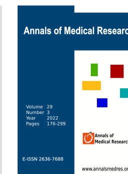
Current issue list available at AnnMedRes

Annals of Medical Research

journal page: www.annalsmedres.org



Combination of daratumumab and doxorubicin loaded polycaprolactone nanoparticles in the treatment of diffuse large B-cell lymphoma

Nazlı Erdoğar[a]

[a] *Hacettepe University Faculty of Pharmacy Department of Pharmaceutical Technology, Sıhhiye, Ankara, Turkey*

ARTICLE INFO

Keywords:
Daratumumab; doxorubicin; polycaprolactone; nanoparticle; B-cell lymphoma

Received: Aug 01, 2021
Accepted: Jan 18, 2022
Available Online: March 18, 2022

DOI:
10.5455/annalsmedres.2021.07.505

Abstract

Aim: The purpose of this study is to evaluate the activity of daratumumab in combination with polycaprolactone (PCL) nanoparticles (NPs) for decreasing toxicity and enhancing the anticancer efficiency of doxorubicin (Dox) for diffuse large B-cell lymphoma (DLBCL).

Material and Methods: Polycaprolactone nanoparticles were developed using double emulsion technique. For characterization of blank and doxorubicin loaded PCL nanoparticles; particle size, polydispersity index and surface charge were determined. Cell culture study was realized to conclude the cytotoxicity of Dox-loaded PCL NPs alone or combination with Daratumumab.

Results: Blank and Dox-loaded PCL nanoparticles remained within 235 nm and 250 nm, respectively. Zeta potential values were -3.9 and -13.3 mV for blank and Dox-loaded PCL nanoparticles, respectively. In cell culture study, whole formulations showed a time-dependent model; the cytotoxicity enhanced with the increase of incubation time. Blank nanoparticles have no toxicity; cell viability was above 85%. Dox-loaded nanoparticles showed more toxicity to A20 cells than Dox solution. Dox-loaded NPs in combination Daratumumab exhibited higher cell toxicity than Dox-loaded NPs and mAb solution, respectively ($P < 0.05$).

Conclusion: Dox-loaded PCL nanoparticles in combination with antibody-targeted chemotherapy could be a considered as promising synergistic strategy for controlling tumor growth by increasing the antitumor efficacy while minimizing toxicity.

Copyright © 2022 The author(s) - Available online at www.annalsmedres.org. This is an Open Access article distributed under the terms of Creative Commons Attribution-NonCommercial-NoDerivatives 4.0 International License.

Introduction

Non-Hodgkin lymphoma (NHL) is a class of hematologic cancer, that explains for 85% of all lymphoma and 4% of whole malignancies [1, 2]. Diffuse large B-cell lymphoma (DLBCL) is the most frequently subtype of NHL. Using standard rituximab and an anthracycline treatment, 66% of patients will be in a disease-free period four years after first diagnosis, and may be considered to be cured [3]. On the other hand, one-third of the patients gets refractory to first-line treatment or finally relapses. Therefore, betterment of the treatment efficiency is an important requirement with novel drug combinations or new therapeutic approach.

In standard chemotherapy, most anticancer drugs are a little selective to cancer cells, and toxicity to healthy tissues can be high, necessitating dose reduction and discontinuance of therapy [4]. Also in clinical NHL treatment, applications are limited due to serious adverse effects and multidrug resistance [2]. Thus, nanotechnology has lately demonstrated to be a favourable approach for lymphoma. Drug encapsulation with nanosystems can enhance circulation time, decrease contact to healthy tissue and correspondingly, increase antitumor efficiency and minimize adverse effects [5, 6]. Nanoparticles have been commonly studied for carrying anticancer agents for lymphoma therapy. Different preclinical/clinical trials related nanosystems for lymphoma therapy are continuing or have recently realized: paclitaxel protein-bound particles, doxorubicin-loaded liposomes, liposomal vincristine, CD22-targeted PEGylated liposomes, rituximab lipid nanoparticles, PEGylated poly(lactic-co-glycolic acid) (PLGA) nanoparticles etc [7, 8].

