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Formulation and in vitro evaluation of telmisartan nanoparticles prepared by emulsion-solvent evaporation technique

Emülsiyon-çözücü buharlaştırma tekniği ile hazırlanan telmisartan nanopartiküllerinin formülasyonu ve in vitro değerlendirilmesi

Naile Ozturk[1], Asli Kara[2], Imran Vural[3]
[1]Department of Pharmaceutical Technology, Faculty of Pharmacy, Inonu University, Malatya, Turkey
[2]Department of Medical Services and Techniques, Sungurlu Vocational High School, Hitit University, Corum, Turkey
[3]Department of Pharmaceutical Technology, Faculty of Pharmacy, Hacettepe University, Ankara, Turkey;
INTRODUCTION: Telmisartan is an antihypertensive drug which has been shown to have antiproliferative effects on cancer cells. It has low solubility, suboptimal oral bioavailability. To investigate potential anticancer effect of telmisartan on breast cancer cells, PLA nanoparticles were formulated with the benefit of improving its solubility.
METHODS: Telmisartan loaded poly(D,L-lactide) nanoparticles were prepared by emulsion solvent evaporation method. Effect of sonication time and polymer: drug ratio on nanoparticle size and drug encapsulation was investigated. TLM loaded nanoparticles were tested against MCF-7 and MDA-MB-231 breast cancer cell lines for anti-proliferative effects.
RESULTS: Nanoparticles with mean particle size 272 nm and 79 % encapsulation efficiency were obtained. Sustained release telmisartan nanoparticles (40% in 24 h) decreased cell viability down to 45% for MCF-7 cells at 72 h, even at lowest telmisartan concentration, indicating better anticancer efficiency than telmisartan solution.
DISCUSSION AND CONCLUSION: It was concluded telmisartan nanoparticles could be potential anticancer agents for breast cancer, deserving further studies.
Keywords: Telmisartan, nanoparticles, anti-cancer effect, drug repositioning

GİRİŞ ve AMAÇ: Telmisartan, kanser hücreleri üzerinde antiproliferatif etkisi olduğu gösterilmiş antihipertansif bir ilaçtır. Düşük çözünürlüğe, düşük oral biyoyararlanıma sahiptir. Telmisartanın meme kanseri hücreleri üzerindeki potansiyel antikanser etkisini araştırmak için, telmisartanın çözünürlüğünü de arttıran PLA nanopartikülleri formüle edilmiştir.
YÖNTEM ve GEREÇLER: Telmisartan yüklü poli (D, L-laktit) nanopartikülleri emülsiyon çözücü buharlaştırma yöntemiyle hazırlanmıştır. Sonikasyon süresi ve polimer: ilaç oranının nanopartikül büyüklüğü ve ilaç enkapsülasyonu üzerindeki etkisi araştırılmıştır. TLM yüklü nanopartiküller, anti-proliferatif etkisini belirlemek için 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. Uzatılmış salım gösteren telmisartan nanopartikülleri (24 saatte % 40) MCF-7 hücrelerinde, telmisartan çözeltisinden daha iyi bir antikanser etkinlik göstererek, en düşük telmisartan konsantrasyonunda bile hücre canlılığını 72 saatte % 45'e düşürmüştür.
TARTIŞMA ve SONUÇ: Telmisartan nanopartiküllerinin, meme kanseri için potansiyel antikanser ajanlar olabileceği ve daha ileri çalışmalarda araştırılmaya değer olduğu sonucuna varılmıştır.
Anahtar Kelimeler: Telmisartan, nanopartiküller, anti-kanser etki, ilaçların yeniden konumlandırılması

