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ORIGINAL ARTICLE

Formulation and *In Vitro* Evaluation of Telmisartan Nanoparticles Prepared by Emulsion-Solvent Evaporation Technique

Emülsiyon-Çözücü Buharlaştırma Tekniği ile Hazırlanan Telmisartan Nanopartiküllerinin Formülasyonu ve *İn Vitro* Değerlendirilmesi

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ABSTRACT

Objectives: Telmisartan (TLM) is an antihypertensive drug that has been shown to have antiproliferative effects on cancer cells. It has low solubility and suboptimal oral bioavailability. To investigate the potential anticancer effect of TLM on breast cancer cells, poly (D, L-lactide) (PLA) nanoparticles were formulated with the benefit of improving its solubility.

Materials and Methods: TLM-loaded PLA nanoparticles were prepared by emulsion solvent evaporation. The effects of sonication time and polymer:drug ratio on nanoparticle size and drug encapsulation were investigated. TLM-loaded nanoparticles were tested against MCF-7 and MDA-MB-231 breast cancer cell lines for antiproliferative effects.

Results: Nanoparticles with mean particle size 272 nm and 79% encapsulation efficiency were obtained. Sustained release TLM nanoparticles (40% in 24 h) decreased cell viability to 45% for MCF-7 cells at 72 h, even at the lowest TLM concentration, indicating better anticancer efficiency than TLM solution.

Conclusion: TLM nanoparticles could be potential anticancer agents for breast cancer and deserve further studies.

Key words: Telmisartan, nanoparticles, anticancer effect, drug repositioning

ÖZ

Amaç: Telmisartan (TLM), kanser hücreleri üzerinde antiproliferatif etkisi olduğu gösterilmiş antihipertansif bir ilaçtır. Düşük çözünürlüğe, suboptimal oral biyoyararlanıma sahiptir. TLM'nin meme kanseri hücreleri üzerindeki potansiyel antikanser etkisini araştırmak için, TLM'nin çözünürlüğünü iyileştiren poli (D, L-laktid) PLA nanopartikülleri formüle edilmiştir.

Gereç ve Yöntemler: TLM yüklü PLA nanopartikülleri emülsiyon hazırlama-çözücü buharlaştırma yöntemiyle hazırlanmıştır. Sonikasyon süresi ve polimer: ilaç oranının nanopartikül büyüklüğü ve ilaç enkapsülasyonu üzerindeki etkisi araştırılmıştır. TLM yüklü nanopartiküllerin, antiproliferatif etkileri MCF-7 ve MDA-MB-231 meme kanseri hücre hatları kullanılarak test edilmiştir.

Bulgular: Ortalama partikül büyüklüğü 272 nm ve %79 enkapsülasyon etkinliğine sahip nanopartiküller elde edilmiştir. Sürekli salım gösteren TLM nanopartikülleri (24 saatte %40) MCF-7 hücrelerinde, TLM çözeltisinden daha iyi antikanser aktivite göstererek, en düşük TLM konsantrasyonunda bile hücre canlılığını 72 saatte %45'e düşürmüştür.

Sonuç: TLM nanopartiküllerinin, meme kanseri için potansiyel antikanser ajanlar olabileceği ve daha ileri çalışmalarda araştırılmaya değer olduğu sonucuna varılmıştır.

Anahtar kelimeler: Telmisartan, nanopartiküller, antikanser etki, ilaç yeniden konumlandırma

