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Research Article
Effect of Tamoxifen Capsulated in Nanoparticles on Serum Antioxidant in Female Wistar Ovariectomized Rats

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Abstract

Background: Recently tamoxifen-loaded solid lipid nanoparticles were introduced as innovative drug delivery system for the treatment of hormone-sensitive breast cancer. An *in vitro* study was conducted to determine the antioxidant status induced by tamoxifen-loaded solid lipid nanoparticles in female Wistar ovariectomized rats. **Materials and Methods:** In this study 36 female Wistar rats between 7-8 weeks old, weighing 159.42±6.43 g were divided randomly into 6 groups of 6 rats each. The 1st group (H) served as non-ovariectomized healthy rats and the 2nd group (C) was ovariectomized as control group. The 3rd (O), 4th (T), 5th (TS) and 6th group (S) were ovariectomized and treated with olive oil, tamoxifen, tamoxifen-loaded solid lipid nanoparticles (TMX-SLN) and solid lipid nanoparticles-free drug (SLN), respectively. The groups 2-5 were treated (2 mg kg^{-1}) for 21 consecutive days using gastric intubations. At the end of the study, the rats were sacrificed and examined for the serum oxidative stress (TAC, TOS and MDA). The endometrial tissues were also evaluated histologically for possible damage. **Results:** The results of the study revealed that either tamoxifen or tamoxifen-loaded SLN during 21 days resulted in increased serum total anti-oxidant and decreased serum total oxidant status insignificantly compared to healthy and non-treated ovariectomized rats. The results also showed that the lipid peroxidation was increased insignificantly in treated groups when compared to the healthy and non-treated ovariectomized rats. No structural abnormalities were observed in endometrial tissues. **Conclusion:** It can be concluded that the effect of tamoxifen on the oxidative stress system is not affected and its bioavailability is increased when it encapsulated in SLN.

Key words: Stress oxidative, solid lipid nanoparticles, tamoxifen

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Breast cancer makes up almost 18% of all malignancies and the prevalent cancer in women worldwide[1]. The antiestrogen molecule, tamoxifen (TMX) is a strong hydrophobic endocrine drug being used widely for the treatment of hormone-sensitive breast cancer and high risk patients[2]. In addition to anticarcinogenic and antioxidant effects, TMX also has toxic side-effects such as increased risk of uterine cancer[3,4]. Endometrial disease such as hyperplasia, polyps, carcinoma and sarcoma has been recognized in approximately 36% of postmenopausal patients with breast cancer and in the cases under TMX treatment. The dose-dependent side-effects of TMX also include liver cancer, increased blood clotting and ocular adverse effects such as retinopathy and corneal opacities. These findings suggest that small doses given through colloidal delivery systems would be useful for long-term therapy of breast cancers[5].

Optimistically progress in nanomedicine regarding cancer can minimize many problems associated with drug delivery in cancer chemotherapy[6]. In recent year's solid lipid nanoparticles (SLNs) have been recommended for drug delivery systems. The main benefit of solid lipid nanoparticles is their lipid matrix composition which is physiologically tolerable and with virtual little acute and chronic toxicity. Additional advantages claimed for SLNs are avoidance of the use of organic solvent for the production of SLNs, widespread application, large-scale production, better bioavailability, protection of drug from degradation agents like water and light and improved controlled drug release[7,8].

Interaction of nanoparticles with biological systems has some un-predictable results, thus understanding their toxicity is essential to prevent their harmful effects on the human body[9-13]. Although, there are some reports about oxidative stress of nanoparticles, yet it is not clear how oxidative state could make cells more sensitive to cytotoxic nanoparticles. Oxidative stress would be increased in some pathological situations such as inflammation. Hence, it is important to know how oxidative stress could change the sensitivity of cells to cytotoxic nanoparticles[14]. Some special features such as high surface area, having 100 nm in size and easy penetration into the cells and proteins, sensing and detection of biological environments, make inorganic nanoparticles as potential candidate for applications in biomedical fields[15].

In this study, tamoxifen encapsulated in solid lipid nanoparticles was administered to adult ovariectomized female rats and the effects of this drug on antioxidant enzymes in endometrial tissue were evaluated.

MATERIALS AND METHODS

Chemicals and methods: Softisan®154 (S154) or hydrogenated palm oil was a gift from CONDEA (Witten, Germany). Lipoid S100 (soy lecithin) was a gift from Lipoid KG (Ludwigshafen, Germany). Thimerosal, sorbitol and tamoxifen were purchased from Sigma.

