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The kinetics of pentoxifylline release in vivo from drug-loaded hydroxyapatite implants

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Abstract

Porous hydroxyapatite implants were evaluated as potential skeletal delivery systems for sustained delivery of drugs. Pentoxifylline (PTX) was employed as a model agent and 50 mg of PTX powder was loaded into hollow cylindrical implants. The kinetics of PTX release from the implants was studied both in vitro, employing phosphate buffer (pH 7.35) at the temperature of 37°C, and in vivo, implanting drug-containing cylinders into rabbit iliac bones. For the sake of comparison rabbits were also administered a single i.v. or i.m. dose of 50 mg PTX. Serum PTX concentration levels were measured using the HPLC method. Results of analyses carried out employing the mercury porosimetry method and the hydrostatic weighing method showed that the investigated biomaterial was characterized by a large number of pores ranging in size from 0.4 to 6 μm and with open and total porosity values of 31.8 and 35.6%, respectively. Studies in vitro revealed typical sigmoid-type drug release patterns with a lag time. After 40 h, the amount of released PTX reached a plateau and equaled 78% of the total amount of drug loaded into an implant. Studies in vivo demonstrated that due to decelerated PTX release from cylinders implanted in rabbit iliac bones, its serum concentration values were maintained at measurable levels almost eight times longer than following the systemic administration of PTX. The serum half-life following PTX administration via implants was significantly higher than the value calculated after systemic administration and equaled 6.3 h. The authors also showed that despite differences in the temporal distribution of PTX concentration values, its bioavailability was similar after i.m. injections and administration via implants. As it follows from the investigations, hydroxyapatite implants manifest positive drug-release patterns both in vitro and in vivo.

Keywords: Skeletal drug delivery system; Hydroxyapatite; In vivo drug release

1. Introduction

Hydroxyapatite-based implantation materials have almost the same chemical composition as an inorganic component of human bone, and demonstrate an excellent biocompatibility with hard tissues and reveal a sufficient mechanical strength [1]. Hydroxyapatite bioceramic materials (HAP) are predominantly employed in bone tissue substitution. Due to their microporous structure, these materials can ensure a slow release of the incorporated drug [2, 3]. Implantable dosage forms are gaining tremendous interest in the treatment of bone cancer, chronic osteomyelitis and in delivery of agents with a low bone penetration and short biological half-life [4]. Such types of drug delivery systems are much more successful in providing the needed local concentration of the drug at/or around the site where they exert their therapeutic effect than it is possible to achieve after conventional systemic administration. The concentration depends on the one hand on the ability of a given drug to penetrate through micropores of the material of which an implant is made and on the rate of such penetration, as well as on the pharmacokinetic profile of the drug on the other.

The investigations aimed at assessment of drug release kinetics in hydroxyapatite cylinders loaded with therapeutic agents and implanted into rabbit iliac bones. The evaluation was based on determinations of serum

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drug levels. Pentoxifylline (1-[5-oxohexyl]-3,7-dimethylxanthine), applied in the treatment of cerebral and peripheral microcirculation disorders, [5] was used as a model drug. The in vivo kinetics of pentoxifylline release from the investigated implants was then compared with the same process occurring in vitro and with time-dependent changes in pentoxifylline serum concentration levels following systemic administration of the drug.