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INTRODUCTION

Drugs are developed by a long and costly development process. However, finding new uses for existing drugs is an alternative strategy that shortens the time to market and lowers the cost for developers. By employing this strategy new anticancer agents could be discovered and more accessible treatments could be presented to patients. Angiotensin II receptor blockers (ARBs) are an example for old drugs that are investigated for potential new activities. Telmisartan (TLM), which is an ARB used for the treatment of hypertension, has been investigated in this context and antiproliferative activity of TLM against various cancer cells such as lung adenocarcinoma cells, urological cancer cells, and endometrial cancer cells has been discovered.[1-3] It was reported that TLM has an antiproliferative effect on leukemia cell lines and *ex vivo* peripheral blood monocytes, and TLM causes autophagy and apoptosis by caspase activation.[4] Besides being an ARB, TLM is also described as a peroxisome proliferator-activated receptor (PPAR)-gamma activator and in some studies its antitumor activity is linked to PPAR-gamma activation.[1-3] Intraperitoneal administration of TLM to human endometrial tumor-bearing nude mice showed that treatment with TLM inhibited tumor growth significantly.[2] In a transgenic rat model for prostate cancer, TLM was administered orally in drinking water (2 or 10 mg/kg/day) for 12 weeks and it was found that TLM suppressed prostate cancer by activation of caspases, inactivation of p38 MAPK, and downregulation of the androgen receptor.[5]

Considering the potential anticancer application of TLM, it is also important to design an optimal formulation for effective therapy. TLM has poor aqueous solubility (0.6 µg/mL) and its solubility is pH-dependent.[6] According to the Biopharmaceutics Classification System (BCS), it is classified as a BCS II drug[7] (low solubility, high permeability) and its oral bioavailability is ~43%.[8] To improve its oral bioavailability, nanoparticle systems are one of the methods that are being investigated.[7,9] Moreover, for effective cancer therapy, accumulation of anticancer agent at the tumor site with minimum distribution in healthy tissues is a major goal. For TLM, its side-effect profile is reported as comparable to that of a placebo;[10] however, using nanoparticles (NPS) to achieve TLM accumulation at the tumor site can enhance the interaction of TLM with cancer cells, which leads to improved cancer therapy.

In the present work TLM was encapsulated with NPS that were formed by biodegradable poly (D, L-lactide) (PLA) polymer. The NPS were prepared by emulsion-solvent evaporation and studies were carried out to optimize particle size. The anticancer effect of TLM-loaded NPS was investigated against MCF-7 and MDA-MB-231 breast cancer cells.

MATERIALS AND METHODS

Materials

TLM was a kind gift from Nobel Pharmaceutical Industry and Trade Inc. Co., Turkey. Dichloromethane, dimethyl sulfoxide (DMSO), PLA (average Mw 75,000-120,000), and poly (vinyl alcohol) (PVA) (average mol wt 30,000-70,000) were purchased from Sigma-Aldrich Co., USA. MCF-7 and MDA-MB-231 cell lines were obtained from the American Type Culture Collection. Fetal bovine serum (FBS) and penicillin/streptomycin, Dulbecco's Modified Eagle Medium (DMEM), and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) were purchased from Biochrom (Germany). All other reagents were analytical grade.

Preparation of nanoparticles

NPS were prepared by a modified emulsion solvent evaporation method.[11] PLA was dissolved in 1 mL of dichloromethane and 10 mL of PVA aqueous solution (0.3% w/v) was added to it. The mixture was vortexed for 1 min and then immediately was sonicated using a probe-type sonicator (Bandelin Sonopuls HD2200). After sonication the dichloromethane was evaporated by a rotary evaporator (IKA RV10). Then the NPS were centrifuged at 15.000 rpm for 15 min (23.656 x g, Hermle Z323K). The precipitated NPS were suspended in ultrapure water and centrifuged twice more for the washing steps. For TLM-loaded NPS (TLM-NP) the same procedure was performed with addition of TLM to dichloromethane. The effect of sonication time on blank and TLM-loaded NP size (polymer:drug ratio, 25:1 mg/mg) was investigated using different sonication times (5, 10, 20 min). After determining the optimal sonication time for obtaining the smallest NPS, two more polymer:drug ratios (25:2, 25:0.5, mg:mg) were studied to obtain TLM-NP and to observe the effect of drug amount on nanoparticle size and encapsulation efficiency (EE).