INTRODUCTION

Drugs are developed by a costly and long development process. However, finding new uses for existing drugs is alternative strategy which shortens the time to market and lower the cost for developers. By employing this strategy new anti-cancer agents could be discovered and more accessible treatments could be presented to patients. Angiotensin II receptor blockers (ARBs) are an example for old drugs which 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 anti-proliferative of activity of TLM against various cancer cells such as lung adenocarcinoma cells, urological cancer cells, endometrial cancer cells have been discovered.[1-3] It was reported that TLM has antiproliferative effect on leukemia cell lines and ex vivo peripheral blood monocytes, and also, 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 anti-tumor activity is linked to PPAR-gamma activation.[1-3] Intra peritoneal 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 down-regulation of the androgen receptor.[5]
Considering, the potential anticancer application of TLM, it is also important to design an optimal formulation for an effective therapy. TLM has poor aqueous solubility (0.6 µg/mL) and its solubility is pH-dependent.[6] According to Biopharmaceutics Classification System (BCS), it is classified as 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 which are being investigated.[7, 9] Also, for an effective cancer therapy, accumulation of anticancer agent at tumor site with minimum distribution to healthy tissues is a major goal. For TLM, its side-effect profile is reported as comparable to placebo[10], however, using nanoparticles to achieve TLM accumulation at tumor site can enhance interaction of TLM with cancer cells, which leads us to improved cancer therapy.
In this work TLM was encapsulated to nanoparticles which were formed by biodegradable poly(D,L-lactide) polymer. Nanoparticles were prepared by emulsion-solvent evaporation technique and studies were carried out to optimize particle size. Anticancer effect of TLM loaded nanoparticles were investigated against MCF-7 MDA-MB-231 breast cancer cells.

MATERIALS AND METHODS

Materials

TLM was a kind gift from Nobel İlaç San. Ve Tic. A.Ş., Turkey. Dichloromethane, dimethyl sulfoxide (DMSO), poly(D,L-lactide) (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 American Type Culture Collection (ATCC, USA). 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

Nanoparticles 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 then immediately was sonicated using a probe type sonicator (Bandelin Sonopuls HD2200). After sonication dichloromethane was evaporated by a rotary evaporator (IKA RV10). Then nanoparticles were centrifuged at 15,000 rpm for 15 min (23,656 g, Hermle Z323K). Precipitated nanoparticles were suspended in ultrapure water and centrifuged twice more for washing steps. For TLM loaded nanoparticles same procedure was performed with addition of TLM to dichloromethane. Effect of sonication time on blank and TLM loaded nanoparticle size (polymer:drug ratio, 25:1 mg/mg) was investigated by performing different sonication times (5,10, 20 min). After determining optimal sonication time for obtaining smallest sized nanoparticles, two more polymer:drug ratios (25:2, 25:0.5, mg:mg) were studied to obtain TLM loaded nanoparticles and to observe the effect of drug amount on nanoparticle size and encapsulation efficiency.

Characterization studies

Particle size and zeta potential measurements

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

Encapsulation efficiency and drug loading capacity

Suspended nanoparticles were frozen at -20 for 16 h then were lyophilized for 48 h (Labconco, Freezone 4.5). The lyophilized nanoparticles were dissolved in DMSO and was analyzed with UV-Vis spectrophotometry at 309 nm to determine encapsulated TLM amount. Spectrums in DMSO were used to show insignificant absorbance of PLA polymer at 309 nm. The concentration range of calibration curve was 1-20 µg/mL. 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 also limit of detection (LOD) 0.3 µg/mL, limit of quantification (LOQ) was 0.8 µg/mL. Encapsulation efficiency (EE) and drug loading capacity (DLC) were calculated using equation 1 and 2, respectively.

EE= mass of the drug in nanoparticles/ /mass of drug used initially Equation 1
DLC=mass of the drug in nanoparticles/total mass of the nanoparticles Equation 2

In vitro drug release

In vitro drug release experiments were carried out using dialysis membrane method in a shaking water bath. Nanoparticles 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 cut off 8000 Da). The dialysis bag was placed in into 50 mL of 0.5 % SLS containing PBS and at specific time points 1 mL of release medium was withdrawn to determine released TLM amount. At each time point, 1 mL of fresh medium was added to the release media after sampling. Also, coarse TLM powder was dispersed in PBS containing 0.5 % SLS and TLM dissolution was determined at the same conditions as nanoparticles.
Samples were analyzed by UV-Vis spectrophotometry using a calibration curve obtained from TLM solutions in 0.5 % SLS containing PBS at 300 nm. Calibration curve was obtained in the concentration range of 0.5-10 µg/mL. 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 LOD and LOQ were 0.1 µg/mL and 0.4 µg/mL respectively.