The SLN was prepared using the high-pressure homogenization (HPH) technique[16]. Briefly, a mixture of palm oil (S154) and soy lecithin (S100) were weighed at ratio of 7:3, respectively heated until a clear yellowish solution was obtained and then mixed with oleyl alcohol, thimerosal, sorbitol and bi-distilled water. The obtained lipid matrix (SLN) then mixed with tamoxifen (TMX) at ratio of 5:1 using an Ultra Turrax® (Ika, Staufen Germany) at 13000 rpm for 10 min. The mixture of TMX-SLN was then incubated at 50-60°C while stirring overnight at 500 rpm and then exposed to air until solidification. The TMX-loaded SLN was then characterized by the particle size, polydispersity index (PI) and zeta potential using a high-performance particle sizer (HPP5001, Malvern Instruments, Worcestershire, UK) and analyzer (Zeta sizer; ZEN-2600, Malvern Instruments, Worcestershire, UK) in triplicate.

Animal study: Thirty six virgin female Wistar rats aged 7-8 weeks, weighing 150-200 g were purchased from Animal Centre of Hamadan University of Medical Sciences, IRAN. The animals were housed two rats per plastic cage and allowed to acclimatize under standard conditions (12 h light/dark cycles) for 1 week. The rats were given free access to distilled water and commercialized food throughout the experiment. The rats were anaesthetized with a mixture of ketamine/xylazin (100/5 mg kg^{-1} b.wt.) by intraperitoneal injection and were bilaterally ovariectomized under standard CAF procedure. To ensure the accuracy of ovarian tissue harvesting, microscopic sections were prepared from tissue harvested and stained using H and E method. After operation, the animals were allowed to recover for 2 weeks before the starting of the study. The rats divided into 6 groups of 6 rats each. The 1st (H) and 2nd (C) groups served as un-ovariectomized healthy and untreated ovariectomized rats, respectively. The 3rd (O), 4th (T), 5th (TS) and 6th (S) groups were ovariectomized and treated with olive oil, tamoxifen, tamoxifen-loaded SLN and

SLN-free drug, respectively. The groups 2-5 were treated (2 mg kg^{-1}) for 21 consecutive days using gastric intubations. At the end of the study, the rats were sacrificed and examined for the serum oxidative stress (TAC, TOS and MDA). The endometrial tissues were also evaluated histologically for possible damage.

Determination of oxidative stress status

Total Antioxidant Capacity (TAC): The TAC in serum samples was assessed using Ferric Reducing Antioxidant Power (FRAP) assay (FRAP)[17].

Malondialdehyde (MDA): The MDA as a lipid peroxidation index was determined using fluorometric thiobarbituric acid method[18].

Total Oxidant Status (TOS): The oxidation of ferrous ion to ferric ion accompanied with a number of oxidant species in acidic pH was used for the measurement of TOS in serum. The ferric ion was determined using xylenol orange[19].

Histopathological study: At the end of the study, the rats were sacrificed and examined for tissue abnormalities. Samples of liver and kidney from all groups were immediately fixed in 10% formalin overnight, embedded in paraffin, cut into 5 mm sections, placed on slides and stained with Hematoxylin-Eosin (H and E). The tissue sections were viewed under a light microscope (Nikon ECLIPSE TS100, Japan).

Statistical analysis: The data were expressed as Mean±Standard Deviation. For statistical analysis, the experimental values were compared with their corresponding control values. One way analysis of variance (ANOVA) incorporated in SPSS software (version 16.0) was used to show the significant difference between the experimental and control groups. The significant difference was considered 0.05 or less.

RESULTS

The SLN and TMX-loaded SLN were characterized *in vitro* for particle size, particle size distribution and zeta potential (Table 1).

The results presented in Table 2 showed that treatment with SLN, TMX and TMX-loaded SLN did not negatively affect the body weights of these animals. The body weight of all animals increased during the study period and there was no significant ($p>0.05$) difference among treatment groups.

Study of the serum oxidant and antioxidant presented in Table 3 revealed that either tamoxifen or tamoxifen-loaded SLN during 21 days resulted in increased serum total anti-oxidant and decreased serum total oxidant status insignificantly compared to healthy and non-treated ovariectomized rats. The results also showed that the lipid peroxidation was increased insignificantly in treated groups with TMX and TMX-loaded SLN when compared to the healthy and non-treated ovariectomized rats.

In this study, the endometrial tissues were also evaluated histologically for possible damage. According to Fig. 1, the endometrial tissue of ovariectomized animals exposed to TMX and TMX-SLN at does 2 mg kg^{-1} for 6 days showed no structural abnormalities.

