ANTI-INFLAMMATORY EVALUATION OF LIPOSOMAL PREDNISOLONE IN WISTAR RATS

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ABSTRACT

The aim of the present study was to evaluate the anti-inflammatory ability of liposomal prednisolone in a carrageenan induced acute inflammatory paw edema model. Drug delivery to inflammatory tissue via liposomal nanoparticles may improve therapeutic agents' risk-benefit ratios. prednisolone encapsulated in long-circulating liposomes can inhibit inflammation after administration. The mechanism by which liposomal prednisolone inhibits Production of inflammatory factor. Variable efficacies have been reported for prednisolone drugs as anti-inflammatory treatment. Liposomal prednisolone was administered to male Wistar rats. carrageenan at a concentration of 1% was injected into hind paw of rats. The thickness of paws was measured by caliper within 0, 1, 2, 3, and 4 hours after the injections to confirm the inflammation. Blood samples were collected immediately after carrageenan injection and also 12 h thereafter. The sera were separated for measurements of interleukin 6 (IL-6). The prednisolone and liposomal prednisolone are efficacious in controlling the paw sizes, as indicators of inflammation; the liposomal prednisolone is remarkably more effective in controlling the inflammation in long term. Both the drug forms were clearly efficient in lowering the IL-6 level. Mostly noteworthy, the liposomal prednisolone was remarkably more efficient in lowering IL-6 levels.

***Key words**: liposomal, long circulation, Inflammation, Interleukin 6*

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Introduction

Inflammation is a complex biological response of the body to cell damage and vascularized tissue, which can be classified as either acute or chronic depending on the time of onset[1]. Acute inflammation is the body's primary response to injurious stimuli, and some of the body's responses are characterized by pain, heat, redness, swelling, and loss of function[2]. Ever since their first description by Bangham more than half a century ago, liposomes have been extensively used for drug delivery applications[3] Because of their relatively straightforward preparation, as well as their excellent biodegradability and biocompatibility, liposomal systems have progressed into one of the most extensively used and clinically most advanced drug delivery platforms[4].

Liposome consist of a number of bilayers, provides the liposome with structural stability, and enables the encapsulation of pharmacologically active agents, either in the layer itself for lipophilic compounds, or, more commonly, in the aqueous core for hydrophilic compounds[5]. When liposomes administered locally, the liposomal formulation allows for prolonged retention of the encapsulated drug at the injected site by limiting its diffusion and degradation ('depot' function). By limiting renal excretion and hepatic degradation, some liposome formulations, especially those with high transition-temperature saturated improve the pharmacokinetics of encapsulated drugs when administered systemically, allowing them to circulate for prolonged periods of time In addition, to the 'Enhanced Permeability and Retention' (EPR) effect[6]. Using the steroidal anti-inflammatory drugs are usual for

inflammatory diseases treatment and prednisolone is a steroid drug index which is a common medicine[7]. Prednisolone generates anti-inflammatory effects by binding to glucocorticoid receptors, thereby triggering signal transduction pathways[9]. prednisolone improves liver function and inhibits pro-inflammatory cytokines and polymorphonuclear neutrophil activation[10]. prednisolone have poor pharmacokinetic profile thereby rendering it ineffective in treatment and necessitates high dosages and frequent administration which, in turn, causes an array of adverse systemic effects, including diabetes mellitus, osteoporosis and hypertension[11]. In order to overcome the above mentioned problems and to improve the pharmacokinetic profile, development of the slow-release dosage forms of the drug is highly desirable[12].

On the other hand, encapsulation of prednisolone in long-circulating liposomes can potentially increase drug levels at the site of the action thus improving the therapeutic efficacy[13]. The aim of this article was to prepare and evaluate the slow-release nanosuspant of prednisolone and investigate its physicochemical properties addition to its stability.

Materials and methods

The ethanolic solutions of lecithin with prednisolone acetate were prepared in different values for find the best concentration densities. Different values volumes of distilled water were poured in beaker under blender with different values speeds. The organic solutions containing Prednisolone and lecithin were added to the beakers through an injection pump in different values speeds to form nanoformulations. At the end of each preparation process, the size of particles and the zeta potential were measured by ZetaSizer. The prepared nanodispersion was, for some parts of the study, freeze-dried after the addition of 0.5 percent lactose using a freeze-dryer (Eyela, model FDU-2100, Tokyo, Japan).

