materials, 34 M.Curie-Sklodowskiej Str., 41-819 Zabrze, Poland
*MAILTO: a.turek75@gmail.com

Abstract

In this work, implantable drug formulation with risperidone on the basis of poly(L-lactide-co-glycolide) (L-PLGA) and poly(D,L-lactide-co-glycolide) (D,L-PLGA) as drug carries was developed. The influence of surface and structural properties on the initial release of risperidone during the first twenty four hours was determined. In this aim, high-performance liquid chromatography, nuclear magnetic resonance spectroscopy, scanning electron microscope and atomic force microscope were used. Significant differences between L-PLGA and D,L-PLGA matrices in all analyzed data were noted. The burst effect was not revealed for any of the studied polymers, however the released drug was almost five times larger for D,L-PLGA matrices. The L-PLGA copolymer revealed a significantly longer average length of the lactidyl and glycolidyl blocks than D,L-PLGA. Moreover, various characters of surface for analyzed matrices were shown, i.e. in case of L-PLGA the surface was porous and in case of D,L-PLGA it was nonporous. Undoubtedly, there were dependences between risperidone's initial release and the topography and the structure of polymeric matrices. We suppose that the larger drug release for L-PLGA was more associated with surface properties and thus structure of matrices. The obtained results showed the great potential of both polymers and possibility to choose the optimal polymer.

Keywords: *risperidone, implantable drug carriers, poly(L-lactide-co-glycolide, poly(D,L-lactide-co-glycolide), initial release*

[Engineering of Biomaterials, 116-117, (2012), 144-146]

Introduction

According to the literature data schizophrenia affects a significant part of the world population, i.e. the range of 0.3-0.7% [1,2]. A lot of neuroleptics in various formulations have found the application in the treatment of schizophrenia [3-6]. One of them was risperidone, clinically used since the late 80's [7,8]. In the last two decades, such formulations containing risperidone as conventional oral tablets and orodispersible tablets, syrups and long-acting injections were proposed to increase the bioavailability of risperidone and to decrease its widely understood side effects [9-12]. Regardless of the point of view, the above-mentioned formulations possess the smaller or larger drawbacks. The biggest drawback of oral formulations seems to be the need of regular daily applications. An alternative to the traditional drug formulations are various long-acting injections. The commercially available medical product containing risperidone in the form of a long-acting injection includes the encapsulated microspheres composed of poly(D,L-lactide-co-glycolide) (D,L-PLGA) and must be applied every two weeks for most patients. In case of this medical product the main release of risperidone begins in the 3rd week, lasts from the 4th to 6th week and ends in the 7th week [13]. However, due to the fact that the microspheres are suspended in an aqueous solution, the drug's administration may be accompanied by pain. Moreover, in the event of any clinical complications microspheres cannot be removed.

In our studies, an implantable drug formulation with risperidone on the basis of poly(L-lactide-co- glycolide) (L-PLGA) and D,L-PLGA as drug carries will be developed. The main aim of the performed studies on a novel formulation is obtaining a medical product which will be applied less frequently and risperidone will be released faster than in the 4th week and longer than for 7 weeks in comparison with a commercial product. However, in this work, at the current stage of studies, the influence of surface and structural properties on the initial release of risperidone from polymeric matrices and the risk of the burst effect will be determined.

Materials and methods

The matrices (10 mm diameter) were obtained from two high molecular copolymers, i.e. L-PLGA 85:15 (100 000 Da) and D,L-PLGA 85:15 (90 300 Da) by solution casting method. In this study, five matrices obtained from L-PLGA with an average weight equal to 60.74±13.60 mg and five matrices from D,L-PLGA with an average weight equal to 71.22±2.47 mg were used. The copolymer was synthesized at Centre of Polymer and Carbon Materials of Polish Academy of Sciences in Zabrze in bulk with the use of $Zr(Acac)_4$ as a low toxic initiator according to the previously developed methodology [14,15].