Scanning electron microscopy (SEM)

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

Cell culture studies

MCF-7 and MDA-MB-231 cell lines were grown in DMEM containing penicillin (50 U/mL), streptomycin (50µg/mL), 10 % FBS and was maintained in a humidified atmosphere of 95% air and 5% CO2 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, medium was removed from wells and serial dilutions of TLM solution (in complete medium containing 1% DMSO), blank nanoparticles (B-NP), TLM loaded nanoparticles (TLM-NP) in complete culture medium were applied to cells. As control, cells were treated with complete medium alone and complete medium containing 1 % DMSO. Following 24, 48 and 72 h incubation 25 µL of MTT solution (5mg/mL in PBS) was added to the wells. After 4 h incubation medium was removed from wells and 200 µL of DMSO was added to the wells to dissolve formazan crystals. Optical densities of plates were measured using a microplate reader (Molecular Devices, USA) at 570 nm. Cell viabilities was calculated as the percentage of control cells.

Statistics

Results were presented as the mean and standard deviation (SD)/standard error (SE). Statistical analysis was performed by GraphPad Prism using one and two way ANOVA analysis and $p < 0.05$ was considered as statistically significant.

RESULTS

Particle size and zeta potential of nanoparticles

Nanoparticles were prepared by emulsion solvent evaporation method and their particle size and zeta potential was measured directly after preparation. Also, to investigate the effect of freeze-drying on particle size, particle size of lyophilized nanoparticles (freeze-dried with 5% trehalose) were measured. Firstly, three different sonication times were studied to investigate effect of sonication time on the size and encapsulation efficiency of nanoparticles. Particle size and zeta potential data of blank and freshly prepared and lyophilized TLM loaded nanoparticles (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 nanoparticle size was between 218-238 nm for blank formulations and 273-333 nm for TLM-NP formulations. Smallest nanoparticle size was obtained by 10 min sonication, therefore, formulations of different polymer:drug ratios were prepared by performing 10 min sonication. After freeze-drying process nanoparticle size was decreased for both blank and TLM formulations which indicates stabilizing effect of trehalose. Zeta potential values of freeze dried formulations were slightly lower than freshly prepared nanoparticles and were between -7.0-9.5 mV.

Table 1. Effect of sonication time on particle size, PDI and zeta potential of freshly prepared and freeze-dried formulations (n=3, mean ±SD)

Sonication time (min)		**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

Particle size and zeta potential values of TLM loaded nanoparticles which were prepared by using different drug amount is given in **Table 2**. Particle size of the formulations with 25:2 and 25:0.5 polymer:drug ratios was smaller than the formulation with 25:1 polymer:drug ratio, for freshly prepared nanoparticles. Freeze-dried formulations showed different behaviors 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.

Table 2. Effect of polymer:drug ratio on particle size, PDI and zeta potential of TLM loaded nanoparticles (n=3, mean±SD).

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

Encapsulation efficiency and drug loading capacity

Three different polymer:drug ratios were studied to investigate to effect on nanoparticle encapsulation efficiency. Encapsulation efficiency and drug loading capacity of TLM loaded nanoparticles is presented at Table 3. Highest drug loading was obtained by the formulation of 25:1 polymer:drug ratio.

Table 3. Encapsulation efficiency and drug loading capacity of TLM loaded nanoparticles (n=3, mean±SD).

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

In vitro drug release

The nanoparticle formulation with highest encapsulation efficiency (polymer:drug ratio, 25:1, mg:mg) was selected for in vitro drug release experiments. Release profiles of TLM loaded nanoparticles 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 nanoparticles showed controlled release. Also, TLM nanoparticles displayed higher dissolution, compared to 17.2 % release of TLM in 24 h.

