IRIS AperTO

UNIVERSITÀ DEGLI STUDI DI TORINO

AperTO - Archivio Istituzionale Open Access dell'Università di Torino

Pentamidine-Loaded Lipid and Polymer Nanocarriers as Tunable Anticancer Drug Delivery Systems

This is a pre print version of the following article:

Original Citation:

Availability:

This version is available http://hdl.handle.net/2318/1765468 since 2020-12-30T16:04:50Z

Published version:

DOI:10.1016/j.xphs.2019.11.011

Terms of use:

Open Access

Anyone can freely access the full text of works made available as "Open Access". Works made available under a Creative Commons license can be used according to the terms and conditions of said license. Use of all other works requires consent of the right holder (author or publisher) if not exempted from copyright protection by the applicable law.

(Article begins on next page)

10 July 2024

Pentamidine-Loaded Lipid and Polymer Nanocarriers as Tunable Anticancer Drug Delivery Systems

Barbara Stella [1,*], Ilaria Andreana [1], Daniele Zonari [1], Silvia Arpicco [1,*]

[1] Dipartimento di Scienza e Tecnologia del Farmaco, Università degli Studi di Torino, Via P. Giuria 9, 10125 Torino, Italy

* *Correspondence to:*

Barbara Stella (Telephone: +39-011-6706660). *Postal address*: Dipartimento di Scienza e Tecnologia del Farmaco, Università degli Studi di Torino, Via P. Giuria 9, 10125 Torino, Italy. *E-mail address*: barbara.stella@unito.it.

Silvia Arpicco (Telephone: +39-011-6706668). *Postal address*: Dipartimento di Scienza e Tecnologia del Farmaco, Università degli Studi di Torino, Via P. Giuria 9, 10125 Torino, Italy. *E-mail address*: silvia.arpicco@unito.it.

ABSTRACT

Initially developed as a synthetic analogue of insulin, pentamidine (PTM) is an antimicrobial drug that has recently shown *in vitro* and *in vivo* anticancer activity. Nevertheless, systemic administration of PTM causes severe side effects, especially nephrotoxicity. Here we propose the association of PTM to different biocompatible nanosystems in order to compare the physico-chemical characteristics of the loaded nanocarriers and their influence on the drug cytotoxicity towards cancer cells. In particular, PTM (as free base or with different counterions) was encapsulated into liposomes and poly(lactide-*co*-glycolide) (PLGA) nanoparticles and all the formulations have been deeply characterized concerning mean diameter, polydispersity index, zeta potential, stability, morphology, PTM loading and drug release profile. The anticancer activity was evaluated on a human ovarian cancer cell line over 72 h. Results showed that PTM is efficiently loaded into liposomes with a transmembrane citrate- or sulfate-gradient; concerning PLGA nanoparticles, important association occurred thanks to ionic interactions between the drug and the polymer. The *in vitro* studies confirmed the anticancer activity of PTM, which was gradually released with different profiles depending on the drug form and the nanocarrier structure.

*Keywords***:** liposomes, nanoparticles, formulation, drug delivery systems, controlled release.

Introduction

Pentamidine (PTM), a diamide composed of two phenyl amidine groups linked by a five-carbon methylene linker, is an FDA-approved drug for the treatment of different parasitic infections, including leishmaniasis, *Pneumocystis carinii* pneumonia, and trypanosomiasis.[1,2] While much is still elusive about its antiparasitic mechanism, some studies have demonstrated its efficacy in cancer. PTM has shown to be a DNA minor groove binder forming noncovalent complexes with DNA.[3] Pathak *et al*. demonstrated for the first time the *in vitro* anticancer potential of PTM showing its growth-inhibitory activity when it links to the phosphatase regenerating liver (PRL) family, a group of oncogenic phosphatases overexpressed in tumor cells; these results were further confirmed in a study in which PTM was combined with chlorpromazine.[4,5] Moreover, PTM is an inhibitor of the DNA endo-exonuclease whose repair processes have a main role in tumor cells. A reduced capacity in DNA repair processes has been reported to take part in cancer cell viability. The action of PTM with the endo-exonuclease may be due to the similar structure of the active site between the endo-exonuclease and PRL family.[6] Other studies proved a different anticancer mechanism of PTM, in particular in melanoma, for which p53 binding site on overexpressed S100B protein has been investigated as a target: PTM inhibits the $Ca(^{+})$-binding protein S100B and disrupts the S100B-p53 protein-protein interaction, thus restoring wild-type p53 tumor suppressor function in melanoma.[7] Moreover, PTM was found to be active against other cancer models, such as prostate, breast, leukemia and renal cancers.[8-10]