Successful cancer therapy is dependant upon safe and efficient delivery of chemotherapeutics to tumor tissue, while keeping away from adverse effects. Nanoparticles are promising systems as they allow localized drug delivery to target site and increased cellular uptake. Nanoparticulate

*Corresponding author:
Email address: nerdogar@hacettepe.edu.tr (Nazlı Erdoğar)

systems can be developed using natural/synthetic polymers. Also, these systems could pass biological barriers or help from enhanced permeability and retention (EPR) effect due to small particle size. Therefore, nanoparticles can protect active agent in biological systems and let targeting to tumor site [9, 10].

One trend to overcome chemotherapy drawback, and enhance efficiency, is to target anticancer agent to tumor site via antibodies that are special for proteins spesifically over-expressed by cancer cells (antibody-mediated chemotherapy, AMC) [11]. Daratumumab (Darzalex®) is a first, human monoclonal antibody (mAB) targeting the CD38. FDA approved in 2015 as a monotherapy for multiple myeloma patients, have taken at least 3 prior therapies [12]. A study demonstrated that daratumumab shows cytotoxic activity in vitro via antibody-dependent cellular cytotoxicity (ADCC) and antibody-dependent cellular phagocytosis (ADCP) in CLL cells and cell lines in chronic lymphocytic leukemia (CLL). In vivo, daratumumab importantly prolongs overall survival of animals in systemic CLL murine models. An another study searched in vitro-in vivo activity of daratumumab on DLBCL cells as monotherapy and in combination with standard therapies. In a DLBCL xenograft model, daratumumab antitumor activity was comparable to chemotherapy regimen and the addition of daratumumab to either chemotherapy or Rituximab-chemotherapy cause tumor regression [13].

The obejctive of this study is to determine in vitro activity of daratumumab on DLBCL cells as monotherapy and in combination with polycaprolactone nanoparticles.

Materials and Methods

Materials

Doxorubicin (Dox, molecular weight (MW): 543.17 g/mol, catalog number D1515), polycaprolactone (PCL) (Mn: 80.000, catalog number 440744), Pluronic F-68 (catalog number P1300) and polyvinylalcohol (PVA, catalog number P8136) were purchased from Sigma-Aldrich, USA. Dichloromethane (DCM) was obtained from J. T. Baker, USA. All other reagents were of reagent grade.

Preparation of Blank and Doxorubicin-Loaded PCL Nanoparticles

Blank and Doxorubicin loaded PCL Nanoparticles (NPs) were developed with double emulsion technique [14, 15]. 1 mL PF68 solution (1%, w/v) containing 2.5 mg Dox (10% of PCL) was emulsified in dichloromethane (5 mL) including 0.5% PCL by ultraturrax (IKA T25 basic) at 13000 rpm for 5 minutes upon an ice. This emulsion was emuslfied into 20 mL aqueous solution containing 0.1% PVA in 1% PF68 solution, then stirred by ultraturrax at 1 000 rpm for five minutes upon an ice. Last, organic solvent was removed using evaporator (IKA RV 10 basic).

Particle Size

Particle size was measured with Malvern NanoZS (Zetasizer NanoSeries ZS, Malvern Instruments, UK) at 25 °C. The results were given as mean diameter (nm)±standard deviation (SD) and polydispersity index (n=3).

Zeta Potential

Surface charge was determined using Malvern NanoZS (Zetasizer NanoSeries ZS, Malvern Instruments, UK) at 25 °C (n=3).

Cell Culture

A20 cell line (ATCC® TIB-208™) was purchased from American Type Culture Collection (ATCC) (Rockville, MD). A20 cells were cultured in RPMI-1640 containing with fetal bovine serum (FBS) (Biochrom, Germany), 100 mg/mL of streptomycin and 100 unit/mL of penicillin (Sigma, USA) and 2-mercaptoethanol at 37°C in CO2 incubator.