Table 1: Characteristics of TMX and TMX-SLN

Formulation	Particle size (nm)	PI	Aspec (m^2 g^{-1})	Zeta potential (mv)
SLN	152.87±9.91[a]	0.22±0.05	19.67±1.24[a]	-15.7±1.120
TMX-SLN	251.65±33.02[a]	0.48±0.11	12.05±1.56[a]	+10.16±0.22

[a]Mean is statistically different ($p<0.05$)

Table 2: Body weight of rats treated with TMX, TMX-SLN, oil and SLN

	H	C	O	T	TS	S
Pre-treatment	152.83±3.19	153.50±1.520	162.00±2.930	166.17±3.310	165.17±4.120	161.17±1.600
Post-treatment	227.33±10.11	245.16±41.80	262.17±18.09	258.33±17.57	257.00±16.78	252.16±19.76

All values are expressed as Mean±Standard Deviation, H: Healthy animal, C: Ovariectomized untreated group, O: Olive oil group, TMX: Tamoxifen, TMX-SLN: Tamoxifen-loaded solid lipid nanoparticles, S: SLN treated group

Table 3: Serum oxidant status of rats treated with TMX, TMX-SLN, oil and SLN

	Groups						
Parameters	H	C	O	T	TS	S	p-value
TAC	0.72±0.10	0.70±0.62	1.14±0.300[a]	0.93±0.09	0.74±0.05	0.72±0.04	0.00
TOS	2.28±0.38	1.94±0.33	2.00±0.56	1.62±0.08	1.62±0.44	1.54±0.35	0.07
MDA	1.73±0.37	1.76±0.34	1.64±0.31	1.94±0.68	2.08±0.98	1.64±0.15	0.679

All values are expressed as Mean±Standard Deviation, TAC: Total antioxidant capacity, MDA: Malondialdehyde, TOS: Total oxidant status, H: Healthy animal, C: Ovariectomized untreated group, O: Olive oil group, TMX: Tamoxifen, TMX-SLN: Tamoxifen-loaded solid lipid nanoparticles, S: SLN treated group, [a]Compared to healthy and control groups

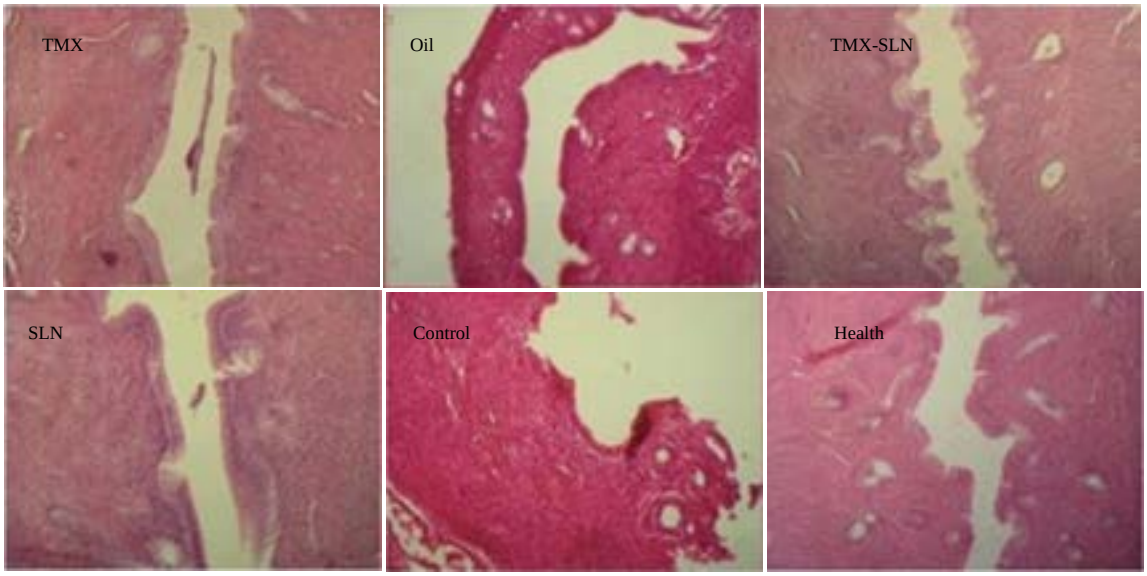

Fig. 1: Histopathological findings of rat's endometrial after treatment with tamoxifen (TMX), tamoxifen-loaded solid lipid nanoparticles (TMX-SLN), oliv oil (O) and solid lipid nanoparticles (SLN)

DISCUSSION

In this study, the average size of TMX-loaded SLNs (251.65±33.02) was significantly larger than that of the free SLNs (152.87±9.91), this may be attributed to the fact that drug is either adsorbed to particle surface or entangled in aliphatic chains of triglycerides[5]. Particle size is an important characteristic for pharmaceutical applications because it significantly affects *in vitro* and *in vivo* studies[20]. When tamoxifen was incorporated into SLNs, the increase in particle size suggested that loaded tamoxifen was either adsorbed onto the particle surface or entangled in the aliphatic chains of triglycerides. The surfaces of TMX-loaded SLNs carried a positive charge (10.16±0.22).