Characterization studies

Particle size and zeta potential measurements

The particle size, polydispersity index (PDI), and zeta potential values of blank and TLM-NP were measured by dynamic light scattering method using a Malvern Zeta Sizer Nano Series ZS instrument (UK). Measurements were performed for both freshly prepared and lyophilized NPS. The initial experiments showed that NPS lyophilized without any additive were not dispersible. Thus they were freeze-dried using 5% trehalose as cryoprotectant to prevent aggregation.

Encapsulation efficiency and drug loading capacity

Suspended NPS were frozen at -20 °C for 16 h and then were lyophilized for 48 h (Labconco, Freezone 4.5). The lyophilized NPS were dissolved in DMSO and were analyzed by ultraviolet-visible (UV-Vis) spectrophotometry at 309 nm to determine the encapsulated TLM amount. Spectrums in DMSO were used to show insignificant absorbance of PLA polymer at 309 nm. The concentration range of the calibration curve was 1-20 µg/mL. The regression equation was y=0.048x-0.006 (standard error (SE) of slope was $8x10^{-5}$, SE of intercept was $2x10^{-3}$, and R^2 was 1) and the limit of detection (LOD) was 0.3 µg/mL and limit of quantification (LOQ) was 0.8 µg/mL.

EE and drug loading capacity (DLC) were calculated using equations 1 and 2, respectively.

EE=mass of the drug in NPS/mass of drug used initially
Equation 1

DLC=mass of the drug in NPS/total mass of the NPS Equation 2

In vitro drug release

In vitro drug release experiments were carried out using the dialysis membrane method in a shaking water bath. NPS were dispersed in phosphate buffered saline (PBS) containing 0.5% (w/v) sodium lauryl sulfate (SLS) and 1 mL of this suspension was placed into a dialysis bag (molecular weight cutoff 8000 Da). The dialysis bag was placed in 50 mL of 0.5% SLS containing PBS and at specific time points 1 mL of release medium was withdrawn to determine the amount of TLM released. At each time point, 1 mL of fresh medium was added to the release medium after sampling. In addition, coarse TLM powder was dispersed in PBS containing 0.5% SLS and TLM dissolution was determined under the same conditions as for NPS.

Samples were analyzed by UV-Vis spectrophotometry using a calibration curve obtained from TLM solutions in 0.5% SLS containing PBS at 300 nm. The calibration curve was obtained in the concentration range of 0.5-10 µg/mL. The regression equation was y=0.048x-0.002 (SE of slope was 0.0002, SE of intercept was 0.0008, and R^2 was 1) and the LOD and LOQ were 0.1 µg/mL and 0.4 µg/mL, respectively.

Scanning electron microscopy (SEM)

NP morphology was observed with SEM. A NP suspension (10 µL) was placed on aluminum foil and was air dried. Then the foil was placed onto an SEM stub and was coated with gold/palladium using a BAL-TEC SCD 050 (Liechtenstein). The coated sample was visualized with a LEO-EVO 40 (UK) SEM unit.

Cell culture studies

MCF-7 and MDA-MB-231 cell lines were grown in DMEM containing penicillin (IU/mL), streptomycin (50 µg/mL), and 10% FBS and were maintained in a humidified atmosphere of 95% air and 5% CO_2 at 37 ˚C. Cells were seeded at a density 5000 cells/well in 100 µL of complete culture medium in a 96-well plate. After overnight incubation, the medium was removed from the wells and serial dilutions of TLM solution (in complete medium containing 1% DMSO), blank-NP (B-NP), and TLM-NP in complete culture medium were applied to the cells. As the control, cells were treated with complete medium alone and complete medium containing 1% DMSO. Following 24, 48, and 72 h of incubation 25 µL of MTT solution (5 mg/mL in PBS) was added to the wells. After 4 h of incubation the medium was removed from the wells and 200 µL of DMSO was added to them to dissolve the formazan crystals. The optical densities of plates were measured using a microplate reader (Molecular Devices, USA) at 570 nm. Cell viability was calculated as the percentage of control cells.

Statistics analysis

The results were presented as the mean and standard deviation/SE. Statistical analysis was performed by GraphPad Prism using One and Two-Way ANOVA and $p<0.05$ was considered statistically significant.