In Vitro Cytotoxicity

A20 cells were seeded into 96-well culture plates at an initial density of 5x104 cells/well in 100 µL RPMI-1640 containing 10% FBS, 1% penicillin-streptomycin. The cultures were maintained at 37 °C in a 5% CO2 incubator. After 24 h of incubation time, fresh medium containing either different concentrations of drug solution (100µM, 50 µM, 25 µM, 12.5 µM, 6.25 µM, 3.12 µM for Dox and 10 µg/mL, 5 µg/mL, 2.5 µg/mL, 1.25 µg/mL, 0.62 µg/mL, 0.31 µg/mL for Daratumumab (mAb solution) was added to determine IC50 value. In order to find cell viability, WST-1 (water soluble tetrazolium salt) assay was applied. For this purpose, WST-1 (10 μL) was added in each well and incubated for 3 hours. Optical densities were determined by a microplate reader (Molecular Devices, CA) at 450 nm. The control group consisted of cells incubated in DMEM alone ($n = 6$).

Also, cells were treated with blank nanoparticle, doxorubicin loaded nanoparticle, Daratumumab (mAb+), Dox-loaded nanoparticle and drug solutions to determine cytotoxicity 24 h and 48 h. At the end of incubation time, cell viability was determined using WST-1 assay at 450 nm as defined before.

Statistical analysis

The obtained results are given as mean ± SD. Data were evaluated with Student's t-test or one-way ANOVA (SPSS Statistics). p value < 0.005 was regarded as statistically significant.

Results

Particle characterization

In this study, Doxorubicin-loaded PCL NPs were used to develop a drug delivery system for targeting of diffuse large B-cell lymphoma. Blank and drug-loaded PCL NPs were obtained with a double emulsion technique. The physicochemical characteristics of blank and drug-loaded formulations are presented in Table 1. Blank and Dox-loaded PCL nanoparticles remained within 235 nm and 250 nm, respectively. Zeta potential values were -3.9 and -13.3 mV for blank and Dox-loaded PCL nanoparticles, respectively. The polydispersity indexes were found < 0.2. No differences were seen in particle size and surface charge when nanoparticles were loaded with Dox (Table 1).

Table 1. Characterization of blank and Dox-loaded PCL nanoparticles (mean±SD, n=3)

Formulations	Blank NPs	Dox-loaded NPs
Particle size (nm)	235±2.6	250±5.4
Polydispersity index	0.16±0.03	0.19±0.02
Zeta potential (mV)	-3.9±1.5	-13.3±2.7

In Vitro Cytotoxicity

The biological efficacy of Dox-loaded PCL NPs in A20 cells was measured using MTT. In vitro toxicity of different formulations was shown as the cell viability of A20 cell line at 24 h and 48 h (Figure 1). Cytotoxicity of Dox-loaded NPs was refered to Doxorubicin solution, mAb solution, Dox-loaded nanoparticles in combination mAb, blank nanoparticles, and no treatment. To determine optimum dose, the IC50 values of Dox and Daratumumab were calculated as 7.36 and 0.64 µM in 24 h, respectively. It can be observed that all formulations showed a time-dependant pattern; cytotoxicity enhanced with the enhance of incubation time. Blank nanoparticles exhibited no toxicity; cell viability was above 85%. Doxorubicin-loaded NPs were shown more toxicity to A20 cells in comparison to Dox solution (Figure 1).

Dox-loaded NPs in combination Daratumumab exhibited higher cell toxicity than Dox-loaded NPs and mAb solution, respectively ($P < 0.05$).