2. Materials and methods

2.1. Preparation of hydroxyapatite carriers

Hydroxyapatite (HAP) was synthesized by the aqueous precipitation method from CaO and H_3PO_4 as the reagents. CaO (p.a.) was initially calcined at 900°C. In the process of preparing a $Ca(OH)_2$ suspension, CaO was added to distilled water, which, in turn, was stirred with a propeller-like stirrer; the stirring was continued for 0.5 h after the addition of the entire portion of the powder. 0.5 M $Ca(OH)_2$ suspension in 1000 ml distilled water was used in the synthesis. A solution of 0.3 M H_3PO_4 in 1000 ml distilled water was prepared. The solution was slowly added to $Ca(OH)_2$ suspension at the rate of 17 ml/min. The reaction mixtures were vigorously stirred during the precipitation process, which was performed in the temperature range of 23–25°C. During powder precipitation, the ammonium solution was added in such a way to maintain pH = 11 until the end of the process. The reaction mixture was stirred for 4 h after the acid had been added. After 2 h, checks were made of the pH value to ensure it was maintained at the estimated level. The resulting gelatinous precipitate was aged for 48 h at room temperature, washed, dried at 90°C and subsequently ground in a rotating/vibrating mill. Porous ceramic implants were prepared using non-calcined powder. Samples of a desired shape were isostatically pressed under 350 MPa. Thirty-seven wt.% flour (0.04–0.2 mm grain sizes) served as a pore-creating medium. Sintering was performed in the air at 1250°C, with a 2 h soaking time [6]. The implants were shaped as hollow cylinders of 8.0–9.0 mm height and 7.4 mm in diameter. The hollows (3.9 mm diameter) for drug placement were drilled in the samples using a high precision drill. The cylinder walls were 2.0 ± 0.3 mm thick.

2.2. X-ray diffraction measurement

The X-ray powder diffraction profiles of sintered samples were measured by a powder X-ray PHILIPS diffractometer (Philips Electronic Instruments, Mahwah, NJ). According to XRD studies, all the investigated samples were single-phase hydroxyapatite material, without any admixtures of TCP or CaO.

2.3. Chemical analysis

The calcium/phosphorous molar ratio of the precipitate was measured by the chemical wet method, i.e. by $KMnO_4$ titration for Ca, and phosphomolybdate technique for phosphate. The calcium/phosphorous molar ratio of the precipitate was 1.67, what corresponds to that of stoichiometric hydroxyapatite–$Ca_{10}(PO_4)_6(OH)_2$.

2.4. Porosity measurement

The total and open porosity values of the carriers were estimated by weighing porous blocks. The volume of the sample compared with its weight makes it possible to calculate total porosity. The micropore distribution was measured by means of mercury porosimetry (AUTO-PORE II 9220, Micromeritics Co.).

2.5. Chemicals

All the solvents and reagents were of HPLC grade. Pentoxifylline (PTX) was kindly supplied by Krka (Slovenia), and [7-(2′-chloroethyl)-3,7-dimethyl-xanthine], used as internal standard, was synthesized in the laboratory of the Department of Pharmaceutical Chemistry, Collegium Medicum, Jagiellonian University.

2.6. Drug loading

The implants were filled with 50 mg of PTX each. Subsequently, the hollows into which the drug was loaded were filled up with hydroxyapatite powder (5 ± 0.18 mg) and sealed tightly with wax (Bone Wax, Ethicon). Drug concentration in particular cylinders was similar. In a separate experiment, the wax was confirmed to be impermeable for PTX. The so-called active drug-release surfaces were of the average area of approximately 252 ± 7 mm^2.

Implants for in vivo experiments were loaded with PTX under sterile conditions using a laminar flow chamber. Completed implants were subjected to 2 h of UV irradiation and stored under sterile conditions.

2.7. Drug release — in vitro study

The implants were introduced into 50 ml of phosphate buffer dissolution medium at pH = 7.35, in round-bottomed flasks which were placed in a water bath (Elpan 357) maintained at 37°C and shaken at 60 strokes/minute. In the course of the release test, 3 ml samples were withdrawn by a pipette at suitable intervals and replaced immediately with 3 ml of fresh dissolution medium, which was accounted for when calculating the amount released. PTX concentrations in the collected samples were measured spectrophotometrically (Helios Beta UV-VIS Spectrophotometer, UNICAM) at a

wavelength of 274 nm. The data represent the average of two measurements from independent experiments.

2.8. Drug release — in vivo study

The experiments were carried out in three New Zealand rabbits with body weight of 2.5±0.3 kg. The study was approved by the Ethics Committee, Collegium Medicum, Jagiellonian University.

Prior to attempting evaluation of the kinetics of PTX release from implants in vivo, the rabbits were administered single intravenous and intramuscular injections of PTX at the dose of 50 mg, with two-week time interval between injections. Blood samples for analysis (1 ml) were drawn from the auricular marginal vein at various time intervals after PTX administration. After centrifuging, the samples were stored at −20°C until further analysis. Two weeks later, cylindrical carriers loaded with 50 mg PTX were implanted under general anesthesia induced by i.v. Vetbutanol injections into the iliac ala of the same rabbits. Each procedure of carrier implantation took approximately 30 min. In the initial two postoperative days, the animals received analgesic agents. Blood samples were collected from the auricular marginal vein every 4 or 12 h for 3 postoperative days. The samples were centrifuged and stored as described above.

