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Slow release of two antibiotics of veterinary interest from PVA hydrogels

A. Moretto, L. Tesolin, F. Marsilio, M. Schiavon, M. Berna, F.M. Veronese *

Department of Pharmaceutical Sciences, University of Padova, Via Marzolo, 5, Padova 35131, Italy

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Abstract

Two antibiotics, tylosin tartrate and oxytetracycline hydrochloride, were entrapped in poly(vinyl alcohol) (PVA) hydrogels (MW 31,000–50,000) by a cryogen procedure obtaining a controlled release system suitable for veterinary application. It was found that at a low drug matrix loading (10 mg/ml), the in vitro release rate of both antibiotics could be reduced by a previous freeze drying of the gel, while no reduction in drug rate took place in heavily loaded matrices (300 mg/ml). When PVA hydrogels containing tylosin were administered to rats per os the drug could not be detected in the blood, but it was found in organs,: liver, kidneys, and muscles, for up to 120 h. On the other hand, when the same amount of drug was administered orally as powder, no appreciable organ accumulation was detected, while the drug was found in faeces and urine. These data show that PVA hydrogels can be a suitable slow release system for tylosin administration. Oxytetracycline could also be quantitatively entrapped and released from PVA hydrogels, but once administered per os to rats, it was not detected in blood or organs.

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Keywords: Poly(vinyl alcohol); Controlled release system; Veterinary release; Tylosin; Oxytetracyclin

1. Introduction

The results reported so far in literature for drug release from poly(vinyl alcohol) (PVA) hydrogels [1–3] are suggesting its application in veterinary field. PVA hydrogels may be conveniently prepared by cryopolymerization, a procedure that consists in freezing and thawing cycles of an aqueous concentrated PVA solution [4]. This method of polymerization has the greatest advantage of not requiring potentially harmful chemicals for the formation of the hydrogels and of being economic and easily scaled up. These proprieties are important for a large-scale application as required for animal treatments. Furthermore, PVA hydrogels have high solvatation degree and biocompatibility, as needed for the in vivo administration of drugs [5–7].

For veterinary therapy application slow release systems are of general interest since the lower frequency of administration leads a cut of labour and costs, advantages that are going also with a reduction in animal trauma [8,9]. Moreover, they decrease the amount of drugs waste when compared to the traditional formulation that usually involves a mixing of the free drug powder with fodder. The controlled release may also reduce and prevent gastrointestinal irritation of those drugs that must be administered at high doses to circumvent the rapid excretion of the free form.

In this paper two antibiotics of veterinary interest, oxytetracycline hydrochloride [10] and tylosin tartrate [11], were considered for a PVA hydrogel release system. The aim was to obtain a suitable method to eventually give an initial drug spike to reach the wanted drug levels and after that to maintain a therapeutic concentrations in the animal body for several hours [12]. The system was devised and tested in vivo for oral delivery, thanks to the easy administration route and also to avoid local antibiotic residues in the injection site, as already demonstrated to take place following intramuscular (i.m.) injection [13].

2. Experimental

2.1. Reagents

Oxytetracycline hydrochloride (OTC) and tylosin tartrate were obtained from L.F.B. Biosint (Campoformido, Italy). PVA (MW 31,000–50,000 99% hydrolysed) was purchased from Sigma (St. Louis, MO, USA). Organic solvents were

* Corresponding author.
E-mail address: francesco.veronese@unipd.it (F.M. Veronese).

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from Carlo Erba (Milan, Italy) and from Lab-Scan LTD (Dublin, Ireland). Sesame oil was obtained from Save (Milan, Italy). Salts used for the preparation of buffers were from Merk (Darmstand, Germany). For in vivo experiments were used Sprague–Dawley rats from Department of Pharmaceutical Science pound.

2.2. Equipment

PVA solution was prepared using a thermostatic air cell: Thermostatic P661 from Bisca (Milan, Italy). In vitro release studies were carried out at 37 °C using a thermostatic bath HAAKE SWB 20 (Sudlohn, Germany). Centrifugations were performed with a mod. 5414 centrifuge by Eppendorf (Milan, Italy) or with a Centrikon T-42K, Kontron Instruments centrifuge. Spectrophotometer measurements were carried out with a LAMBDA 5 Spectrophotometer from Perkin-Elmer (Norwolk, CT, USA). High performance liquid chromatography (HPLC) was performed with a LiChro-prep RP-8 guard column (25–40 μm) and a LiChroCART 250-4 column (LiChrosorb RP-18, cat. 15539) from Merk (Darmstandt, Germany). Columns were run by a Gilson instrument (Martin le Bell, France) equipped with a Gilson 117 UV detector (Middleton, USA). For organ homogenation a Sorvall inc. (Newtown, CT, USA) homogenizator was used.