The matrices containing 10% of risperidone (Teva Kutno S.A.) were incubated in a PBS buffer (pH 7.4) in the ratio of 15 mg of matrix: 1 ml of PBS during 24 hours under constant agitation (240 revs per minute) at the temperature of 37ºC. After this period the buffer was examined for the presence of risperidone. Surface and structural properties of polymeric matrices were determined before their incubation in the PBS buffer.

Determination of risperidone concentration was done by high-performance liquid chromatography – HPLC using a column oven (L-2450, VWR Hitachi, Merck), a UV detector (L-2355 diode array detector, VWR Hitachi, Merck) and Elite LaChrom chromatograph (VWR Hitachi, Merck).

The composition and chain structure of used copolymers (the average length of the glycolidyl (l_{GG}) and lactidyl blocks (l_{LL}), randomization ratio (R)) were determined by ^{1}H and ^{13}C nuclear magnetic resonance spectroscopy (NMR). ^{1}H NMR data were recorded at 600 MHz and ^{13}C NMR at 125 MHz using AVANCE II Ultra Shield Plus Bruker 600 MHz spectrometer and a 5-mm sample tube. $DMSO_{d6}$ was used as a solvent.

The morphological study of the matrices' surface was performed by a scanning electron microscope (SEM) and an atomic force microscope (AFM). SEM Quanta 250 FEG (FEI Company) was employed in this study. Prior to observation the samples were sputter coated with a 3 nm layer of gold and investigated at low vacuum. The accelerating voltage

was 5 kV while scanning. AFM imaging was performed using MultiMode 3 (di-Veeco, CA) working in the tapping mode under atmospheric conditions. Before measurements, the matrices were dried on air at room temperature in the laminar box and then under reduced pressure. All AFM images were processed using the software package WSxM (Nanotec Electronica) [16].

Results and discussions

During risperidone release from the implantable biodegradable formulations various unfavorable phenomena may take place. One of them is burst effect, widely described in literature for other drugs and various drug carriers [17,18]. In this work, novel formulations which may release risperidone longer than for 7 weeks will be developed. In this aspect, the limitation or elimination of the burst effect is significantly important. On the one hand, uncontrolled release of drug substance may influence toxic effect in vivo. On the over hand, excessive release of drug substance directly affects the dose reduction in the polymeric matrices.

Undoubtedly, there are dependences between risperidone's initial release and the topography and structure of polymeric matrices. In this study it was confirmed. Significant differences between L-PLGA and D,L-PLGA matrices in the level of risperidone release, SEM and AFM images and NMR data were observed.

The initial risperidone release was not very significant, so the burst effect did not occur in any of the studied polymers. However, significant differences between copolymers in released drug amount were noted - in case of L-PLGA, 30.94 μg ± 3.16 (i.e. 0.51%) of risperidone was released, while in case of D,L-PLGA, the amount of 167.07±19.25 μg (i.e. 2.35%) of risperidone was noted after the first day of the matrices' incubation in the PBS buffer. The level of risperidone release during the first twenty four hours may give information about the amount of substance which was not incorporated into matrix. It should be emphasized that in fact during the first twenty four hours the hydrolytic degradation does not take place in case of high molecular mass aliphatic polyesters. In this period mainly surface phenomena may decide on risperidone release. However, surface properties are largely a reflection of structural properties.

In the NMR study, significant differences in the average l_{LL} and l_{GG} blocks as well as in R between L-PLGA and D,L-PLGA matrices were shown. The L-PLGA copolymer revealed significantly longer average l_{LL} and l_{GG} blocks than D,L-PLGA (TABLE 1). The average l_{LL} and l_{GG} blocks allowed determining the kind of the copolymers' structure. Considering that the block structure of polymers occurs at the value of randomization ratio equal to 0, the semi-block structure at the value of 0.5 and the random structure at the value of 1, the structure of L-PLGA was more blocky than that of D,L-PLGA (TABLE 1).