2.9. Serum drug assay

Serum concentrations of PTX were measured by a high-performance liquid chromatography (HPLC) with solid-phase extraction (SPE) [7]. For the sample preparation a 0.2 ml volume of each serum sample was loaded into SPE cartridges containing reversed-phase octadecylsilane (C_{18}) bonded to silica sorbent, 100 mg in 1-ml tube (J.T. Baker, Philipsburg, USA).The high-performance liquid chromatograph (Spectra-Physic, San Jose, USA) consisted of an isocratic solvent delivery system (IsoChrom LC Pump), an injector (Rheodyne Model 7125) equipped with a 20-μl loop, and a variable-wavelength UV detector (Model Spectra 100) set at 275 nm. The analytical column was a 125×4 mm i.d. reversed-phase LiChrospher 100RP — 18.5 μm particle size (Merck, Darmstadt, Germany) at ambient temperature. The mobile phase was water–dioxan–acetonitrile (87:6.5:6.5 v/v/v), acidified to pH = 3 with glacial acetic acid (0.6% v/v), vacuum-degassed before use, and pumped at a flow rate of 1.2 ml min^{-1}. The limit of detection with this assay was 20 ng ml^{-1} and the recovery — 93.5 and 93.3% for pentoxifylline and internal standard, respectively . The accuracy and the precision of the method were expressed by the coefficient of variation values, which were less than 10%. No interference between chromatograms of the analyzed compound and employed analgesic drugs were observed.

2.10. Calculation and statistical analysis

The concentrations of PTX in blood serum were calculated from the respective regression equation relating peak-area ratios (PTX standard vs. internal standard) to its concentrations. Pharmacokinetic parameters of PTX, such as: serum half-life ($t_{0.5}$), total area under the serum concentration-time curve (AUC) and bioavailability (F) were calculated employing appropriate equations [8] using the WinNonlin software.

The pharmacokinetic parameters obtained after systemic or implantable dosage form of PTX were compared by Student's t test for paired data (the Statistica software) and the significant differences were established at $P < 0.05$.

3. Results and discussion

Local delivery application is one way of targeting a drug to a desired site. However, to prolong the therapeutic level of drug at the site, a local sustained release device is required. In bone tissue, sustained drug release can be achieved using hydroxyapatite implants loaded with given drugs [9,10]. These porous ceramic samples that fulfill a double role of a carrier and filling material for a bone deficit constituted the object of present experiments.

The investigated hydroxyapatite drug carries showed open and total porosity of 31.8 and 35.6%, respectively. In the drug delivery system, a very important parameter is size distribution of micropores, which in the case of our material was in the range of 0.4–6.0 μm.

As it follows from in vitro studies and as reported by other authors [11,12], the curve which described the amount of released PTX over time had a typically sigmoid-like pattern with a lag time of approximately 3 h. After 40 h, the amount of released PTX reached a plateau and equaled 78% of the total amount of drug loaded into an implant (Fig. 1).

As it can be seen in the graph presented in Fig. 2, the rate of PTX release in vitro increased with time, reaching the peak value of 1 mg/h after 25 h. The rate was maintained at the steady level between 25 and 40 h after the beginning of the experiment and then decreased to approximately 0.4 mg/h after 110 h.

Fig. 3 illustrates changes in serum pentoxifylline concentrations following single intravenous or intramuscular injections of 50 mg PTX. The curve describing these changes shows that the initial blood PTX level was high, amounting to 25 μg/ml, but its value was practically undetectable 6–8 h following an i.v. or i.m. injection. This resulted from the fast elimination of PTX, what is supported by its low serum half-life of 2.1 and 1.2 h in the case of intravenous and intramuscular administration, respectively (Table 1).