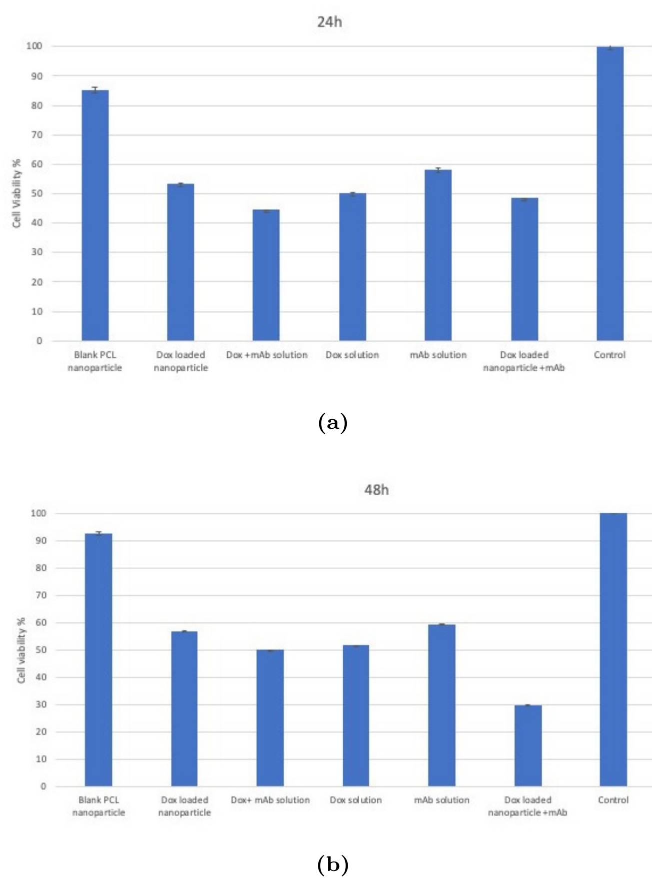


Figure 1. The in vitro cytotoxicity of Doxorubicin, mAb solution, blank NPs, Dox-loaded NPs and Dox-loaded NPs in combination mAb in A20 cells for a) 24 h and b) 48 h (n=6, mean±SD).

Discussion

To improve anticancer drug efficiency a variety of drug delivery systems (DDS) have been studied in the literature. Nano-DDS can help to overcome insolubility of hydrophobic drugs, prolong the time of blood circulation, decrease the toxicity and increase drug concentration in the tumor tissue due to the enhanced permeability and retention (EPR) effect [16]. Moreover, passive accumulation of DDS in the tumor tissue can be enhanced by various targeting moieties including monoclonal antibodies and their fragments, saccharides etc [17]. Lai et al. conjugated anti-CD30 antibody on Doxil for the treatment of anaplastic large cell lymphoma (ALCL). The results showed that CD30 functionalization greatly improved the affinity and inhibitory effect of Doxil to ALCL cells in vitro and inhibition of K299 ALCL tumor growth in vivo [18]. Lidicky et al. studied a novel actively-targeted mAb-polymer-drug conjugate (APDC) composed from central mAb decorated with biocompatible polymer chains carrying doxorubicin. Anti-lymphoma efficacy of APDC nanotherapeutics was evaluated in vitro and in vivo lymphoma xenograft model. The obtained data showed that daratumumab appears especially promising, especially in the subgroup of patients with relapsed, CD38-positive B-NHL. The anti-lymphoma efficiency of APDCs is mediated by targeted delivery of doxorubicin to the lymphoma with limited immunologic mode-of-action [19].

In this study, blank and drug loaded PCL NPs were prepared by double emulsion technique and their physical characterization was analysed by dynamic light scattering technique. Double emulsion method is a suitable preparation method to encapsulate hydrophilic drugs such as doxorubicin, resulting enhanced efficacy and controlled size distribution [20]. Particle size was found between 235-250 nm, thus demonstrating being a good method for hydrophilic drug encapsulation. Also, formulations were below 400 nm indicating passive targeting and accumulation in tumor sites [21]. Zeta potential is important to display the physical stability of the nanoparticles and can depend on concentration and polymer MW and surfactants in aqueous phase. In this study, negative zeta potential value was measured suggesting kinetically stable formulations. Also, the negative charge of nanoparticles can be attributed to no doxorubicin on nanoparticles surface.

Blank nanoparticles exhibit non-toxic behaviour; so this findings showed that polymer could be used as a suitable carrier for drug delivery [14]. Doxorubicin loaded NPs showed more toxicity to A20 cells than Dox solution. Moreover, co-administration of Dox-loaded nanoparticles with Daratumumab did reduce the cell viability % (Figure 1), indicating that there is a synergistic cytotoxic effect among Dox-loaded NPs and Daratumumab. Hence, it can be concluded that these effect of drug loaded PCL NPs can not only be dependent upon free drug released into surrounding media from degrading nanoparticles but must depend on penetration of Daratumumab by lymphoma cells

and increased efficacy of doxorubicin (8). Vidal-Crespo et al. showed that daratumumab, at clinically appliable dose, efficiently penetrates a in vitro lymphoma organoid model. Also, a significant reduction of the sphere volume was observed with daratumumab in 3D lymphoma model. These results agree with daratumumab efficiency in diffuse large B-cell lymphoma [13].