However, PTM has a poor oral bioavailability and pharmacokinetics studies after PTM administration by the intravenous route showed interindividual differences in rates of metabolism;

moreover, the renal drug clearance was associated to acute kidney injury.[11,12] Thus, several drug delivery strategies have been developed to overcome adverse PTM pharmacokinetics and toxicity, such as by synthesizing PTM bioconjugates[13] or through the association to nanocarriers, but mainly in the treatment of leishmaniasis. In this field, among the different drug delivery nanosystems, liposomes and nanoparticles have been proposed to carry PTM[14,15] and even a recent study using both polycaprolactone nanoparticles and phosphatidylcholine liposomes was performed to compare the *in vitro* transportation of PTM across the blood-brain barrier.[16] On the contrary, only a minor effort has been devoted to the use of nanocarriers to deliver PTM in anticancer therapy. Concerning liposomes in this field, Mérian *et al*. tested some liposomal PTM formulations in order to enhance tumor drug accumulation and lower drug exposure to other tissues. Liposomes were prepared with saturated/unsaturated phospholipids of different chain lengths, tuning cholesterol content, and with or without the presence of PEG on the outer surface in order to optimize the physico-chemical characteristics of liposomes. In tumor-bearing mice, liposomal delivery decreased PTM kidney drug levels and increased tumor drug exposure compared to free drug.[17]

Regarding the use of non-liposomal nanocarriers with PTM in anticancer therapy, PTM was associated to PEG-stabilized gold nanoparticles in order to enhance the effect of radiotherapy on triple negative breast cancer. The results showed that the adsorption of PTM onto the PEG-gold nanoparticles surface increased their cellular uptake. In addition, the combination resulted in a significantly greater number of residual DNA double-strand breaks compared to that of single agents.[18] Moreover, we have recently proposed functionalized mesoporous silica nanoparticles for PTM delivery. Results showed that the tuning of the nanocarrier characteristics may greatly affect the efficacy of PTM encapsulation.[19] Nevertheless, despite the growing interest in the applications

of gold and silica nanoparticles in drug delivery technologies, the long term effects of inorganic materials for nanoparticle preparation is still an open issue.[20]

Besides to the reported studies, to the best of our knowledge, a comparative study between PTM-loaded liposomes and polymer nanoparticles for anticancer therapy has not been performed yet. To this aim, here we describe the preparation and the characterization of different PTM-loaded non-toxic, biocompatible and biodegradable nanocarriers (*i.e.*, liposomes and poly(lactide-*co*-glycolide) (PLGA) nanoparticles). PLGA is an attractive material for biomedical uses because it is approved for medical application by both FDA and EMA and is already widely employed in the biomedical field.[21,22] The cytotoxicity of PTM-loaded nanocarriers was evaluated on the A2780 human ovarian carcinoma cell line. Results showed that the encapsulation of different forms of PTM into lipid or polymer nanosystems allowed the drug release (and, thus, the cytotoxicity) to be tuned and controlled.

Materials and Methods

Materials

All the phospholipids, cholesterol (CHOL), Sepharose CL-4B, PLGA 50:50 (Resomer® RG 502 H), PLGA 75:25 (Resomer® RG 752 H), PEG_{2000}-PLGA 50:50, PTM isethionate, and solvents (analytical grade) were purchased from Sigma-Aldrich (Milan, Italy).