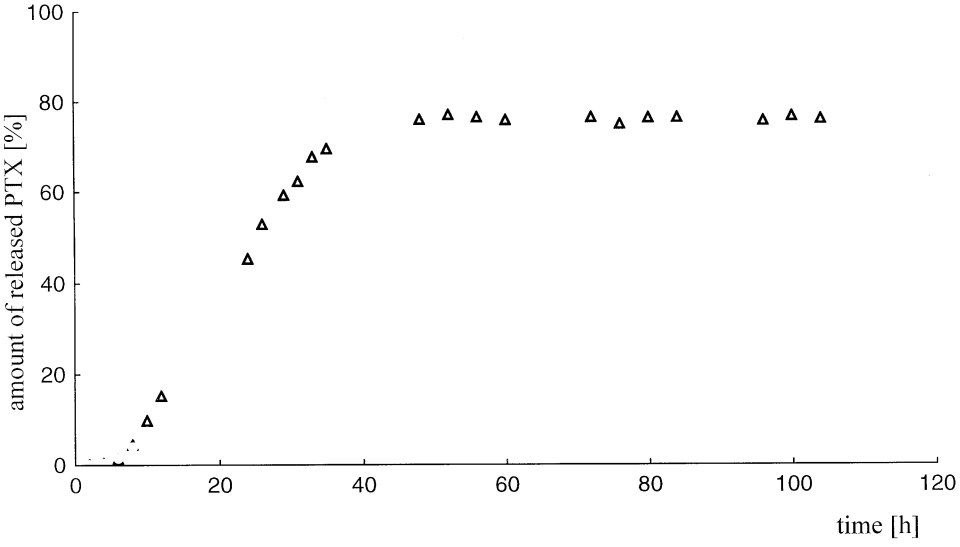

Fig. 1. In vitro drug-release profile of PTX-loaded HAP carriers ($n=2$).

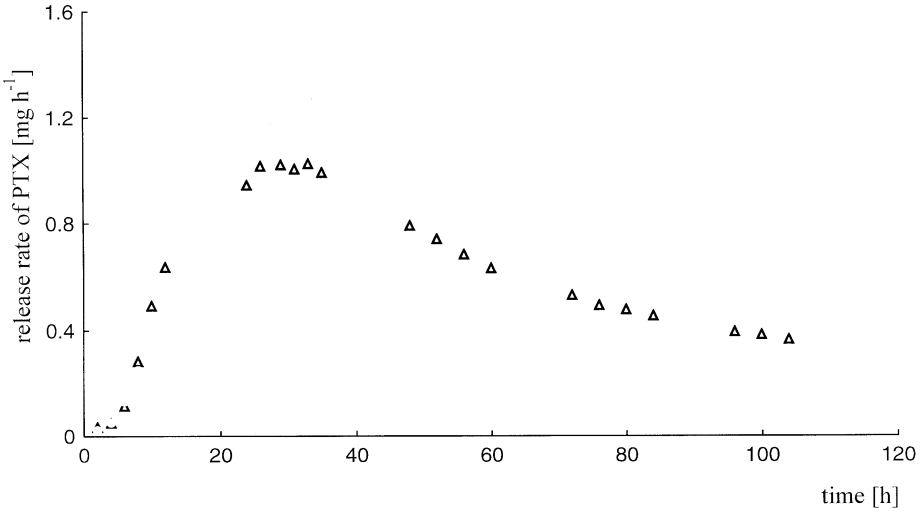

Fig. 2. In vitro drug-release rate profile of PTX-loaded carriers ($n=2$).

Table 1
Pharmacokinetic parameters of PTX after different routes of administration in rabbits[a]

Parameter	i.v.	i.m.	Implant
$t_{0.5}$ (h)	2.1±0.3	1.2±0.5	6.3±0.8
AUC (μg h/ml)	21.96±1.38	11.61±1.80	8.00±0.75
F (%)[b]	100	53.23±11.43	36.40±2.88

[a] Data expressed as mean±S.D. ($n=3$).
[b] F values after i.m. or implants administration were calculated as a ratio $AUC_{i.m.}/AUC_{i.v.}$ or $AUC_{implant}/AUC_{i.v.}$ respectively.