On the basis of average l_{LL}, IGG blocks and R it is difficult to clearly explain how the matrix structure influenced the initial release of risperidone from L-PLGA and D,L-PLGA matrices. Therefore, it is not known on the basis of the NMR study why the initial release of risperidone from the L-PLGA matrix was noted on a significantly lower level. More explanations may be obtained from the analysis of surface properties.

SEM and AFM images reflected significant differences in the morphology of the analyzed surface between the L-PLGA and D,L-PLGA matrices.

TABLE 1. Structure of L-PLGA and D,L-PLGA determined by NMR study before incubation in the PBS buffer. Content of lactidyl (F_{LL}) and glycolidyl (F_{GG}) segment; the average length of lactidyl (l_{LL}) and glycolidyl (l_{GG}) blocks; R – randomization ratio.

Copolymers	F_{LL} [%]	F_{GG} [%]	l_{LL}	l_{GG}	R
L-PLGA	85	15	11.34	2.08	0.33
D,L-PLGA	85	15	8.8	1.6	0.43

In case of L-PLGA, the SEM image showed strong porosity of the matrix. The pores were uniformly distributed and possessed oval shape (FIG.1a). The AFM analysis of the matrix revealed numerous hills and hollows on the surface, which might be related to the presence of the pores (FIG. 1b). In our opinion, generally porosity may influence initial release in various ways. It may be confirmed only in an experimental way. Pores localized inside the matrix may be responsible for entrapping drugs and influence the release of drugs in higher amount at the later stage of degradation. However, the pores localized on the surface which do not contain drug or contain little amount of drug may cause insignificant risperidone release at the initial stage of degradation. This phenomenon might take place in case of the studied L-PLGA matrices which revealed 0.51% release of risperidone during the first twenty four hours.

The observed porous surface was formed during the obtaining of matrices. It seems that it is a result of copolymer composition. The effect of solvent addition during processing might also result in differences between surface of polymers with various structure. Differences in solvent induced conformational and morphological changes on the surface of L-PLGA and D,L-PLA were determined. Other authors showed that the contact angle increases after solvent treat-

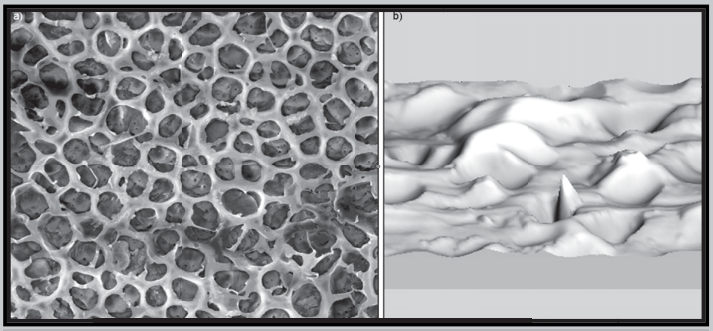

FIG. 1. Morphology of the L-PLGA matrix containing risperidone before incubation in the PBS buffer: SEM image (magnification x 9356) (a), AFM height 3D image (14.5 μm x 14.5 μm x 1.13 μm) (b).

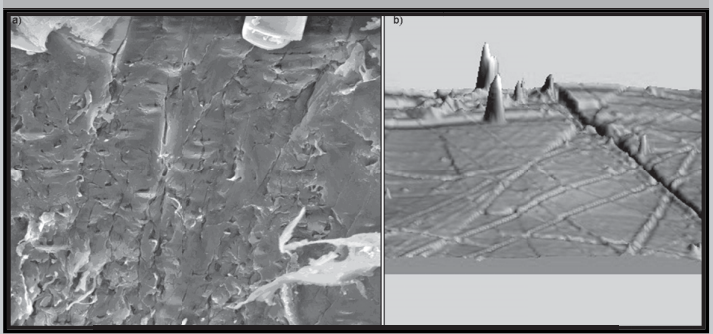

FIG. 2. Morphology of the D,L-PLGA matrix containing risperidone before incubation in the PBS buffer: SEM image (magnification x 9402) (a), AFM height 3D image (10 μm x 10 μm x 1.13 μm) (b).