Methods

Preparation of Liposomes and Polymer Nanoparticles Containing PTM

Preparation of Different PTM Forms

Free base form of PTM (PTM-B) was obtained by precipitation of PTM isethionate (PTM-I) solution in alkaline medium and characterized as previously reported.[19] PTM citrate (PTM-C) and PTM sulfate (PTM-S) were obtained by adding dropwise a solution of citrate buffer (150 mM, pH 4.5) or of ammonium sulfate (150 mM, pH 4.0), respectively, to a MilliQ® water solution of PTM-I (100 mg/mL) until the formation of a precipitate. The solutions were then filtered and the resulting precipitates were washed three times with MilliQ® water and dried under vacuum. Melting points of PTM-C and PTM-S were determined using a BUCHI Melting Point B-450 (set point: 165 °C, heating rate: 2 °C/min) and were 207 °C and more than 300 °C, respectively. The conversion of PTM-I into the desired PTM forms was confirmed by mass spectrometry analysis using electrospray ionization (ESI) or by atmospheric pressure chemical ionization (APCI), in positive ion mode, on a Micromass ZQ spectrometer (Waters, Milan, Italy).

PTM-B-Containing Liposomes

PTM-B-containing liposomes (Lipo PTM-B) were prepared using the thin lipid film-hydration method mixing together 1,2-distearoil-*sn*-glycero-3-phosphocoline (DSPC), CHOL and 1,2-distearoyl-*sn*-glycero-3-phosphoethanolamine-N-[amino(polyethylene glycol)-2000] (mPEG2000-DSPE) in 82:12:6 molar ratio. PTM-B was added to the lipid mixture in 40% ratio (mol drug/mol lipid). Lipids were dissolved in chloroform and evaporated by rotary evaporator. The resulting lipid film was hydrated with 900 µL of HEPES buffer pH 7.4. The suspension was vortex mixed for 10 min and bath sonicated. The formulations were extruded (Extruder, Lipex, Vancouver, Canada) at 60 °C

under nitrogen through 200 nm polycarbonate membrane (Costar, Corning Incorporated, NY) and then purified by gel filtration using Sepharose CL-4B columns, eluting with HEPES buffer. Liposomes were stored at 4 °C.

PTM-C and PTM-S-Containing Liposomes

The liposomes (Lipo PTM-C and Lipo PTM-S) were composed of DSPC, CHOL, and mPEG2000-DSPE in 75:20:5 molar ratio. The lipid film was hydrated with 1 mL of citrate buffer for Lipo PTM-C or ammonium sulfate solution for Lipo PTM-S, vortex mixed and incubated for 45 min at 60 °C. Liposomes were then extruded as previously reported and passed through a Sepharose CL-4B column equilibrated with HEPES buffer to replace the extra-liposomal solution. Subsequently, a solution of PTM-I in HEPES buffer (0.75 mg/50 µL for Lipo PTM-C and 0.5 mg/100 µL for Lipo PTM-S) was added to liposome suspension and incubated for 60 min at 60 °C. Finally, liposomal preparations were purified by gel filtration as previously reported and stored at 4 °C.

PTM-B-Containing PLGA Nanoparticles

PTM-B-loaded PLGA nanoparticles were prepared by the nanoprecipitation technique.[23] Practically, for each preparation, to 12 mg of PLGA 50:50 or 75:25 dissolved in acetone, an aliquot of an ethanolic stock solution of PTM-B (5 mg/mL) was added until a total volume of 1 mL. This organic solution was then poured into 2 mL of MilliQ® water under magnetic stirring. Precipitation of nanoparticles occurred spontaneously. After solvent evaporation under reduced pressure, an aqueous suspension was obtained. Further nanoparticle batches were prepared by 1:1 or 1:0.5 (w:w) polymeric blends between PLGA (50:50 or 75:25) and PEG_{2000}-PLGA 50:50[24] or by PEG_{2000}-

PLGA 50:50 alone. To purify the nanoparticles from non-incorporated drug, PTM-B-loaded nanoparticles were extensively dialyzed against MilliQ® water at 4 °C (Spectra/Por® 3500 MWCO dialysis membrane, Spectrum, Huston, TX). Unloaded PLGA nanoparticles (*i.e.*, without adding PTM-B) were prepared as well. The particles were then stored at 4 °C.