As it is shown in Fig. 4, the placement of PTX within HAP carriers that were subsequently implanted into rabbit iliac bones resulted in blood PTX concentrations reaching peak values approximately 25 h following the procedure. As seen in Fig. 2, in vitro this corresponded to the period of maximum drug release rate. Despite low PTX concentrations, but thanks to the process occurring over a prolonged period, the total area contained below the concentration-time curve plotted for PTX administration via implants was demonstrated to be similar to the area determined following intramuscular PTX administration (Table 1). Therefore, the absolute bioavailability (F) of PTX administered via i.m. injections or implants and calculated based on the above data were similar ($P>0.05$).

A limited number of experimental data necessitates caution in concluding that PTX administration via hydroxyapatite carriers changes the pharmacokinetic profile of the drug in comparison to data obtained after a single intramuscular injection, but no differences are observed in PTX systemic bioavailability. Moreover, the altered pharmacokinetic profile of PTX achieved in

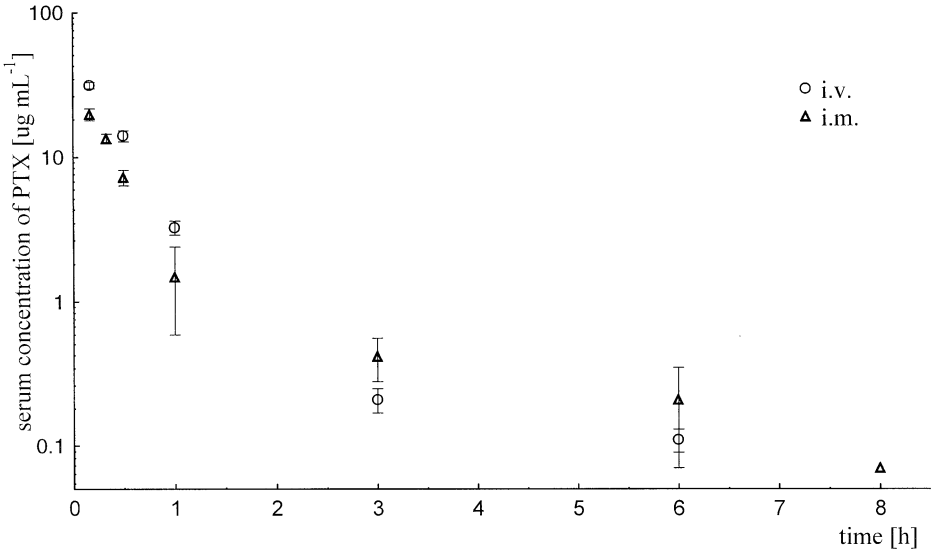

Fig. 3. Mean serum concentration-time profiles of PTX after injecting 3 rabbits with a single 50 mg i.v. or i.m. dose.

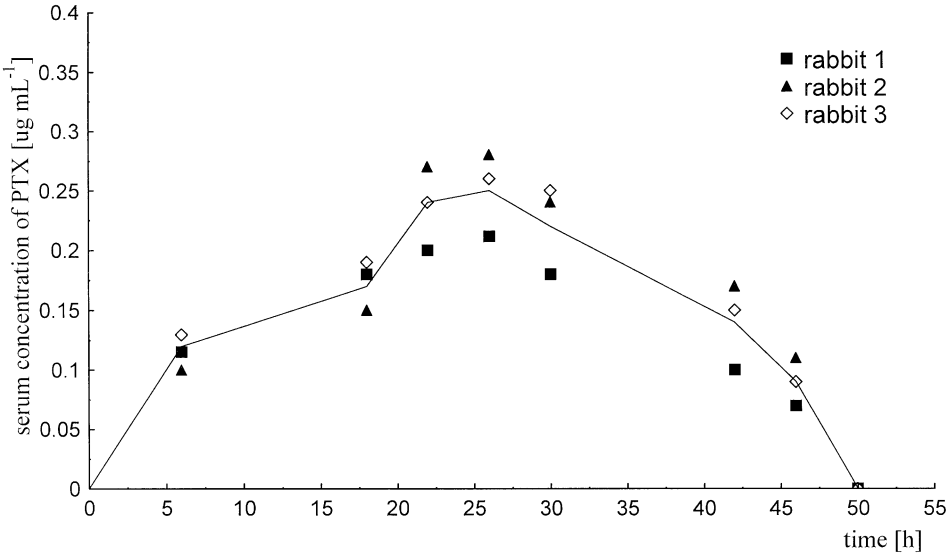

Fig. 4. Serum concentration-time profiles of PTX after the implantation of 50 mg PTX-loaded HAP carriers into rabbit iliac bones.

our studies may be beneficial from the clinical point of view in view of the possibility of decreasing the frequency of such drug administration and achieving appropriate local concentration of the drug. To confirm the above presented suggestion it is necessary to analyze the drug concentrations at the immediate site following both its systemic administration and delivery via surgically implanted HAP carriers.