RESULTS

Particle size and zeta potential of nanoparticles

NPS were prepared by emulsion solvent evaporation and their particle size and zeta potential were measured directly after preparation. In addition, to investigate the effect of freeze-drying on particle size, the particle size of lyophilized NPS (freeze-dried with 5% trehalose) was measured. Firstly, three different sonication times were used to investigate the effect of sonication time on the size and EE of NPS. Particle size and zeta potential data of the freshly prepared and lyophilized B-NP, TLM-NP with different sonication times are given in Table 1. Particle size and zeta potential data of the blank and freshly prepared and lyophilized B-NP, TLM-NP with different sonication times are given in Table 1. Zeta potential values were negative and between -10 and -20 mV for all formulations and NP size was between 218 and 238 nm for blank formulations and between 273 and 333 nm for the TLM-NP formulations. The smallest NP size was obtained with 10 min sonication; therefore, formulations of different polymer:drug ratios were prepared by performing 10 min sonication. After the freeze-drying process nanoparticle size was decreased for both blank and TLM formulations, which indicates the stabilizing effect of trehalose. Zeta potential values of freeze-dried formulations were slightly lower than those of freshly prepared NPS and were between -7.0 and -9.5 mV.

Particle size and zeta potential values of TLM-NP, which were prepared using different drug amounts, are given in

Table 1. Effect of sonication time on the particle size, polydispersity index, and zeta potential of freshly prepared and freeze-dried formulations (n=3, mean ± standard deviation)

Sonication time (minute)		B-NP			TLM-NP		
		Particle size (nm)	PDI	Zeta potential (mV)	Particle size (nm)	PDI	Zeta potential (mV)
5	Fresh	237.8±1.1	0.1±0.0	-15.2±1.3	332.6±3.8	0.3±0.0	-10.5±2.1
10		218.1±3.6	0.1±0.0	-18.8±0.5	272.6±1.6	0.2±0.0	-15.0±2.6
20		223.6±1.5	0.1±0.0	-15.7±2.0	302.2±3.1	0.3±0.0	-15.7±0.4
5	Freeze-dried	210.1±1.7	0.1±0.0	-7.0±0.2	227.1±8.2	0.2±0.0	-7.9±0.2
10		205.9±3.9	0.1±0.0	-9.5±0.4	215.5±3.7	0.1±0.0	-8.1±0.2
20		212.4±1.6	0.1±0.0	-8.5±0.5	286.7±9.1	0.2±0.0	-7.4±0.2

PDI: Polydispersity index, TLM-NP: Telmisartan-loaded nanoparticles, B-NP: Blank-nanoparticles

Table 2. Particle size of the formulations with 25:2 and 25:0.5 polymer:drug ratios was smaller than that of the formulation with 25:1 polymer:drug ratio for freshly prepared NPS. Freeze-dried formulations showed different behavior in terms of particle size. While for the formulations of 25:1 and 25:0.5 polymer:drug ratios particle size decreased with freeze-drying, for the formulation of 25:2 polymer:drug ratio the opposite was true. The heterogeneity of the particle size distribution of this formulation (25:2 polymer:drug ratio) is thought to be the reason behind the further aggregation during lyophilization.

Encapsulation efficiency and drug loading capacity

Three different polymer:drug ratios were studied to investigate the effect on NP EE. The EE and DLC of TLM-NP are presented in Table 3. The highest drug loading was obtained with the formulation of 25:1 polymer:drug ratio.

In vitro drug release

The NP formulation with the highest EE (polymer:drug ratio, 25:1, mg:mg) was selected for *in vitro* drug release experiments. The release profiles of TLM-NP and TLM in PBS with 0.5% SLS are given in Figure 1. It was clear from the TLM release profile (around 40% release at the end of 24 h) that the NPS showed controlled release. Moreover, the TLM NPS displayed higher dissolution, compared to 17.2% release of TLM in 24 h.