Daratumumab has shown a targeted therapeutic effect and an obvious anti-multiple myeloma curative effect. Despite, the clinical application is not yet widespread. Also, when combined with lenalidomide and dexamethasone, infusion reactions are likely to occur (22). Different studies have been ongoing to test the combination of daratumumab with chemotherapeutic drugs like Ara-C and doxorubicin in lymphoma models (23). In our study, a nanoparticulate drug delivery system of doxorubicin in combination with Daratumumab was evaluated in the cell culture level. As a limitation, the results of cell culture studies may also be misleading due to cells do not have the same genotypic and/or phenotypic characteristics as in the human body [24, 25]. Hence, the obtained data should be supported by animal studies in the future in order to be more reliable. To obtain the maximum benefit clinically, we believe that ongoing studies on the advancement of combinatorial nanocarriers will lead to the ideal clinical combination therapy sought after by physicians.

Conclusion

In the light of obtained data, it was possible to develop a nanoparticulate drug delivery system of anticancer drug doxorubicin in combination with Daratumumab for the treatment of DLBCL. Polymeric nanoparticles were developed using double emulsion technique successfully. Nanoparticles were able to exhibit a suitable particle size, surface charge and higher cytotoxicity in A20 cell line. Also, Dox-loaded nanoparticles in combination Daratumumab exhibited higher cell toxicity in lymphoma cells. At the end of characterization studies, the results rendered visionary concepts for in vivo and clinical studies planned to be carried out with further studies.

Herein, it is considered that in vitro characterization and cell culture studies showed that Doxorubicin-loaded nanoparticles in combination with Daratumumab can supply encouraging results in further animal studies. In conclusion, Dox-loaded PCL nanoparticles in combination with antibody-targeted chemotherapy could be a considered as promising synergistic strategy for controlling tumor growth by improving the efficacy while reducing toxicity.