Characterization of PTM-Containing Liposomes and Nanoparticles

The mean particle size and the polydispersity index of liposomes and nanoparticles were determined at 25 °C by quasi-elastic light scattering (QELS) using a nanosizer (Nanosizer Nano Z, Malvern Inst., Malvern, UK). The selected angle was 90° and the measurement was made after dilution of the particulate suspensions in MilliQ® water. The surface charge of liposomes and nanoparticles was evaluated by zeta potential measurements after dilution of the suspensions in 10 mM KCl using the Nanosizer Nano Z. Each measurement was carried out in triplicate.

The amount of PTM incorporated into liposomes and nanoparticles was determined spectrophotometrically (DU 730 UV-vis spectrophotometer, Beckman Coulter, Brea, CA) at 264 nm (PTM-B) or 267 nm (PTM-C and PTM-S). Each sample was analyzed in triplicate. To this aim, 100 μL of liposomal suspensions were diluted in 400 μL of ethanol, vortexed and centrifuged for 5 min at 6,000 rpm and the supernatants were analyzed spectrophotometrically. Phospholipid phosphorous was assessed in each liposome preparation by phosphate assay after destruction with perchloric acid.[25] For PLGA nanoparticles, the formulations were lyophilized and then 4-mg samples were dissolved in 200 μL of dichloromethane. To the solution, methanol (2 mL) was added followed by centrifugation (6,000 rpm for 15 min) to completely separate the precipitated polymer.[26] The

supernatants were then measured at 264 nm. The concentration of the polymer in the suspension was based on dry weight analysis.

To evaluate the PTM release from the suspensions, the formulations were incubated at pH 7.4 and 37 °C in buffer (HEPES 20 mM for liposomes and PBS 10 mM for nanoparticles) for various periods of time; drug leakage was determined after purification of liposomes by chromatography on Sepharose CL-4B columns, eluting with HEPES buffer, or PLGA nanoparticles by dialysis against MilliQ® water. Then, the drug content was measured as previously described and compared with initial values.

The physical stability of liposome and nanoparticle suspensions in the storage conditions (at 4 °C) was determined by evaluating at different interval times the mean diameter, the zeta potential and the drug leakage.

The nanocarrier morphology was determined by cryogenic-transmission electron microscopy (cryo-TEM) analysis. The diluted samples were dropped onto 300 Mesh holey carbon films (Quantifoil R2/1) and quench-frozen in liquid ethane using a cryo-plunge workstation (made at LPS Orsay). The specimens were then mounted on a precooled Gatan 62 specimen holder, transferred in the microscope (Philips CM120) and observed at an accelerating voltage of 120 kV (Centre Technologique des Microstructures (CTµ), platform of the Université Claude Bernard Lyon 1, Villeurbanne, France).

In Vitro Cell Studies

Tumor Cell Line Culture

A2780 (human ovarian carcinoma) cells were cultured in RPMI 1640 medium containing 10% fetal bovine serum (FBS), 0.03% of L-glutamine, 2% penicillin and streptomycin, and 25 μg/mL of gentamicin sulfate. Cells were maintained in a humidified incubator at 37 °C in 5% CO_2.

Cytotoxicity

A2780 cells were seeded at 1×10^4 cells/well in 96 wells microtiter plates and incubated overnight to allow cellular adhesion. Various dilutions of PTM-loaded liposomes and selected PLGA nanoparticles were added to the cells in the culture medium in triplicate, and incubated for 24, 48 and 72 h. Empty liposomes and nanoparticles, as well as free PTM-B, PTM-C, and PTM-S, were also tested. Cell growth inhibition was evaluated by sulforhodamine B (SRB) colorimetric proliferation assay, modified by Vichai and Kirtikara.[27]