Scanning electron microscopy

Freshly prepared NP suspension (polymer:drug ratio, 25:1, mg:mg) was placed on aluminum foil and after drying in atmospheric conditions the NPS were visualized by SEM as presented in Figure 2.

Table 2. Effect of polymer:drug ratio on the particle size, polydispersity index, and zeta potential of telmisartan-loaded nanoparticles (n=3, mean ± standard deviation)

Polymer:drug ratio (mg/mg)		Particle size (nm)	PDI	Zeta potential (mV)
25:0.5	Fresh NP	233.8±2.0	0.2±0.0	-15.2±0.4
25:1		272.6±1.6	0.2±0.0	-15.0±2.6
25:2		226.2±9.4	0.3±0.1	-11.1±0.5
25:0.5	Freeze-dried NP	228.0±4.6	0.2±0.0	-12.2±0.2
25:1		215.5±3.7	0.1±0.0	-8.1±0.2
25:2		267.8±3.3	0.3±0.0	-7.5±0.2

PDI: Polydispersity index, NP: Nanoparticles

Table 3. Encapsulation efficiency and drug loading capacity of telmisartan-loaded nanoparticles (n=3, mean ± standard deviation)

Polymer:drug ratio (mg/mg)	Sonication time (min)	EE (%)	DLC (%)
25:0.5	10	40.0±1.2	1.1±0.1
25:1	5	79.1±3.5	5.4±0.1
25:1	10	37.7±0.2	1.7±0.1
25:1	20	97.4±0.9	4.8±0.1
25:2	10	60.3±2.1	5.3±0.3

EE: Encapsulation efficiency, DLC: Drug loading capacity

Cell culture studies

MCF-7 and MDA-MB-231 cells were used as model cell lines to investigate whether there is an antiproliferative effect of TLM and TLM-NP on breast cancer cells. The cell viability results for 24, 48, and 72 h incubation periods are given in Figures 3-5, respectively.

For MCF-7 cells, B-NP did not reduce cell viability below 80% at 24 h incubation period and at 48 and 72 h the highest concentration resulted in 73% and 67% cell viability, respectively (Figures 3a, 4a, and 5a). TLM solution reduced cell viability to 75% at 24 h, to 61% at 48 h, and to 60% at 72 h (Figures 3a, 4a, and 5a). However, there was not a dramatic difference between the viability results of different concentrations of TLM treatments at each time period. The TLM-NP formulation decreased cell viability in a concentration-dependent manner and at the highest TLM concentration viability decreased to 45% at 24 h and 72 h (Figures 3a and 5a). As the incubation time increased lower TLM concentrations caused lower cell viability results.

For MDA-MB-231 cells, B-NP did not reduce cell viability below 76% even at 72 h incubation (Figures 3b, 4b, and 5b). TLM solution reduced cell viability in a dose-dependent manner;

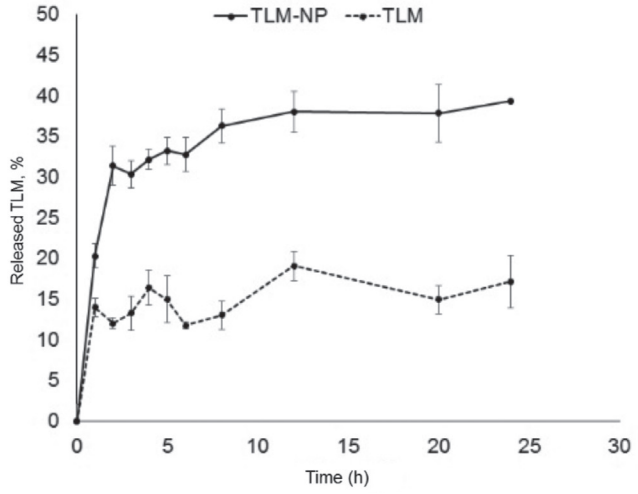


Figure 1. In vitro release profile of TLM-loaded nanoparticles and TLM (n=3, mean ± SE)
TLM-NP: Telmisartan-loaded nanoparticles, SE: Standard error