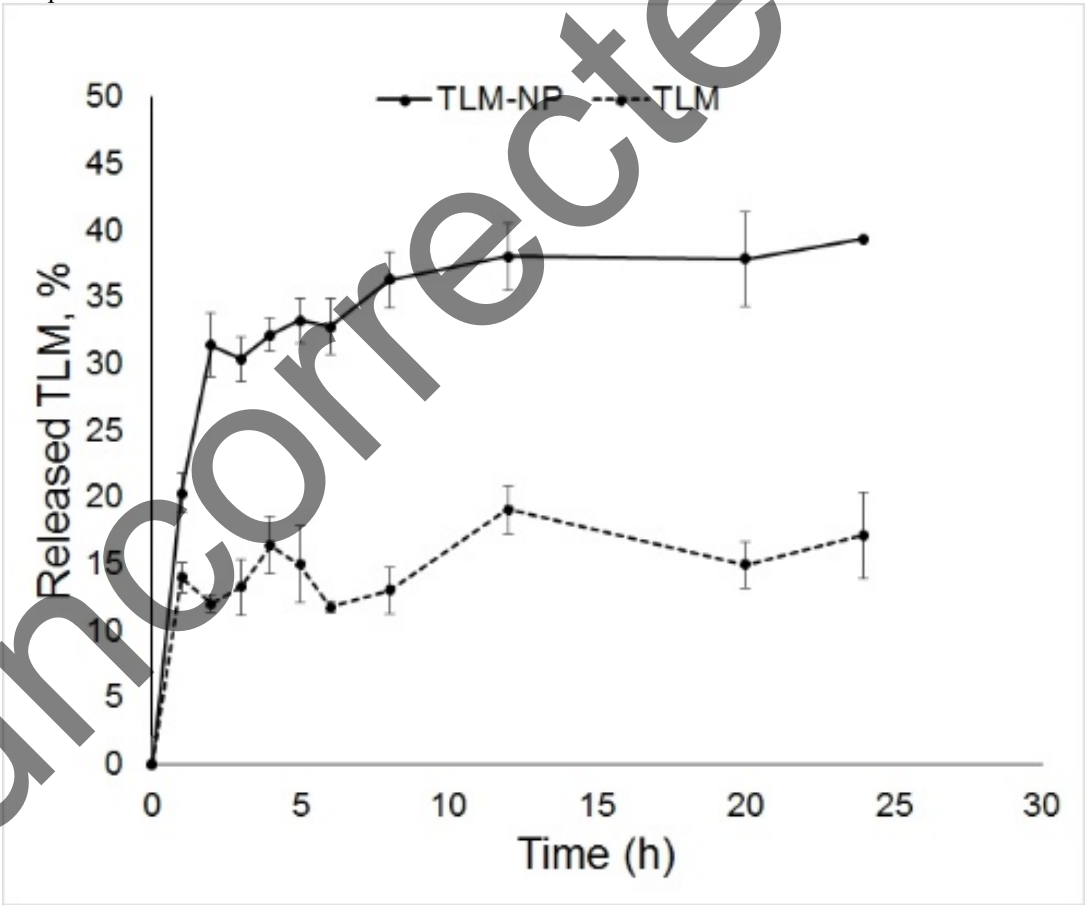

Figure 1. In vitro release profile of TLM loaded nanoparticles and TLM (n=3, mean±SE).

Scanning electron microscopy (SEM)

Freshly prepared nanoparticle suspension (polymer:drug ratio, 25:1, mg:mg) was placed on an aluminum foil and after drying at atmospheric conditions nanoparticles were visualized by SEM as presented in Figure 3.