4. Conclusions

The experiments have demonstrated that porous bioceramic hydroxyapatite implants are characterized by a beneficial drug release profile both in vitro and in vivo. For this very reason, hydroxyapatite may find a potential use as a novel skeletal drug delivery system. This type of drug administration prolongs the time of drug disposition processes, and thus it prolongs the exposure to drug activity at the site at which it exerts its therapeutic result. At the same time, low serum concentrations observed following drug administration via implants might be of special importance in the case of agents with high systemic toxicity. Moreover, employing the methods of pharmacokinetic studies, the authors have demonstrated that despite an altered profile of changes in PTX concentrations over time, the absolute bioavailability of PTX administered via implants did not significantly differ from the value of this parameter calculated for intramuscular administration.

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References

[1] R.Z. LeGeros, Calcium Phosphates in Oral Biology and Medicine, Karger, New York, 1991.
[2] M. Otsuka, Y. Matsuda, Y. Suwa, J.L. Fox, W.I. Higuchi, A novel skeletal drug delivery system using a self-setting calcium phosphate cement. 5. Drug release behavior from a heterogeneous drug-loaded cement containing an anticancer drug, J. Pharm. Sci. 83 (11) (1994) 1565–1568.
[3] M. Otsuka, Y. Nakahigashi, Y. Matsuda, J.L. Fox, W.I. Higuchi, In vitro drug release behaviors from self-setting calcium phosphate cement loaded on bovine bone, in: Proceedings of Second International Symposium on Apatite, Japanese Association of Apatite Science, June 1997.
[4] A.K. Dash, G.C. Cudworth, II. Therapeutic applications of implantable drug delivery systems, J. Pharm. Tox. Methods 41 (1998) 1–12.
[5] A. Ward, S. Clissold, Pentoxifylline. A review of its pharmacodynamic and pharmacokinetic properties and its therapeutic efficacy, Drugs 34 (1987) 50–97.
[6] A. Ślósarczyk, E. Stobierska, Z. Paszkiewicz, Porous hydroxyapatite ceramics, J. Mater. Sci. Lett. 8 (1999) 1163–1165.
[7] J. Szymura-Oleksiak, J. Bury, R. Lauterbach, M. Pawlowski, Serum concentrations of pentoxifylline and its metabolites in premature infants with sepsis when administered by continuous intravenous infusion, Pharm. Sci. 3 (1997) 367–371.
[8] L. Shargel, A.B.C. Yu, Applied Biopharmaceutics and Pharmacokinetics, Appleton & Lange, Stamford, CT, 1993.
[9] Y. Shinto, A. Uchida, F. Korkusuz, N. Araki, K. Ono, Calcium hydroxyapatite ceramic used as a delivery system for antibiotics, J. Bone Joint Surg. 74-B (1992) 600–604.
[10] A.K. Dash, R. Suryanarayanan, An implantable dosage form the treatment of bone infections, Pharm. Res. 9 (1992) 993–1002.
[11] M. Otsuka, Y. Matsuda, Y. Suwa, J.L. Fox, W.I. Higuchi, A novel skeletal drug-delivery system using self-setting calcium phosphate cement. 4. Effects of the mixing solution volume on the drug-release rate of heterogeneous aspirin-loaded cement, J. Pharm. Sci. 83 (2) (1994) 259–263.
[12] M. Otsuka, Y. Matsuda, T. Kokubo, S. Yoshihara, T. Nakamura, T. Yamamuro, A novel skeletal drug delivery system using self-setting bioactive glass bone cement. III: the in vitro drug release from bone cement containing indomethacin and its physicochemical properties, J. Controlled Release 31 (1994) 111–119.