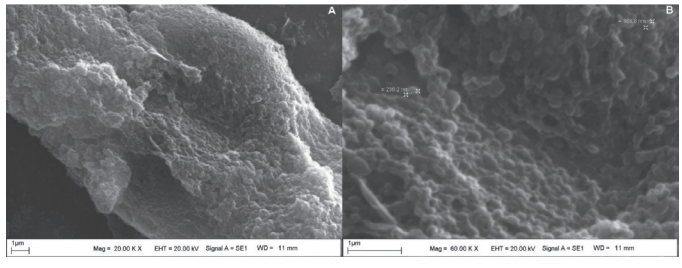


Figure 2. SEM images of TLM-loaded nanoparticles at A: 20K magnification B: 60K magnification
SEM: Scanning electron microscopy, TLM: Telmisartan

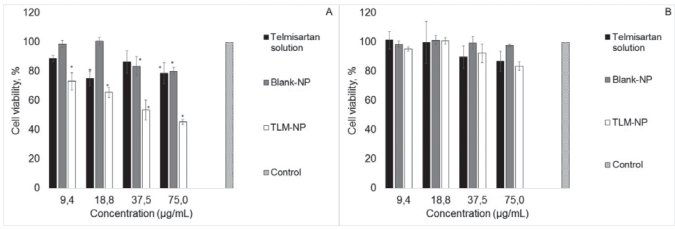


Figure 3. Cell viability of formulations after 24 h incubation. A: MCF-7 cells, B: MDA-MB-231 cells (n=3, mean ± SE). *Indicates significantly different from control ($p<0.05$)

SE: Standard error, TLM-NP: Telmisartan-loaded nanoparticles

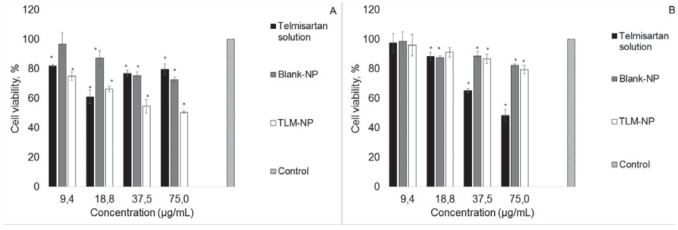


Figure 4. Cell viability of formulations after 48 h incubation. A: MCF-7 cells, B: MDA-MB-231 cells (n=3, mean ± SE). *Indicates significantly different from control ($p<0.05$)

SE: Standard error, TLM-NP: Telmisartan-loaded nanoparticles

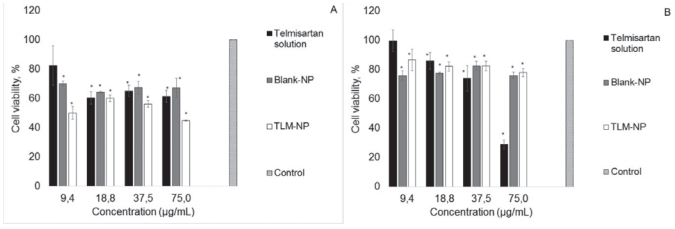


Figure 5. Cell viability of formulations after 72 h incubation. A: MCF-7 cells, B: MDA-MB-231 cells (n=3, mean ± SE). *Indicates significantly different from control ($p<0.05$)

SE: Standard error, TLM-NP: Telmisartan-loaded nanoparticles

it was especially pronounced at 48 and 72 h (Figures 4b and 5b). Interestingly the TLM-NP formulation did not cause any significant loss of viability; 78% was the lowest viability, obtained with the TLM-NP formulation at 72 h.