Results and Discussion

Preparation and Characterization of PTM-Containing Liposomes and Nanoparticles

Different forms of PTM (as free base and citrate and sulfate salts) were obtained and incorporated into lipid or polymer nanocarriers to obtain non-toxic, biocompatible and biodegradable formulations of PTM with favorable characteristics if compared with previously reported nanosystems. In particular, the liposomal formulations described in the literature were obtained using PTM-I and were characterized by a quite low amount of encapsulated drug.[17,28] With the aim to improve the amount of entrapped drug, initially in this work the lipophilic derivative PTM-B was used and nine different liposomal formulations were prepared changing the phospholipidic mixture composition

and the amount of PTM-B added in the lipid film. A liposomal formulation (Lipo PTM-B) characterized by an encapsulation efficiency (EE%) value of 20%, higher than that found in the literature, was obtained. However, to further improve the drug loading into the liposomes, the active drug encapsulation technique was exploited *via* citrate and ammonium sulphate method to prepare Lipo PTM-C and Lipo PTM-S, respectively.[29] PTM-C and PTM-S were also prepared to confirm their low water solubility (that was reported to be 0.6 and 1.4 mg/mL, respectively[30]) and to evaluate their UV spectra useful for the quantification of the drug loaded in liposomes. The EE% was around 30% for Lipo PTM-C and around 45% for Lipo PTM-S, confirming that this encapsulation method allows the amount of drug loaded into the liposomes to be increased. These two formulations were then used for further *in vitro* tests and showed similar mean diameter and zeta potential values (Tab. 1).

PTM-B was also incorporated into PLGA nanoparticles, which were prepared in a single step by nanoprecipitation. Even if PTM was previously associated to poly(D,L-lactide) (PLA) nanoparticles[31] and polymethacrylate nanoparticles[32] for anti-*Leishmania* treatment, here we obtained monodispersed PTM-loaded PLGA nanoparticles without adding any surface-active agent and with high EE% values (95% and 83% for PLGA 50:50 and 75:25, respectively, for a maximum of 30 μg of drug/mg of polymer). These results can be ascribed to the molecular structure of PTM and PLGA: indeed, at physiological pH 7.4 PTM (Fig. 1) has two positively charged amidines (pKa=12), while PLGA has negatively charged carboxylic groups. Thus, PTM and PLGA in the nanoparticles interact and stabilize each other by ionic interactions; this phenomenon probably also leads to a decrease in the mean diameter of loaded PLGA nanoparticles if compared with empty carriers (Tab. 1). Moreover, as previously demonstrated,[32] in this condition PTM release is pH-dependent: at acidic pH values, such as in lysosomes, PTM can be released from the polymer to reach its target.

As reported for PLGA nanoparticles prepared for active targeting of African trypanosomiasis,[33] we also obtained PEGylated PLGA nanoparticles; nevertheless, we did not covalently attach PEG to PLGA, but we co-nanoprecipitated two PLGA-based polymers (PLGA and PEG_{2000}-PLGA), as previously described.[24] When PEG_{2000}-PLGA was added to the formulations at 1:1 ratio, the mean diameter was lower than 100 nm, while it was higher at 1:0.5 ratio (Tab. 1). Concerning PTM content, while the EE% for PEG_{2000}-PLGA alone was very low (28%), for the polymeric blends PLGA/PEG_{2000}-PLGA it was only slightly lower (88% and 79% for 1:0.5 and 1:1 ratio, respectively) than that of non-PEGylated PLGA nanoparticles. The PLGA nanoparticle zeta potential was negative for all the formulations; however, the incorporation of PTM increased the values in all loaded nanoparticles (Tab. 1).

After 4-weeks storage at 4 °C, all the formulations still conserved at least 90-95% of the initial PTM content and over this period no appreciable size and/or zeta potential change and no precipitation or aggregation were observed, except for non-PEGylated PLGA nanoparticles, whose diameter showed a tendency to increase after 4 weeks until 200-230 nm.

The drug release profile of liposomes in HEPES buffer at 37 °C showed the good stability of the formulations: it was observed that after 24 h only 7% of the less water soluble PTM-C and 10% of PTM-S were released; after 72 h these values reached 14% for PTM-C and 30% for PTM-S. These results confirm that the precipitation of the drug in the aqueous core of liposomes allows to obtain an important controlled drug release, as previously observed for Doxil®, the commercial liposomal formulation of doxorubicin, in which the drug release was around 5% in buffer at 37 °C after 24 h.[34]

For PLGA nanoparticles, the PTM-B release was faster, as a consequence of the higher water solubility of PTM-B compared to that of PTM-C and PTM-S: after 24 h the 70% and 63% of the

initial amount of PTM-B was released from PLGA 50:50 and PLGA 75:25 nanoparticles, respectively, until about 90% after 72 h. PEGylated PLGA nanoparticles showed a similar release profile, with about 75% after 24 h and 100% after 72 h.