2.3. Matrices preparation for in vitro and in vivo release studies

PVA water solutions at 15% and 20% (w/v) were obtained by dispersion of the polymer in distilled water followed by heating at 105 °C for 6 h for complete dissolution [2]. After solution cooling, drugs were added and the resulting mixture were poured into cylindrical plastic moulds. Both low and high drug loaded matrices were prepared: the low matrices contained 10 mg/ml of drug in 15% (w/v) PVA solution while the high loaded ones 300 mg/ml of drug in 20% (w/v) PVA solution. Matrices underwent into freezing and thawing cycles: at –25 °C for 2 h followed by half an hour at room temperature. The freezing and thawing cycle was repeated for five times. The resulting 15% PVA matrices had a 0.8 cm diameter and 1.2 cm thick while the 20% ones, used in the case of high loading, had 0.8 cm diameter and 2.5 cm thick. The matrices could be later conveniently cut for easier administration.

2.4. In vitro release studies

In vitro release studies were performed with both hydrated and dried matrices; the dried matrices were obtained by drying the gels under vacuum. The releasing buffer, 25 or 500 ml, for low or high loaded matrices respectively, was 0.15 M NaCl, 0.025 M $Na_2HPO_4/Na_2H_2PO_4$, at pH 7.4 maintained at 37 °C for all the experimental time [14]. At scheduled times the supernatant was taken for analysis and replaced with fresh buffer in order to maintain a “sink” condition. Drug concentration was evaluated by spectroscopic analysis and expressed as M_t/M_0 ratio, where M_t means the amount of drug released at time t and M_0 is the amount at time zero.

2.5. In vivo release study of PVA hydrogel

For oral administration, the 20% PVA cylinder matrices, loaded with 300 mg of tylosin, were conveniently cut and dipped into sesame oil to make easier the sliding along the oesophagus. At scheduled times, animals were anaesthetized with ethyl ether and bled by intracardiac injection with a heparinized syringe. The blood samples were centrifuged at 12,000 rpm for 4 min and the supernatant were analysed in HPLC-C4 column using a gradient of MilliQ water (eluent A) and acetonitrile (eluent B): 20% of acetonitrile, to rich 60% in 8 min and 80% in more 8 min at a flow rate of 1 ml/min. The eluate was monitored by an UV detector at 292 nm for tylosin and at 353 nm for oxytetracycline.

2.6. In vivo study of free drug

Tylosin buffer solutions were administered in rats by intraperitoneal (i.p.) injection. At predetermined time points the animals were bled by intracardiac injection and blood samples were processed as reported above. From the area of the drug’s pick the plasma concentration/time profile was calculated on the basis of a calibration curve prepared from the same drug.

2.7. Oral administration of drug powder

For the powder administration, a needle of size suitable to reach the rat gut was connected to a syringe; 300 mg of tylosin was suspended in the minimum water volume needed for sliding along the needle (usually 1 ml). A needle of 1.5 mm diameter, with a smooth tip to avoid animal pain was used. At scheduled times the animals were sacrificed and the organs were extracted, treated and analysed as reported below. Four rats were analysed for each point and the data were expressed as a mean of the obtained concentration value as μg/g.

2.8. Drug distribution studies in organs after oral matrices administration

For the organ distribution investigation liver, leg muscles and kidneys of treated rats were analysed. Organs obtained from animals, sacrificed at scheduled times, were rinsed with isotonic solution (0.7%, w/v, NaCl), dipped in 50 ml of CH_3CN/CH_3OH (50:50), and immediately homogenized in ice bath. The homogenate was centrifuged at 6000 rpm for 12 min. Supernatant was evaporated under vacuum and the residue was recovered with 10 ml of distilled water. The aqueous solution was divided into two equal parts that were separately extracted three times with 5 ml of chloroform.

Organic solvent was evaporated and the residue was dissolved in 2.5 ml of CH_3CN; the obtained solution was evaporated again and taken up with CH_3CN to reach 0.5 ml of final volume. The solution was filtered with Millipore 0.25 μm and analysed by HPLC using the conditions reported above for blood analysis. Analytical data are expressed as μg of drug/g of organ. For each time the value of drug concentration reported in the plot is the mean of drug concentration found in three animals.