Animals: 60 Male Wistar rats (6 - 8 weeks and the mean weight of 250±20 gr; Razi Institute, Karaj, Iran) were housed under control conditions (12 hour light-dark cycles, 22°C, and 60% humidity) with free access to food and water on recycled paper pellet bedding. The animals were cared for in accordance with the guidelines of Research branch, Islamic Azad University, Tehran, Iran (NO:890975409).

Grouping of animals:

Group A: Healthy control: intramuscular (IM) injection of Dimethyl sulfoxide (DMSO) plus 100 μL SC injection of sterile distilled water (SDW) in paw;

Group B: Healthy control: intramuscular (IM) injection of SDW plus 100 μL subcutaneous (SC) injection of SDW in paw;

Group C: Inflammation with no treatment: IM injection of DMSO plus 100 μL SC injection of carrageenan (0.1% in saline) in paw;

Group D: Inflammation with no treatment: IM injection of SDW plus 100 μL SC injection of carrageenan (0.1% in saline) in paw;

Group E: Inflammation with low-dose prednisolone: IM injection of 2.5 mg/kg prednisolone solution in DMSO plus 100 μL SC injection of carrageenan (0.1% in saline) in paw;

Group F: Inflammation with mid-dose prednisolone: IM injection of 5 mg/kg prednisolone solution in DMSO plus 100 μL SC injection of carrageenan (0.1% in saline) in paw

Group G: Inflammation with high-dose prednisolone: IM injection of 10 mg/kg prednisolone solution in DMSO plus 100 μL SC injection of carrageenan (0.1% in saline) in paw

Group H: Inflammation with low-dose nanoformulation: IM injection of the nanoformulation equivalent to 2.5 mg/kg prednisolone dispersed in SDW plus 100 μL SC injection of carrageenan (0.1% in saline) in paw;

Group I: Inflammation with mid-dose nanoformulation: IM injection of the nano formulation equivalent to 5 mg/kg prednisolone dispersed in SDW plus 100 μL SC injection of carrageenan (0.1% in saline) in paw;

Group J: Inflammation with high-dose nanoformulation: IM injection of the nano formulation equivalent to 10 mg/kg prednisolone dispersed in SDW plus 100 μL SC injection of carrageenan (0.1% in saline) in paw.

Inflammatory stimulus

Carrageenan induced inflammatory model was used to assess the anti-inflammatory of prednisolon and nanoformulation[15].100 μl of freshly prepared carrageenan solution (1%) was Subcutaneous injected into the Left hind paw of each rat excluding the control group (A) which was maintained as vehicle control. To gauge the extent of inflammation, paw thickness was measured before and after injection of edematogenic agent T0: carrageenan

injection time. T1:1 hour after inflammation, T2: 2 hour after inflammation, T3: 3 hour after inflammation, T4: 4 hour after inflammation. with the help of digital vernier caliper (Mitotoyu, Series 500, Japan). The drug was injected IM two times: once 12 hours, and then one hour before induced inflammation in three doses 2.5, 5 and 10 mg/kg. prednisolone was dissolved in DMSO and nanoformulation dispersing in sterile deionized water. The dorsal-ventral thickness of rat left hind paw at the central plantar surface was determined with a digital vernier caliper (Mitotoyu, Series 500, Japan) following the subcutaneous injection of carrageenan. The paw edema was presented as the increase of thickness from the baseline values measured before the injection of the carrageenan. For each time point, three times were measured and then averaged[15].

Bloods were also sampled two times: first time before carrageenan injection and also 12 hour thereafter carrageenan injection, the rats were anesthetized with Diethyl ether, and the blood was collected from the orbital sinus in heparinized tubes. The blood was centrifuged at 1500 ×g for 10 min (4◦C); the plasma was aliquot and stored at -70◦C until use. The level of IL-6 in the plasma was determined by a rat enzyme-linked immunosorbent assay (IL-6 ELISA) kit (R&D Systems Europe Ltd. Abingdon, Oxon OX14 3NB, UK), according to the manufacturer instructions. All samples were analyzed in duplicate and read at 450 nm[16].