For PLGA nanoparticles, the cryo-TEM microscopy analysis confirmed the diameter found with QELS (Fig. 2); moreover, the observed rounded shape was similar for both empty and PTM-loaded nanoparticles (PEGylated or uncoated). For liposomes, the analysis was applied to assess the physical state of PTM inside the vesicles; to this aim, empty and drug-loaded liposomes were analyzed and the resulting images have been summarized in Figure 3. PTM precipitation was observed inside the liposomes prepared either *via* citrate buffer method or sulfate gradient.

Cytotoxicity

The cytotoxic activity of the different forms of PTM and the loaded nanocarriers was tested on A2780 (human ovarian carcinoma) cells at different incubation times (24, 48, and 72 h). Interestingly, to the best of our knowledge, this study reports for the first time an *in vitro* anticancer activity evaluation of PTM encapsulated in liposomes and polymer nanoparticles. Results reported in Table 2 show that the different forms of PTM (*i.e.*, PTM-B, PTM-C, and PTM-S) have similar cytotoxicity profiles. Once encapsulated into the nanocarriers (both liposomes and nanoparticles), PTM maintains its anticancer activity, even if it is delayed due to the gradual release from the nanosystems, which show different cytotoxicity profiles. In particular, loaded PLGA nanoparticle cytotoxicity is higher than that of liposomes; this can be due to the kinetics of matrix degradation that influences the drug release, but also to the higher water solubility of PTM-B (29.2 mg/L at 25 °C) than that of PTM-C and PTM-S (49-fold and 21-fold higher, respectively). Moreover, in PLGA

nanoparticles PTM is dispersed within the polymer matrix, while in liposomes it is located in the central aqueous core.

Unloaded liposomes and nanoparticles did not show any cytotoxic activity at the considered concentration range.

Conclusions

In conclusion, this study highlights the possibility to formulate PTM in liposomes and polymer nanoparticles for anticancer therapy. Moreover, it has been shown that the cytotoxic activity of PTM can be tuned depending on the type of nanocarriers considered and the form of PTM encapsulated. Thus, the nanocarriers here proposed could be considered as a platform for PTM delivery. Moreover, the encapsulation of PTM in lipid and polymer nanocarriers is also a proof of concept that could enlarge the field of application of these PTM drug delivery systems to other pathologies for which PTM has been shown to be active (other than *Leishmania*), such as myotonic dystrophy type 1, with different administration routes.

Funding

This work was supported by the Italian Ministry for University and Research (MIUR) – Università degli Studi di Torino, “Fondi Ricerca Locale (ex-60%)”.

Acknowledgments

We would like to acknowledge the contribution of Pierre-Yves Dugas (University of Lyon 1, C2P2 UMR 5265) for cryo-TEM observations at the “Centre Technologique des Microstructures” (CTµ - University of Lyon 1).

Declarations of interest: none.