2.9. In vivo elimination studies

2.9.1. Urine

At predetermined time points, urine samples were treated with CH_3CN at a ratio of 3:2 (v/v). The samples were centrifuged at 4000 rpm for 10 min, the supernatant concentrated to 1 ml by vacuum evaporation and after filtration analysed by HPLC.

2.9.2. Faeces

Faeces were homogenized with a pestle in a volume of water equal to four times the weight of the solid material. After centrifugation at 6000 rpm for 10 min, the supernatant was treated with CH_3CN (CH_3CN/supernatant equal to 3/2) and centrifuged again at 4000 rpm for 5 min. Supernatant was filtered through a 0.25-μm Millipore filter and analysed by HPLC.

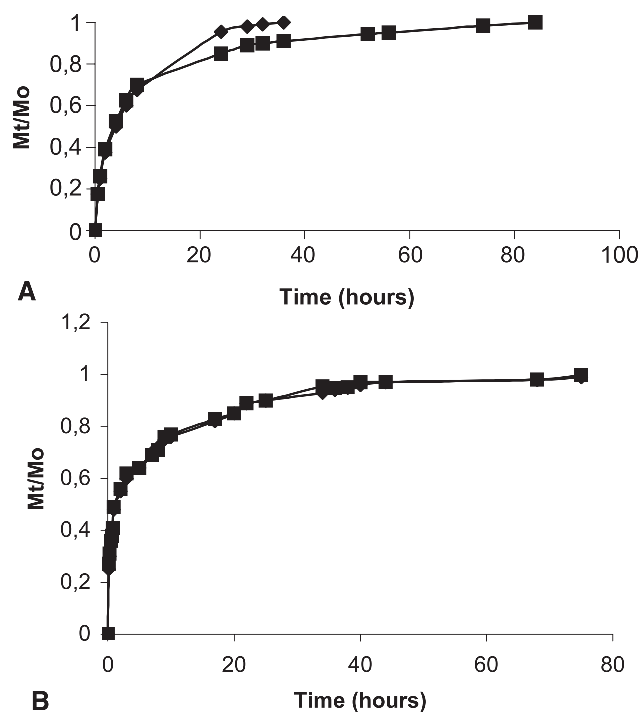

Fig. 1. (a) Oxitetracycline release from 15% (w/v) PVA matrices containing 10 mg of drug. The release evaluation was carried out starting from hydrated (◇) or dried (■) matrices. (b) Oxytetracycline release from 20% (w/v) PVA matrices containing 300 mg of drugs. Hydrated (◇) or dried (■) matrices were used.

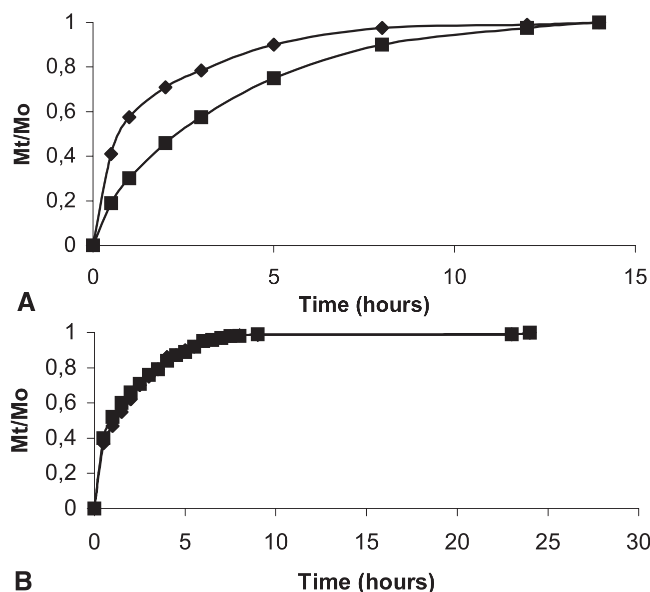

Fig. 2. (a) Tylosin release from 15% (w/v) PVA matrices containing 10 mg of drug. The release evaluation was carried out starting from hydrated (◇) or dried (■) matrices. (b) Tylosin release from 20% (w/v) PVA matrices containing 300 mg of drug. The release evaluation was carried out starting from hydrated (◇) or dried (■) matrices.

3. Results

3.1. In vitro release studies

The rate of drug release from PVA hydrogels of both tylosin and oxytetracycline was studied as a function of loading and of characteristics of starting hydrogel matrices: hydrated vs. dried [14].