DISCUSSION

Novel strategies are being investigated for cancer therapy by repositioning various drugs that have different action mechanisms. Recently, these strategies have become extremely popular for the development of new potential cancer treatment modalities. Angiotensin II receptor antagonists are widely used for the treatment of hypertension and their anticancer activity was reported in various scientific papers.[12,13] Several tumor cells were reported to express angiotensin II receptors;[12-14] therefore, the anticancer effect of ARBs has been associated with inhibition of tumor angiogenesis.[15-17] One of the ARBs, TLM, is generally used for treatment of cardiovascular diseases including hypertension and coronary artery diseases.[18] However, recent studies have drawn attention to the anticancer effect of TLM. As indicated in different studies, TLM showed antitumoral activities in various cancer types such as lung,[1] prostate,[19] and endometrium.[2] Researchers have revealed that TLM has the potential to inhibit the proliferation of cancer cells through apoptosis by PPAR-gamma activation.[1,3,20] PPAR-gamma activation by using TLM as a PPAR-gamma ligand may serve as an anticancer therapy model.[21]

Due to properties mentioned above, TLM has the potential to be used in cancer therapy as a part of drug repositioning studies. However, TLM is a BCS class II model drug and has some limitations like poor solubility influencing its bioavailability.[22] Therefore, to improve the solubility of TLM and to deliver it to the target site specifically, it is important to design an effective drug delivery system. TLM encapsulation within polymeric carriers is an alternative way to protect the drug from degradation, to ensure controlled release, and to increase bioavailability because of increased solubility.

Based on this idea, in the present study we prepared a polymeric nanoparticulate system by encapsulating TLM in PLA NPS by a modified emulsion solvent evaporation method to improve drug solubility. In addition, we aimed to investigate the anticancer efficiency of an antihypertensive drug nanoparticulate system as a potential, novel treatment modality for breast cancer. To the best of our knowledge this is the first study in which a novel drug delivery system was developed using PLA NPS to deliver the poorly soluble drug TLM as a model anticancer drug for breast cancer therapy.

In the present study, TLM-loaded and B-NP were prepared with biodegradable PLA polymer. As indicated in the literature, PLA is a biocompatible, biodegradable polymer that shows low toxicity and high mechanic strength, and so in the present study PLA polymer was chosen for these advantages.[23,24] NPS were prepared by emulsion solvent evaporation, which involves emulsification of polymer solution and then evaporation of solvent, which precipitates the polymer as NPS. In order to obtain an optimized formulation, sonication time and polymer:drug ratio were evaluated in terms of particle size, PDI, EE, DLC. The particle size and PDI were measured by dynamic light scattering. Several parameters like sonication time and polymer:drug ratio highly affect the physicochemical properties of NPS, including particle size, EE, and drug loading ratio. Therefore, these parameters were studied to determine the optimum formulation. Different sonication times were tested to determine particle size as the first parameter and the results indicated that while the size of B-NP was in the range of 218-238 nm, the size of TLM-NP formulations was in the range of 273-333 nm. During the emulsification process sonication leads to dispersion of the organic phase into small nanodroplets, and so it is expected that by increasing sonication time more energy will be released and smaller droplets will be dispersed; consequently smaller NPS will be obtained.[25] Increasing sonication time from 5 min to 10 min reduced particle size but nanoparticle size was higher when 20 min sonication was performed, compared to 10 min sonication. The decrease in particle size as the sonication

time increases is expected, but several scientific papers report that there is a threshold value.[26,27] After that threshold value is reached, increasing sonication time does not reduce particle size significantly. Furthermore, it should be noted that sonication is a very dynamic process; while sonication energy disrupts droplets into smaller droplets, some of the droplets may collide due to this energy and form larger droplets, which may explain why 20 min sonication caused a slight increase in particle size. The difference between the size of TLM-NP (formulations prepared with different sonication times and drug amounts) was statistically significant ($p<0.05$). As expected, encapsulating drug into PLA NPS caused an increase in particle size. The PDI results of the blank and TLM-NP were in the range of 0.1-0.3, indicating a narrow and homogeneous size distribution. As well as the sonication time, the second important parameter, different drug:polymer ratios, was tested. Increasing drug amount from 0.5 to 1 mg in the formulation caused an increase in particle size. In contrast, by using 2 mg of drug in the formulation, a smaller mean particle size was obtained. However, PDI was increased for this formulation, indicating that size distribution was not as narrow as in other formulations in which 0.5 and 1 mg TLM were used. The effect of freeze-drying on the particle size of NPS was also investigated. Addition of trehalose as cryoprotectant to the NP suspension before the freeze-drying process resulted in decreased particle size generally. Similarly, Fonte et al.[28] reported that the particle size of polymeric NPS lyophilized with trehalose was decreased and this may be related to adsorption of cryoprotectants on the nanoparticle surface and their particular behavior during freeze-drying. The negative zeta potential values of blank and TLM-NP decreased after lyophilization. This is attributed to the rearrangement of surfactants on the NP surface. Moreover, trehalose may mask the surface of NP due to hydrogen bonding between nanoparticle and cryoprotectant.[29]