References

1. Bray PG, Barrett MP, Ward SA, de Koning HP. Pentamidine uptake and resistance in pathogenic protozoa: past, present and future. Trends Parasitol 2003;19(5):232-239.
2. Bruni N, Stella B, Giraudo L, Della Pepa C, Gastaldi D, Dosio F. Nanostructured delivery systems with improved leishmanicidal activity: a critical review. Int J Nanomedicine 2017;12:5289-5311.
3. Baraldi PG, Bovero A, Fruttarolo F, Preti D, Tabrizi MA, Pavani MG, Romagnoli R. DNA minor groove binders as potential antitumor and antimicrobial agents. Med Res Rev 2004;24(4):475-528.
4. Pathak MK, Dhawan D, Lindner DJ, Borden EC, Farver C, Yi T. Pentamidine is an inhibitor of PRL phosphatases with anticancer activity. Mol Cancer Ther 2002;1(14):1255-1264.
5. Lee MS, Johansen L, Zhang Y, Wilson A, Keegan M, Avery W, Elliott P, Borisy AA, Keith CT. The novel combination of chlorpromazine and pentamidine exerts synergistic antiproliferative effects through dual mitotic action. Cancer Res 2007;67(23):11359-11367.
6. Chow TY, Alaoui-Jamali MA, Yeh C, Yuen L, Griller D. The DNA double-stranded break repair protein endo-exonuclease as a therapeutic target for cancer. Mol Cancer Ther 2004;3(8):911-919.
7. Smith J, Stewart BJ, Glaysher S, Peregrin K, Knight LA, Weber DJ, Cree IA. The effect of pentamidine on melanoma ex vivo. Anticancer Drugs 2010;21(2):181-185.
8. Jung HJ, Suh SI, Suh MH, Baek WK, Park JW. Pentamidine reduces expression of hypoxia-inducible factor-1alpha in DU145 and MDA-MB-231 cancer cells. Cancer Lett 2011;303(1):39-46.
9. Qiu G, Jiang J, Liu XS. Pentamidine sensitizes chronic myelogenous leukemia K562 cells to TRAIL-induced apoptosis. Leuk Res 2012;36(11):1417-1421.
10. Zerbini LF, Bhasin MK, de Vasconcellos JF, Paccez JD, Gu X, Kung AL, Libermann TA. Computational repositioning and preclinical validation of pentamidine for renal cell cancer. Mol Cancer Ther 2014;13(7):1929-1941.

11. Lidman C, Bronner U, Gustafsson LL, Rombo L. Plasma pentamidine concentrations vary between individuals with Pneumocystis carinii pneumonia and the drug is actively secreted by the kidney. J Antimicrob Chemother 1994;33(4):803-810.

12. Prabhavalkar S, Masengu A, O'Rourke D, Shields J, Courtney A. Nebulized pentamidine-induced acute renal allograft dysfunction. Case Rep Transplant 2013;2013:907593.

13. Scala A, Piperno A, Micale N, Mineo PG, Abbadessa A, Risoluti R, Castelli G, Bruno F, Vitale F, Cascio A, Grassi G. "Click" on PLGA-PEG and hyaluronic acid: Gaining access to anti-leishmanial pentamidine bioconjugates. J Biomed Mater Res B Appl Biomater 2018;106(8):2778-2785.

14. Ortega V, Giorgio S, de Paula E. Liposomal formulations in the pharmacological treatment of leishmaniasis: a review. J Liposome Res 2017;27(3):234-248.

15. de Almeida L, Terumi Fujimura A, Del Cistia ML, Fonseca-Santos B, Braga Imamura K, Michels PAM, Chorilli M, Graminha MAS. Nanotechnological Strategies for Treatment of Leishmaniasis--A Review. J Biomed Nanotechnol 2017;13(2):117-133.

16. Omarch G, Kippie Y, Mentor S, Ebrahim N, Fisher D, Murilla G, Swai H, Dube A. Comparative in vitro transportation of pentamidine across the blood-brain barrier using polycaprolactone nanoparticles and phosphatidylcholine liposomes. Artif Cells Nanomed Biotechnol 2019;47(1):1428-1436.

17. Merian J, De Souza R, Dou Y, Ekdawi SN, Ravenelle F, Allen C. Development of a liposome formulation for improved biodistribution and tumor accumulation of pentamidine for oncology applications. Int J Pharm 2015;488(1-2):154-164.

18. Her S, Cui L, Bristow RG, Allen C. Dual Action Enhancement of Gold Nanoparticle Radiosensitization by Pentamidine in Triple Negative Breast Cancer. Radiat Res 2016;185(5):549-562.

19. Peretti E, Miletto I, Stella B, Rocco F, Berlier G, Arpicco S. Strategies to Obtain Encapsulation and Controlled Release of Pentamidine in Mesoporous Silica Nanoparticles. Pharmaceutics 2018;10(4).