ment, so the solvent has tremendous effect onto the surface characteristics of the polymer. The increase in the water contact angle is also in a relationship with the segregation of the methyl hydrophobic groups at the polymer surface. It was also reported that the glass transition temperature (Tg) has a great influence onto the surface segregation of methyl groups – polymer with lower Tg value showed higher surface restructuring extent in comparison with polymers with higher Tg value. Thereafter there might be the differences in surface morphology between L-PLGA and D,L-PLGA, which differ in glass transition temperature [19].

The matrices deprived of pores, i.e. D,L-PLGA (FIG.2), revealed the release of risperidone which was almost five times larger. The SEM (FIG.2a) and AFM (FIG. 2b) images of the D,L-PLGA matrix showed also a solid surface composed of morphologic elements with various sizes. D,L-PLGA is a more amorphous material as a result of the atactic structure of the polylactide segments. Copolymer chains were distributed irregularly which might have influence on the creating of the solid surface. Ipso facto the surface of interaction and the adhesion forces between the molecules of risperidone and D,L-PLGA might be lower. We suppose that the higher release of risperidone is closely associated with this feature. Moreover, the increased chain mobility which characterizes amorphous polymers may favor easier drug release from the outer part of matrix.

Conclusions

Concluding, none of the studied polymers exhibited the burst release, however in case of L-PLGA lower amount of drug released during first twenty four hours was noted than in case of D,L-PLGA. However, generally D,L-PLGA is a more amorphous than L-PLGA. D,L-PLGA will degrade faster, which may influence bigger release of risperidone in a shorter period. In turn, more crystalline L-PLGA may influence longer and less even release of risperidone. A proper and the simplest way to avoid the burst effect in case of a medical product seems to be washing the implant during its preparation. However, this will affect the decrease in risperidone dose. It seems that there is a strong correlation of the surface and structural properties with the level of the released drug. A 10% content in the D,L-PLGA matrix might be too high from the point of view of the higher amount of released drug. It is possible that a lower dose (e.g. 5% or 7.5%) will be more optimal for this copolymer. Then, adhesion forces between the molecules of risperidone and D,L-PLGA may be sufficient for the lowering of released drug in the initial phase. Much lower amount of drug was released from L-PLGA, which found a reflection in different surface morphology, i.e. the presence of pores. As was mentioned before, pores localized inside the matrix may be responsible for entrapping drugs and influence the release of drugs in higher amount in the later stage of degradation. However, the pores localized on the surface which do not contain drug or contain little amount of drug cause insignificant risperidone release in the initial stage of degradation. Undoubtedly, further study is necessary to determine the whole drug release profile, however the obtained results showed the lack of burst effect for both of the studied polymers, so they exhibited potential in developing implantable delivery system of risperidone. Moreover, they showed different drug release in the first twenty four hours, which gives the possibility to choose the optimal polymer for the treatment and demanded initial dose.

Acknowledgments

This work was financially supported by Medical University of Silesia, grant no KNW-1-023/P/2/0.

The authors thank Teva Kutno S.A. for providing the drug substance, i.e. risperidone.