EE was increased as higher drug amounts were used and the highest drug loading was achieved with 25:1 polymer:drug ratio. *In vitro* drug release experiments showed that TLM was released from the NPS slowly and in a sustained manner (40% drug release at 24 h). The drug's partitioning between polymer and aqueous release medium influences the release rate.[30] Slow TLM (a hydrophobic drug) release from hydrophobic PLA NPS is explained by the solubility of drug in the polymer and its lower partitioning to the aqueous phase. Furthermore, coarse TLM powder dissolution (17.2% in 24 h) was significantly slower than the TLM-NP formulation ($p<0.05$), indicating increased solubility of TLM. The release medium was 50 mL of PBS with 0.5% SLS (pH 7.4) and coarse TLM powder and TLM-NP equivalent to 0.15 mg TLM were dispersed in PBS with 0.5% SLS and were placed in a dialysis membrane. As solubility of TLM in PBS with 0.5% SLS (pH 7.4) was reported as 0.108±0.04 mg/mL,[31] sink conditions were maintained during the experiment.

SEM images of NPS (Figure 2) show that they were clustered together during the drying process but individual spherical NPS were easily distinguished in images obtained at higher magnifications as expected.

The cell viability results indicate that although TLM solution decreased the cell viability of MCF-7 cells as the incubation time increased, the concentration viability relationship was erratic. TLM-NP decreased cell viability in a concentration-dependent manner to 45%, even at the lowest concentrations at 72 h incubation. By using NPS, solubility can be increased and cellular uptake of drugs can be modified. The superior antiproliferative results of the TLM-NP formulation compared to TLM solution is thought to be linked to these properties of TLM-NP formulations. TLM solution decreased the cell viability of MDA-MB-231 cells as the incubation time increased in a dose-dependent manner. However, the TLM-NP formulation did not cause the same dramatic loss of viability. It is clear for MDA-MB-231 cells that there should be a certain drug dose present in solution to see a significant loss of viability. Considering the slow drug release from TLM NPS, the reason for the high viability results obtained with them is attributed to the low amount of drug released. MCF-7 is an estrogen-dependent cell line and MDA-MB-231 is a triple negative cell line (cells that do not express estrogen receptor or progesterone receptor, and do not have HER-2/Neu amplification). It was reported that TLM induced the stimulation of collagen biosynthesis (which may influence cell growth and metabolism) in MCF-7 cells cultured in the absence of estrogen and there was crosstalk between PPAR-gamma and estrogen receptor. However, collagen biosynthesis was not influenced by TLM in estrogen-independent MDA-MB-231 cells when cultured in the same conditions.[32] As Kociecka et al.[32] reported, the different responses of cells to TLM could be receptor related and need to be investigated further.

Study limitations

TLM NP formulations were prepared and their antiproliferative effect was investigated, but investigation of anticancer effect mechanisms was not within the scope of this study.

CONCLUSION

In our study TLM-loaded biodegradable PLA NPS were prepared and it was observed that sonication time and drug amount could impact NP size. It was demonstrated that sustained release TLM-NP inhibit proliferation of MCF-7 breast cancer cells better than TLM solution, indicating their potential use as an anticancer system. Further studies should be conducted to elucidate the anticancer mechanisms of TLM NPS on breast cancer cells.

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