20. De Matteis V. Exposure to Inorganic Nanoparticles: Routes of Entry, Immune Response, Biodistribution and In Vitro/In Vivo Toxicity Evaluation. Toxics 2017;5(4).

21. Ulery BD, Nair LS, Laurencin CT. Biomedical Applications of Biodegradable Polymers. J Polym Sci B Polym Phys 2011;49(12):832-864.

22. Danhier F, Ansorena E, Silva JM, Coco R, Le Breton A, Preat V. PLGA-based nanoparticles: an overview of biomedical applications. J Control Release 2012;161(2):505-522.

23. Fessi H, Puisieux F, Devissaguet JP, Ammoury N, Benita S. Nanocapsule formation by interfacial polymer deposition following solvent displacement. Int J Pharm 1989;55(1):R1-R4.

24. Colzani B, Speranza G, Dorati R, Conti B, Modena T, Bruni G, Zagato E, Vermeulen L, Dakwar GR, Braeckmans K, Genta I. Design of smart GE11-PLGA/PEG-PLGA blend nanoparticulate platforms for parenteral administration of hydrophilic macromolecular drugs: synthesis, preparation and in vitro/ex vivo characterization. Int J Pharm 2016;511(2):1112-1123.

25. Bartlett GR. Phosphorus assay in column chromatography. J Biol Chem 1959;234(3):466-468.

26. Mandal TK, Bostanian LA, Graves RA, Chapman SR. Poly(D,L-lactide-co-glycolide) encapsulated poly(vinyl alcohol) hydrogel as a drug delivery system. Pharm Res 2002;19(11):1713-1719.

27. Vichai V, Kirtikara K. Sulforhodamine B colorimetric assay for cytotoxicity screening. Nat Protoc 2006;1(3):1112-1116.

28. Banerjee G, Nandi G, Mahato SB, Pakrashi A, Basu MK. Drug delivery system: targeting of pentamidines to specific sites using sugar grafted liposomes. J Antimicrob Chemother 1996;38(1):145-150.

29. Gubernator J. Active methods of drug loading into liposomes: recent strategies for stable drug entrapment and increased in vivo activity. Expert Opin Drug Deliv 2011;8(5):565-580.

30. Pai S, Alam AS, Kapoor JN. Pentamidine salts useful in the treatment and prophylaxis of pneumocystis carinii pneumonia. 1989. EP19880310395.

31. Paul M, Fessi H, Laatiris A, Boulard Y, Durand R, Deniau M, Astier A. Pentamidine-loaded poly(D,L-lactide) nanoparticles: Physicochemical properties and stability work. Int J Pharm 1997;159:223-232.

32. Paul M, Durand R, Boulard Y, Fusai T, Fernandez C, Rivollet D, Deniau M, Astier A. Physicochemical characteristics of pentamidine-loaded polymethacrylate nanoparticles: implication

in the intracellular drug release in Leishmania major infected mice. J Drug Target 1998;5(6):481-490.

33. Arias JL, Unciti-Broceta JD, Maceira J, Del Castillo T, Hernandez-Quero J, Magez S, Soriano M, Garcia-Salcedo JA. Nanobody conjugated PLGA nanoparticles for active targeting of African Trypanosomiasis. J Control Release 2015;197:190-198.

34. Ohnishi N, Tomida H, Ito Y, Tahara K, Takeuchi H. Characterization of a doxorubicin liposome formulation by a novel in vitro release test methodology using column-switching high-performance liquid chromatography. Chem Pharm Bull (Tokyo) 2014;62(6):538-544.

Figure captions

Figure 1. Chemical structure of PTM.

Figure 2. Representative cryo-TEM images of: (**a**) unloaded PLGA nanoparticles; (**b**) PTM-B-loaded PLGA nanoparticles; (**c**) PTM-B-loaded PLGA/PEG_{2000}-PLGA nanoparticles. Bar, 200 nm.

Figure 3. Representative cryo-TEM images of: (a) unloaded liposomes; (b) Lipo PTM-C; (c) Lipo PTM-S. Bar, 200 nm.