Results

Effects of prednisolone on carrageenan-induced rat paw edema

Carrageenan-induced paw edema is a routine, useful model to assess the contribution of mediators involved in vascular changes associated with acute inflammation. Paw volume was used as an indicator of the anti-inflammatory efficacy of the prednisolone-encapsulated liposomes formulation, at 0 hours, 4 hours. A comparison between the inflammations occurred in different groups of rats in different times following the drug administration is made in (Fig.1, 2 and 3). According these data, firstly, the inflammation has occurred in all test groups, secondly both the free and nanoliposomal drugs were efficacious in controlling the paw sizes, as indicators of inflammation, thirdly and most importantly, the nanoformulation was remarkably more effective in controlling the inflammation both in short-term and long-term, and fourthly, increasing the drug dose was advantageous for anti-inflammatory effect until the dose of 10 mg/kg in both free and liposomal drug.

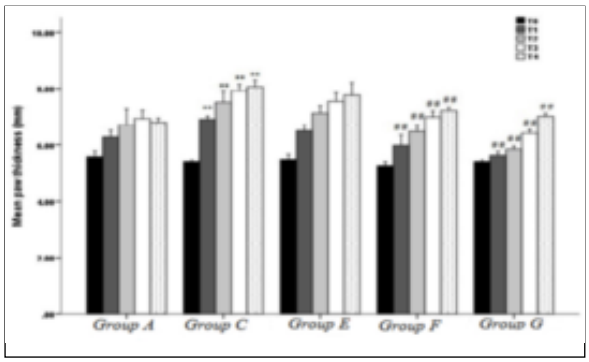

Figure 1: The comparison the paw thickness averages between control, inflammation and doses prednisolone in 5 different times.

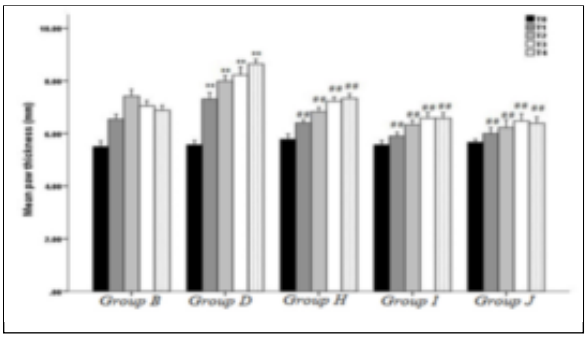

Figure 2: The comparison of the paw thickness in different groups Control and nanoformulations.

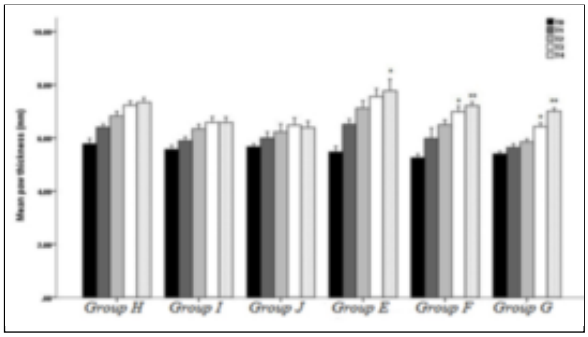

Figure 3: The comparison the paw thickness averages between, doses prednisolone and nanoformulation in 5 different times.

As shown in Fig. 1, paw thickness in the group D compared with group B. The paw in 4 time 1 hour after inflammation (T1), 2 hour after inflammation (T2), 3 hour after inflammation (T3), 4 hour after inflammation (T4) increase the paw thickness which is statistically significant (**P<0.001). Paw thickness in the group E compared with group D decrease in the paw thickness in 4 times T1, T2, T3,

and T4 statistically was not significant. paw thickness in the group F compared with group D decrease the paw thickness in 4 time T1, T2, T3, T4 which is statistically significant (##P< 0.001). Paw thickness in the group, G compared with group D decrease in the paw thickness in 4 time T1, T2, T3, T4 which is statistically significant (##P<0.001). As shown in Fig. 2, paw thickness in the group, C compared with group A The paw thickness in 4 time T1, T2, T3, T4 increase in the paw thickness which is statistically significant(**P<0.001). Thickening in the group, H compared with group C decrease the paw thickness in 4 time T1, T2, T3, T4 which is statistically significant (##P<0.001).