References

[1] http://www.who.int/mental_health/management/schizophrenia/en/.
[2] McGrath J., Saha S., Chant D., Welham J.: Schizophrenia: a concise overview of incidence, prevalence, and mortality. Epidemiol Rev 30 (2008) 67–76.
[3] Asenjo Lobos C., Komossa K., Rummel-Kluge C., Hunger H., Schmid F., Schwarz S., Leucht S.: Clozapine versus other atypical antipsychotics for schizophrenia. Cochrane Database Syst Rev 11 (2010) CD006633.
[4] Leucht C., Heres S., Kane J.M., Kissling W., Davis J.M., Leucht S.: Oral versus depot antipsychotic drugs for schizophrenia. A critical systematic review and meta-analysis of randomised long-term trials. Schizophr Res 127 (2011) 83-92.
[5] Leucht S., Tardy M., Komossa K., Heres S., Kissling W., Davis J.M.: Maintenance treatment with antipsychotic drugs for schizophrenia. Cochrane Database Syst Rev 5 (2012) CD008016.
[6] Miyamoto S., Miyake N., Jarskog L.F., Fleischhacker W.W., Lieberman J.A.: Pharmacological treatment of schizophrenia: a critical review of the pharmacology and clinical effects of current and future therapeutic agents. Mol Psychiatr 10 (2012) 1038/mp.2012.47.
[7] Roose K., Gelders Y., Heylen S.: Risperidone (R64 766) in psychotic patients. A first clinical therapeutic exploration. Acta Psychiatr Belg 88 (1988) 233-241.
[8] Castelão J.F., Ferreira L., Gelders Y.G., Heylen S.L.: The efficacy of the D2 and 5-HT2 antagonist risperidone (R 64,766) in the treatment of chronic psychosis. An open dose-finding study. Schizophr Res 2 (1989) 411-415.
[9] Gutierrez R., Lee P.I., Huang M.L., Woestenborghs R.: Risperidone: effects of formulations on oral bioavailability. Pharmacotherapy 17(1997) 599-605.
[10] Kane J.M., Eerdekens M., Lindenmayer J.P., Keith S.J., Lesem M., Karcher K.: Long-acting injectable risperidone: efficacy and safety of the first long-acting atypical antipsychotic. Am J Psychiat 160 (2003) 1125-1132.
[11] Tassaneeyakul W., Kumar S., Gaysonsiri D., Kaewkamson T., Khuroo A., Tangsucharit P., Phunikhom K., Vannaprasaht S., Kanjanawart S., Rao Thudi N., Jain R., Reyar S., Monif T.: Comparative bioavailability of two risperidone orodispersible tablet products after single dose administration. Int J Clin Pharm Th 48 (2010) 614-620.
[12] Huang M., Shen-Tu J., Hu X., Chen J., Liu J., Wu L.: Comparative fasting bioavailability of dispersible and conventional tablets of risperidone: a single-dose, randomized-sequence, open-label, two-period crossover study in healthy male chinese volunteers. Clin Ther 34 (2012) 1432-1439.
[13] http://www.ema.europa.eu/docs/pl_PL/document_library/Referrals_document/Risperdal_Consta_30/WC5000 08170.pdf.
[14] Dobrzyński P., Bero M., Kasperczyk J.: Sposób wytwarzania bioresorbowalnych polimerów. Opis patentowy PL 191846 B1 (2000).
[15] Dobrzyński P., Kasperczyk J., Janeczek H., Bero M.: Synthesis of biodegradable copolymers with the use of low toxic zirconium compounds. 1: Copolymerization of glycolide with L-lactide initiated by Zr(Acac)4. Macromolecules 34 (2001) 5090 -5098.
[16] Horcas I., Fernández R., Gómez-Rodríguez J.M., Colchero J., Gómez-Herrero J., Baro A.M. WSXM: a software for scanning probe microscopy and a tool for nanotechnology. Rev Sci Instrum 78 (2007) 013705.
[17] Trindade R.A., Kiyohara P.K., de Araujo P.S., Bueno da Costa M.H.: PLGA microspheres containing bee venom proteins for preventive immunotherapy. Int J Pharm 423 (2012) 124-133.
[18] Rafati A., Boussahel A., Shakesheff K.M., Shard A.G., Roberts C.J., Chen X., Scurr D.J., Rigby-Singleton S., Whiteside P., Alexander M.R., Davies M.C.: Chemical and spatial analysis of protein loaded PLGA microspheres for drug delivery applications. J Control Release (2012) [Epub ahead of print].
[19] Thanki P.N., Dellacherie E., Six J.L.: Surface characteristics of PLA and PLGA films. Appl Surf Sci 253 (2006) 2758–2764.