It was found that for both drugs the freeze drying procedure of the matrices decreased the drug release rate only in the case of low loading degree (10 mg drug/ml in 15% PVA, w/v, matrices). This effect being much more pronounced for tylosin (see Fig. 1a,b) may suggest interaction of this drug with the matrix. However, in both cases, the release was complete, indicating that non-reversible binding of drugs to PVA matrix did not take place during the freezing and thawing process of gelation. A slow release rate from PVA hydrogels following freeze drying was already observed in our laboratory for incorporation of amino acids as tryptophan or proteins as ribonuclease or superoxide dismutase [14]. This behaviour may be due to a further cross-linking of the gel since lyophilization may represent an additional freezing and thawing step of the cryopolymerization process.

No difference in release rate between the hydrated and the dried matrices was observed (see Fig. 2a,b for OTC and tylosin, respectively) when the matrices were highly loaded with drug (300 mg/ml in 20%, w/v, PVA matrix). It is noteworthy that in this case a higher concentration of PVA, compared to the one necessary for the low loaded matrices, was needed to entrap the drug, otherwise the cross-linking of PVA could not be achieved by the freezing and thawing

procedure. Also, in this case a complete release of the entrapped drug could be achieved. The stability of the dry PVA gel system was evaluated by carrying out the release from high loaded dry matrices after 2 months storage at room temperature. No loss of drug could be revealed under these conditions.

3.2. In vivo investigations

3.2.1. Tylosin

Solutions of tylosin were administered i.p. to rats and the concentration in plasma and organs was evaluated by HPLC after CH_3CN extraction. A rapid decrease of the drug in plasma was found; in fact the drug was below the limit of evaluation, already 2.5 after the administration (see Fig. 3). This result is in agreement with the data reported in literature on tylosin pharmacokinetic and justifies the reported repeated administration for achieving a therapeutic efficacy. In our case a direct HPLC procedure was employed for tylosin evaluation, similar to those reported by other researches [11]. In different investigations, on the contrary, microbiological assays [15], liquid chromatography or liquid chromatography–mass spectroscopy [16] were employed. Furthermore, the drug was undetectable in the three organs that we examined as a reference of body localization: liver, kidneys and muscles.

When tylosin was administered per os as powder, although at a higher amount, the drug was below the limit of evaluation in blood, while it was found in faeces for 48 h and, at low amount, in urine for the first 24 h after administration. Also in this case tylosin was below the limit for detection in the three organs that were examined, liver, kidneys and muscles. These results are in favour of incomplete absorption and rapid elimination of the drug through oral administration and demonstrates the limits of this administration route.

On the other hand, when the same amount of drug (300 mg) was entrapped into PVA hydrogel and administered per os, it reached kidneys and muscles. In any of these three organs a detectable peak of drug was found between 25 and 50 h, to slowly decrease and disappear after 5 d (see Fig. 4a–c). In this case, the drug could not be detected in blood, a result in agreement with its rapid elimination and the fact

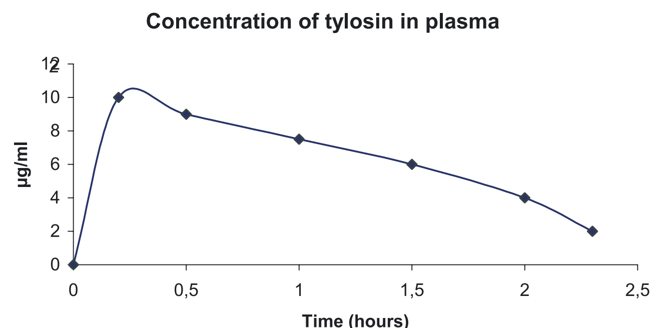

Fig. 3. Tylosin concentration vs. time in plasma when the drug solution was administered i.p. to rats (30 mg in 2 ml). The drug amount was evaluated by HPLC after suitable precipitation and extraction (see experimental section).