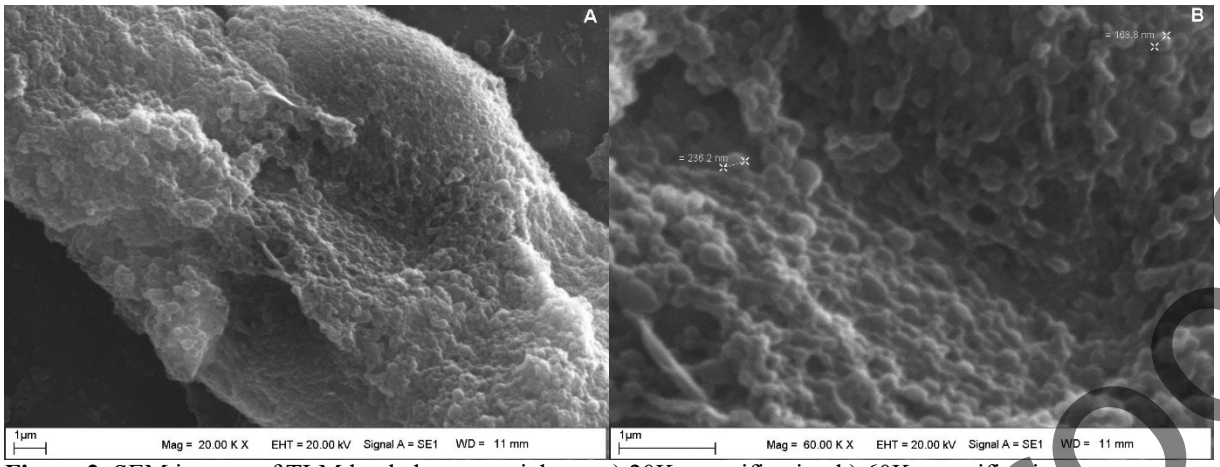

Figure 2. SEM images of TLM loaded nanoparticles at a) 20K magnification b) 60K magnification.

Cell culture studies

MCF-7 and MDA-MB-231 cells were used as model cell lines to investigate whether there is an anti-proliferative effect of TLM and TLM loaded nanoparticles on breast cancer cells. The cell viability results for 24, 48 and 72 h incubation periods were given in Figure 3, 4 and 5, respectively.

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

For MDA-MB-231 cells, blank nanoparticles did not reduce cell viability below 76% even at 72 h incubation period (Figure 3b, 4b 5b). TLM solution reduced cell viability in a dose dependent manner, especially it was pronounced in 48 and 72 h (Figure 4b, 5b). Interestingly TLM-NP formulation did not cause any significant loss of viability, 78% was the lowest viability was obtained by TLM-NP formulation at 72 h.

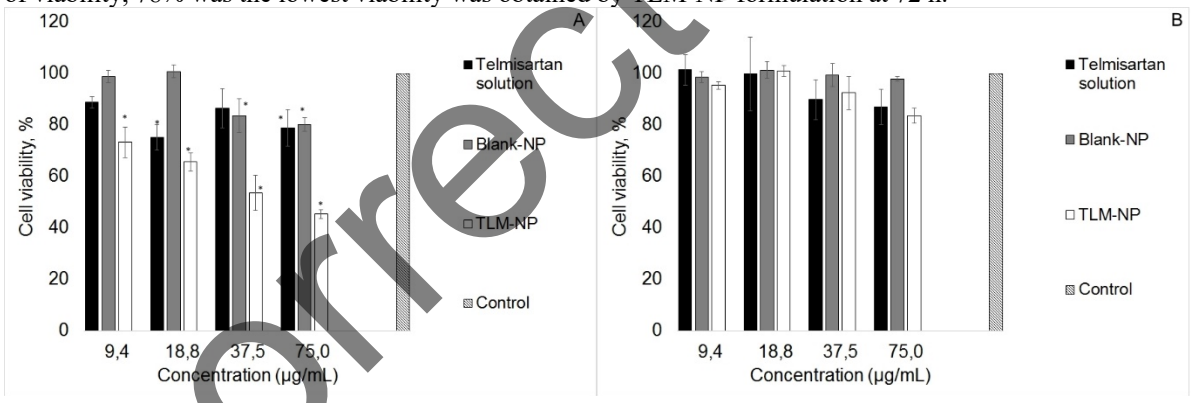

Figure 3. Cell viability of formulations after 24 h incubation. A: MCF-7 cells, B: MDA-MB-231 cells (n=3, mean±SE). * indicate significantly different from control ($p<0.05$).