Paw thickness in the group, I compared with group F (inflammation with no treatment) decrease in the paw thickness in 4 time T1, T2, T3, T4 which is statistically significant (##P<0.001). paw thickness in the group, J compared with group C decrease in the paw thickness in 4 time T1, T2, T3, T4 which is statistically significant (##P<0.001).

As shown in Fig. 3, paw thickness in the group H compared with group, E decrease in the paw thickness in Fourth time (T4) which is statistically significant (*P<0.05). Paw thickness in the group I compared with group, F decrease in the paw thickness in in 2 time T3 and T4 which is statistically significant (*P<0.05) and (**P<0.001). Paw thickness in the group J compared with group, G decrease in the paw thickness in in 2 time T3 and T4 which is statistically significant(*P<0.05) and (**P<0.001).

Effects of prednisolone on carrageenan-induced rat Serum IL-6 levels

Serum IL-6 levels in different groups of rats in two time points of 0 and 12 hours are shown in (Fig. 4, 5, and 6). As indicated, firstly the IL-6 levels show no significant differences between groups before inflammation induction.

Secondly the occurrence of inflammation is strongly confirmed by the huge elevations in IL-6 levels in all groups but no inflammation controls. Thirdly, both the drug forms were clearly efficient in lowering the IL-6 level. Fourthly and noteworthy, the nanoliposomal drug was remarkable more efficient in lowering IL-6 levels.

As shown in Fig. 4, serum levels of IL-6 in the group inflammation with no treatment compared with group healthy control in the second time increase the Serum levels of IL-6 which is statistically significant (**P<0.001). Serum levels of IL-6 in the group E (547.83±9.1) compared with group D (613.80±7.2) decrease the IL-6 in after inflammation statistically was significant (##P<0.001). Serum levels of IL-6 in the group F (341.83±4.9) compared with group D (613.80±7.2) decrease the IL-6. After inflammation which is statistically significant (##P< 0.001). Serum levels of IL-6 in the group, G (249.66±4.7) compared with group D (613.80±7.2) decrease in the IL-6 in after inflammation which is statistically significant (##P< 0.001). As shown in Fig. 5, Serum levels of IL-6 in the group, C (622.00±5.7) compared with group A (2.50±0.7) the IL-6.

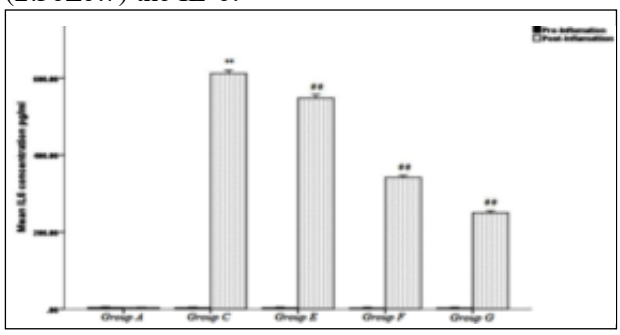

Figure 4: The comparison IL6 averages between control, inflammation and doses prednisolone in 2 time ❍: Before inflammation, ❍:12 h after the induction of inflammation.

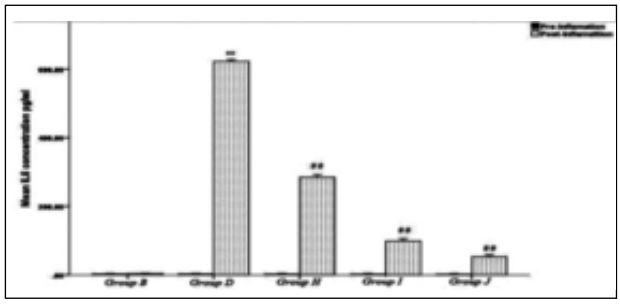

Figure 5: The comparison IL6 averages between control, inflammation and doses nanoformulation in 2 Time ❍: Before inflammation, ❍:12 h after the induction of inflammation.