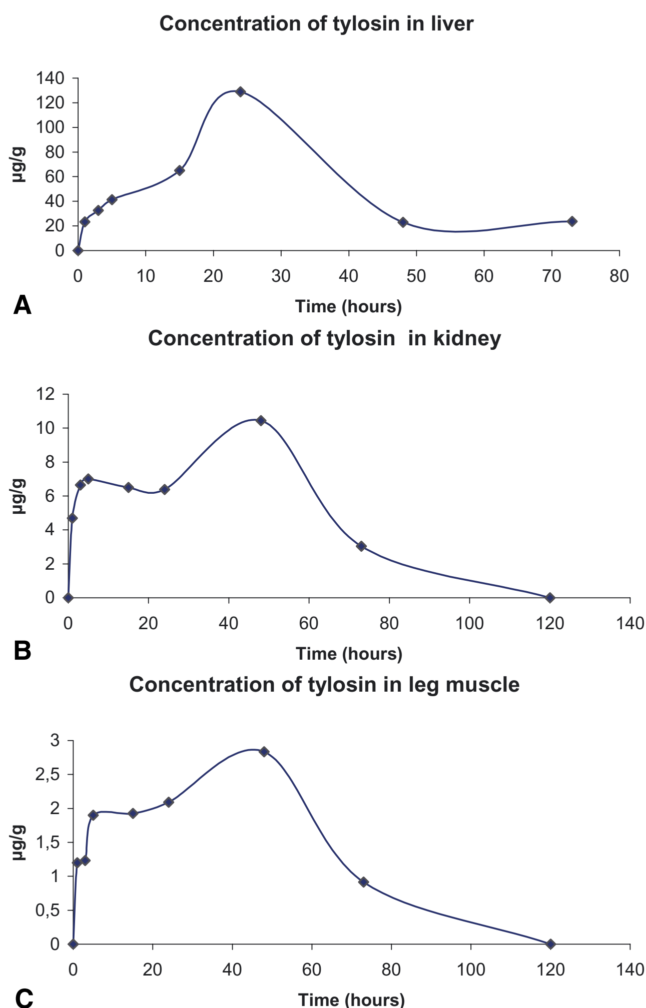

Fig. 4. Tylosin concentration vs. time in organs when the drug was administered into dried hydrogel matrices to rats. Tylosin, 300 mg, was entrapped into 20% (w/v) PVA matrices. The drug was evaluated in: liver (a), kidney (b) and muscle (c). For each time, groups the three animals were employed and the concentration value reported in plot is the mean of separate evaluation of the three (for the analytical details, see experimental section).

that, by the slow release from the gel, it reaches slowly the circulation also. We may therefore conclude that, following this procedure of administration, thanks to the slow and continuous release from the matrix, tylosin could reach and accumulate into organs where it may exploit the therapeutic activity.

3.2.2. Oxytetracyclin

The same in vivo investigation was carried out for oxytetracycline. In this case, when it was administered orally as powder, it could not be detected in blood and also when it was given orally entrapped in PVA matrices, it was not detected in blood and in the organs.

Other authors evaluated the residence time of oxytetracyclin in sheep plasma, but only after repeated subcutaneous administration, or after a single intravenous (i.v.) injections [17]. They could also demonstrate that a prolonged body residence time was achieved following subcutaneus injec-

tions of a special slow release biodegradable formulation and the concentration found in blood was considered suitable for therapy. Low amount was found in kidneys while in liver or other organs the concentration was below the detection limit. It is believable that our difficulty in detecting and evaluating oxytetracyclin is due to the different drug absorption in our administration route (oral, vs. i.p. or i.v.), and to the low accumulation of the drug in the organs.

4. Conclusions

The potential application of PVA, a biocompatible polymer that may yield strong non-covalent cross-linking by repeated freezing and thawing steps, was investigated for a slow release of products of veterinary interest. It was found that two drugs chosen as models, tylosin and oxytetracycline, entrapped in viscous PVA water solution before the cryopolymerization steps, were completely incorporated into the hydrogel and totally released by water incubation.

Hydrogels containing tylosin, an antibiotic largely used in veterinary field, once administered per os to rats allowed to reach organs. The antibiotic could be detected for up to 5 d in kidneys and muscles and in the highest accumulation was found in liver. On the other hand the same amount of drug, administered orally as powder, could not be detected in organs, but it was found mostly in faeces, while a small amount was found in urine also. This is demonstrating the higher bioavailability from the controlled released formulation, paving the way to a more convenient drug administration procedure.

The results were different for oxytetracycline which, on the contrary of tylosin, could not be evaluated in blood or in other organs although it was administered, entrapped in our drug delivery system, at high dose. This may be due to a low oral absorption for one-shot administration used. Other authors in fact could study the pharmacokinetics of this drug, but only after i.p. or i.m. administration.

These results are in favour of a tylosin entrapment into PVA hydrogels for veterinary application, since a convenient targeting and localization into organs may be achieved. Further advantage of this PVA formulation is that the matrices, when maintained in dry state, are very stable over time, allowing the mixing with fodder for animals feeding as needed for an easy administration procedure. The slow release from the hydrogel may therefore reduce the administration problems related to injections and the very large loss of drug commonly observed when the dry powder, the usual form of administration in veterinary field, is employed.

Acknowledgements

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