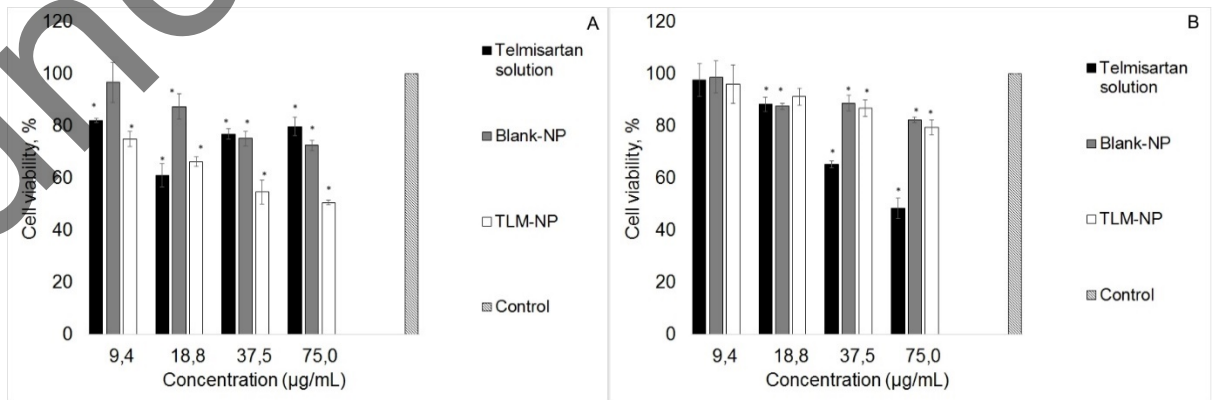

Figure 4. Cell viability of formulations after 48 h incubation. A: MCF-7 cells, B: MDA-MB-231 cells (n=3, mean±SE). * indicate significantly different from control ($p<0.05$).

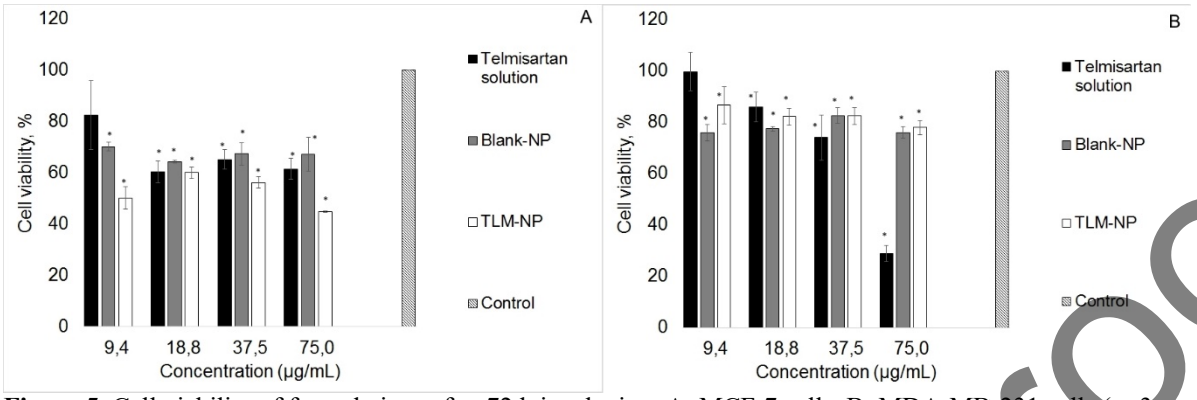

Figure 5. Cell viability of formulations after 72 h incubation. A: MCF-7 cells, B: MDA-MB-231 cells (n=3, mean±SE). * indicate significantly different from control ($p<0.05$).