Zeta potential is also an important factor when evaluating the stability of colloidal system[21]. In the presence of 1 mg of tamoxifen, some of the negative charges were neutralized by the complex formation, thus leading to a less negative or positive zeta potential (Table 1). The positive charge also might be raised by the tamoxifen amino group and by tamoxifen localization on the surface of SLNs[22].

Oxidative stress is the result of an imbalance between the free radicals and reactive oxygen species such as superoxide anion, hydroxyl radical, hydrogen peroxide and body antioxidant defense system. In aerobic biological systems, to deal with free radicals and reactive oxygen species, defense mechanisms designed to neutralize the deleterious effects of these factors or to minimize striker. Some components of the immune system contain enzymes (such as superoxide dismutase, glutathione peroxidase, catalase, etc.) that are synthesized within the body but some other system components, such as vitamin E, β-carotene and have to go through their diets. Oxidative stress causes harmful effects on macromolecules such as DNA, proteins and lipids[23].

In this study, oxidative stress-caused by free tamoxifen and tamoxifen encapsulated in solid lipid nanoparticles in ovariectomized female Wistar rats were studied by determining maolndialdehyde, total antioxidant capacity and total antioxidant status. The results showed that in group treated with olive oil (group O) oxidative stress was reducedand serum antioxidant capacitywas significantly increased compared to the control group (C). This could indicate that olive oil can be a suitable solvent for water-insoluble drugs such as tamoxifen. Treatment with drug free SLN (group S) resulted in slightly reduced MDA and TOS in comparison to control group. There was no difference between two groups, S and C in TAC. It is reported that SLN with 30% lecithin and 1% oleyl alcohol as nonionic co-surfactant in aqueous phase displayed no significant cytotoxicity effect on breast cancer cell lines. In the light of these findings, SLN was found to be safe and acceptable for the incorporation of lipophilic drugs such as tamoxifen[24].

The results of this study also revealed that administration of TMX and TAM-SLN at concentration 2 mg kg^{-1} b.wt., of animals for 21 days increased TAC and decreased TOS compared to group C and H. However, there are not significant. A study showed that tamoxifen at 5 mg kg^{-1} did not changed antioxidant status after a short period of 24 h

study[25]. According to the results obtained in the current study, although TMX and TMX-SLN increased serum MDA level compared to the groups H and C, this was not significant. The other study reported the oxidative stress in MCF-7, receptor positive breast cancer cell line, treated with tamoxifen for long time[26]. However, a study showed the antioxidant and protection effect of tamoxifen on the animal heart[27]. In addition, there are side-effects to the use of tamoxifen; among them are development of liver cancers, increasing blood clothing, retinopathy and corneal opacities[3-5]. Due to these side-effects, the colloidal delivery systems were suggested to be the best way of delivery of tamoxifen for long-term chemotherapy of breast cancers. Solid lipid nanoparticles (SLNs) have been recommended as the carrier in these drug delivery systems. It was previously reported that loading of tamoxifen inside SLN enhanced the treatment efficacy of tamoxifen and decreased its side effect on liver and biochemical serum parameters[28].

From the current study, TMX-loaded SLN like free TMX displayed antioxidant activity. It means the biological availability of drug is not affected when it is encapsulated inside SLN. Therefore, when TMX is incorporated into the SLN as carrier system, its antioxidant properties and decreasing effect of oxidant activity is still preserved, suggesting that SLN is a good carrier for the drug insoluble in water. Therefore, SLN could be applied as a drug delivery system for cancer treatments. In addition, the TMX-loaded SLN, because of its small size, could not be easily phagocytized by macrophages and therefore the nanoparticles could be potentially used in long-term circulating carrier system for breast cancer therapy. As a result, when TMX encapsulated inside the SLN, its adverse impacts are reduced as well as the antioxidant effect are maintained. However, more studies are warranted to further develop and optimize drug-loaded SLN in the treatment of cancers.

CONCLUSION

The main challenge in cancer chemotherapy is toxic side-effects induced by chemotherapeutic drugs. The use of biodegradable nano-sized particles for long-term or life-time therapy may produce other serious side-effects. Increasing the encapsulation efficiency of poorly water-soluble molecules will lead to the development of improved SLN formulations. In the near future, it is expected more studies will focus on improving SLN and drug-loaded SLN formulations to increase the efficacy and reduce the side-effects of chemotherapeutic drugs for anticancer treatment. However, further studies using clinical trials will be needed to determine if the results obtained in this study can be extrapolated to humans.

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