In the second time increase in the IL-6 which is statistically significant (**P<0.001). Serum levels of IL-6 in the group, H (284.66±6.9) compared with group C (622.00±5.7) decrease the IL-6 in after inflammation which is statistically significant (##P<0.001). Serum levels of IL-6 in the group, I (98.66±6.8) compared with group C(622.00±5.7) decrease in the IL-6 in After inflammation which is statistically significant (##P<0.001). Serum levels of IL-6 in the group, J (52.83±6.0) compared with group C (622.00±5.7) decrease in the IL-6 in after inflammation which is statistically significant (##P<0.001).

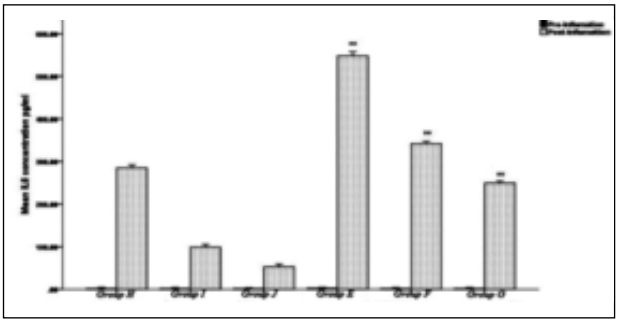

Figure 6: The comparison IL6 averages between and doses prednisolone and nanoformulation in 2 different times ○: Before inflammation, ●:12 h after the induction of inflammation.

As shown in Fig. 6 after inflammation serum levels of IL-6 in group E was 613.80±7.2 pg/ml and group H was 284.66±6.9 pg/ml, the difference statistically significant(**P<0.001). After inflammation serum levels of IL-6 in group F was 341.83±4.9 pg/ml and group I was 98.66±6.8 pg/ml, the difference statistically significant (**P<0.001). After inflammation Serum levels of IL-6 in group G was 249.66±4.7 pg/ml and group J was 52.83±6.0 pg/ml, the difference statistically significant (**P<0.001). The comparison the IL6 averages between different were interviews in 2 blood sample. The comparison between the IL6 averages of all rats before inflammation in all of the groups which is statistically no significant.

Also, dose increment from 2.5 mg/kg to 10 mg/kg was caused significantly more reduction in IL-6 levels.

Discussion

Clinical applications of nanotechnology are now on the verge of becoming a reality, the promising data of experimental findings awaiting confirmation in clinical trials[17]. Although prednisolone are highly potent drugs, and although they have been proven to be useful for the treatment of many different diseases, the severe side effects associated with their prolonged and/or high-dose use have somewhat limited their broad clinical applicability(18). Consequently, in order to improve drug efficacy and at the same time reduce toxicity, significant research efforts have focused on the development of drug delivery systems for prednisolone[19]. Recently, drug delivery systems using liposomes as drug carriers have been well studied to achieve controlled and site-specific delivery of drugs[20]. Liposomes are solid, water insoluble nano and microparticles composed of a solid hydrophobic core containing a layer of a phospholipid embedded on the surface of the corea layer of a phospholipid embedded on the surface of the core. The hydrophobic core is made of solid triglycerides or fatty acid esters containing the active agent[18].

Researches have shown that the processing methods for liposome preparation have significant effects on its properties such as size and efficiency[21]. The prednisolone and liposomal prednisolone are efficacious in controlling the paw sizes, as indicators of inflammation; the liposomal prednisolone is remarkably more effective in controlling the inflammation in long term[13]. Both the drug forms were clearly efficient in lowering the IL-6 level. Mostly noteworthy, the liposomal prednisolone was remarkably more efficient in lowering IL-6 levels[22].

Using nanoliposome structure and designing prednisolone nanoformulation lead to an increase in drug delivery and additionally, it leads to slow-release in comparison with the ordinary prednisolone. Using the slow release form eventually leads to an increase in the medication distances, reduces the value of medicine, and finally reduces the concentration of the drug in body and drug presence in target tissue for longer times[22]. It also leads to an increase in the therapeutic index of drug, i.e., drug usage become easier and its side effects will be reduced and it becomes economically affordable.

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