DISCUSSION

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

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

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

In this study, TLM loaded and blank nanoparticles were prepared by biodegradable PLA polymer. As indicated in literature PLA is a biocompatible, biodegradable polymer, shows low toxicity and high mechanic strength so that in this study PLA polymer were chosen for these several advantages.[23, 24] Nanoparticles were prepared by emulsion solvent evaporation method which involves emulsification of polymer solution and then evaporation of solvent which precipitates the polymer as nanoparticles. In order to obtain an optimized formulation, sonication time and polymer:drug ratio were evaluated in terms of particle size, polydispersity index, encapsulation efficiency and drug loading capacity. The particle size and polydispersity index were measured by dynamic light scattering method. Several parameters like sonication time and polymer: drug ratio highly effect the physicochemical properties of nanoparticles including particle size, encapsulation efficiency and drug loading ratio. So that, 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 blank nanoparticles in the range of 218-238 nm, the size of TLM-NP formulations was in the range of 273-333 nm. During emulsification process sonication leads to dispersion of organic phase into small nanodroplets, so it is expected that by increasing sonication time, more energy will be released and smaller droplets will be dispersed so consequently smaller nanoparticles will be obtained.[25] Increasing sonication time from 5 min to 10 min reduced particle size but nanoparticle size was increased when 20 min sonication performed, compared to 10 min sonication. The decrease in particle size as the sonication time increases is expected, however several scientific papers reports that there is a threshold value.[26,27] After that threshold value is reached, increasing sonication time does not reduce particle size significantly. Also, it should be noted that sonication process 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 loaded nanoparticles (formulations prepared by different sonication times and drug amounts) was found statistically significant ($p<0.05$). As expected, encapsulated drug into the PLA nanoparticles, caused the increase of particle size. Polydispersity index results of blank and TLM loaded nanoparticles were in the range of 0.1-0.3, indicating the narrow and homogenous size distribution. Besides the sonication time, the second important parameter, different drug: polymer ratios were tested. Increasing drug amount from 0.5 to 1 mg in formulation, caused the increase of particle size. But by using 2 mg drug in the formulation, smaller sized mean particle size was obtained. However, PDI was increased for this formulation, indicating size distribution was not narrow as other formulations where 0.5 and 1 mg TLM was used. The effect of freeze-drying on the particle size of nanoparticles was also investigated. Addition of trehalose as cryoprotectant to nanoparticle suspension before freeze-drying process resulted in decreased particle size generally. Similarly, Fonte et al.[28] reported that particle size of polymeric nanoparticles lyophilized with trehalose was decreased and this may be related to adsorption of cryoprotectants to nanoparticle surface and their particular behavior during freeze-drying. Negative zeta potential values of blank and TLM loaded nanoparticles were decreased after lyophilization. This is attributed to the rearrangement of surfactants on nanoparticle surface. Also, trehalose may mask the surface of nanoparticles due to hydrogen bonding between nanoparticle and cryoprotectant.[29]

Encapsulation efficiency was increased as higher drug amounts was used and highest drug loading was achieved by 25:1 polymer:drug ratio. In vitro drug release experiments showed that TLM was released from the nanoparticles slowly and in a sustained manner (40% drug release at 24 h). Drug's partitioning between polymer and aqueous release medium influences the release rate.[30] Slow TLM (a hydrophobic drug) release from hydrophobic PLA nanoparticles is explained by the solubility of drug in the polymer and its lower partitioning to aqueous phase. Also, coarse TLM powder dissolution (17.2 % in 24 h) was significantly slower than TLM-NP formulation ($p<0.05$), indicating increased solubility of TLM. 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 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 nanoparticles (Figure 2) shows that nanoparticles were clustered together during drying process but individual spherical nanoparticles were easily distinguished in images which are obtained at higher magnifications as expected.

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

STUDY LIMITATIONS

TLM nanoparticle formulations were prepared and their antiproliferative effect was investigated, however investigation of anticancer effect mechanisms was not in the scope of this study.

CONCLUSION

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

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Conflict of interest

No conflict of interest was declared by the authors.

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