References

1. Knapp CM, Whitehead KA. In pursuit of a moving target: nanotherapeutics for the treatment of non-Hodgkin B-cell lymphoma. Expert opinion on drug delivery. 2014;11(12):1923-37.
2. Li H, Guo K, Wu C, Shu L, Guo S, Hou J, et al. Controlled and Targeted Drug Delivery by a UV-responsive Liposome for Overcoming Chemo-resistance in Non-Hodgkin Lymphoma. Chem Biol Drug Des. 2015;86(4):783-94.
3. Sehn LH, Donaldson J, Chhanabhai M, Fitzgerald C, Gill K, Klasa R, et al. Introduction of combined CHOP plus rituximab therapy dramatically improved outcome of diffuse large B-cell lymphoma in British Columbia. Journal of clinical oncology : official journal of the American Society of Clinical Oncology. 2005;23(22):5027-33.
4. Allen TM. Ligand-targeted therapeutics in anticancer therapy. Nature reviews Cancer. 2002;2(10):750-63.
5. Barenholz Y. Doxil(R)–the first FDA-approved nano-drug: lessons learned. Journal of controlled release : official journal of the Controlled Release Society. 2012;160(2):117-34.
6. Gabizon A, Catane R, Uziely B, Kaufman B, Safra T, Cohen R, et al. Prolonged circulation time and enhanced accumulation in malignant exudates of doxorubicin encapsulated in polyethylene-glycol coated liposomes. Cancer research. 1994;54(4):987-92.
7. Chen WC, Completo GC, Sigal DS, Crocker PR, Saven A, Paulson JC. In vivo targeting of B-cell lymphoma with glycan ligands of CD22. Blood. 2010;115(23):4778-86.
8. Park J, Fong PM, Lu J, Russell KS, Booth CJ, Saltzman WM, et al. PEGylated PLGA nanoparticles for the improved delivery of doxorubicin. Nanomedicine : nanotechnology, biology, and medicine. 2009;5(4):410-8.
9. Kingsley JD, Dou H, Morehead J, Rabinow B, Gendelman HE, Destache CJ. Nanotechnology: a focus on nanoparticles as a drug delivery system. J Neuroimmune Pharmacol. 2006;1(3):340-50.
10. Bei D, Meng J, Youan BB. Engineering nanomedicines for improved melanoma therapy: progress and promises. Nanomedicine (Lond). 2010;5(9):1385-99.
11. Nevala WK, Buhrow SA, Knauer DJ, Reid JM, Atanasova EA, Markovic SN. Antibody-Targeted Chemotherapy for the Treatment of Melanoma. Cancer research. 2016;76(13):3954-64.
12. McKeage K. Daratumumab: First Global Approval. Drugs. 2016;76(2):275-81.
13. Vidal-Crespo A, Matas-Cespedes A, Rodriguez V, Rossi C, Valero JG, Serrat N, et al. Daratumumab displays in vitro and in vivo anti-tumor activity in models of B-cell non-Hodgkin lymphoma and improves responses to standard chemo-immunotherapy regimens. Haematologica. 2020;105(4):1032-41.
14. Varan C, Bilensoy E. Cationic PEGylated polycaprolactone nanoparticles carrying post-operation docetaxel for glioma treatment. Beilstein J Nanotechnol. 2017;8:1446-56.
15. Erdogar N, Iskit AB, Eroglu H, Sargon MF, Mungan NA, Bilensoy E. Cationic core-shell nanoparticles for intravesical chemotherapy in tumor-induced rat model: Safety and efficacy. International journal of pharmaceutics. 2014;471(1-2):1-9.
16. Maeda H, Nakamura H, Fang J. The EPR effect for macromolecular drug delivery to solid tumors: Improvement of tumor uptake, lowering of systemic toxicity, and distinct tumor imaging in vivo. Advanced drug delivery reviews. 2013;65(1):71-9.
17. Bertrand N, Wu J, Xu X, Kamaly N, Farokhzad OC. Cancer nanotechnology: the impact of passive and active targeting in the era of modern cancer biology. Advanced drug delivery reviews. 2014;66:2-25.
18. Molavi O, Xiong X-B, Douglas D, Kneteman N, Nagata S, Pastan I, et al. Anti-CD30 antibody conjugated liposomal doxorubicin with significantly improved therapeutic efficacy against anaplastic large cell lymphoma. Biomaterials. 2013;34(34):8718-25.
19. Lidicky O, Klener P, Machova D, Vockova P, Pokorna E, Helman K, et al. Overcoming resistance to rituximab in relapsed non-Hodgkin lymphomas by antibody-polymer drug conjugates actively targeted by anti-CD38 daratumumab. Journal of controlled release : official journal of the Controlled Release Society. 2020;328:160-70.
20. Martinez NY, Andrade PF, Duran N, Cavalitto S. Development of double emulsion nanoparticles for the encapsulation of bovine serum albumin. Colloids Surf B Biointerfaces. 2017;158:190-6.
21. Erdogar N, Iskit AB, Mungan NA, Bilensoy E. Prolonged retention and in vivo evaluation of cationic nanoparticles loaded with Mitomycin C designed for intravesical chemotherapy of bladder tumours. Journal of microencapsulation. 2012;29(6):576-82.
22. Zhong W, Zhang X, Zhao M, Wu J, Lin D. Advancements in nanotechnology for the diagnosis and treatment of multiple myeloma. Biomater Sci. 2020;8(17):4692-711.
23. Ghosh S, Lalani R, Patel V, Bardoliwala D, Maiti K, Banerjee S, et al. Combinatorial nanocarriers against drug resistance in hematological cancers: Opportunities and emerging strategies. Journal of controlled release : official journal of the Controlled Release Society. 2019;296:114-39.
24. Karaarslan N, Yilmaz I, Sirin DY. Toxicity of the acetyl-para-aminophenol group of medicines to intact intervertebral disc tissue cells. Experimental and therapeutic medicine.

2021;21(2):147.

25. Kaya YE, Karaarslan N, Sirin DY, Ozbek H, Kaplan N, Yilmaz I. Investigation of the Effects of Methylphenidate, an Amphetamine Derivative, on Intervertebral Disc Tissue Cell Cultures and Matrix Structures. Turk Neurosurg. 2019;29(5):734-42.