

Nanoparticles Containing Dexamethasone: Physicochemical Properties and Anti-Inflammatory Activity

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SUMMARY. The purpose of this study was to develop and characterize formulations of nanoparticles containing dexamethasone and to evaluate their anti-inflammatory activity in rats. Nanoparticles were prepared according to the procedure of nanoprecipitation, using poly (DL-lactide) and poly (ϵ -caprolactone) and dexamethasone, both in its base form and as acetate ester. The anti-inflammatory activity of the formulations was evaluated using two *in vivo* methodologies: inhibition of pellet cotton granuloma formation and of acute edema produced by injection of carragenin. The drug entrapment efficiency was about 80% and the formulations containing dexamethasone acetate was unstable. The association of this drug with nanoparticles improve its pharmacological activity in comparison to a commercial formulation.

RESUMO. "Nanopartículas contendo dexametasona: propriedades físico-químicas e atividade antiinflamatória". O principal objetivo deste trabalho foi desenvolver e caracterizar formulações de nanopartículas contendo dexametasona e avaliar a atividade antiinflamatória em ratos. As nanopartículas foram preparadas pelo método da nanoprecipitação, utilizando o poli(ácido láctico) e a poli(ϵ -caprolactona) como polímeros, e a dexametasona, como fármaco modelo, tanto na sua forma livre, quanto de éster acetato. A atividade antiinflamatória das formulações foi avaliada através de duas metodologias: a inibição da formação do granuloma e a inibição do edema de pata de rato induzido por carragenina. A eficiência de incorporação foi em torno de 80% e as formulações contendo acetato de dexametasona apresentaram-se instáveis. A associação do fármaco às nanopartículas melhorou a sua atividade farmacológica em relação a formulação comercial.

INTRODUCTION

Dexamethasone is a glucocorticoid with a relevant clinical use mainly due to its anti-inflammatory and immunosuppressive effects. However, the great number of side effects, such as: hypertension, hydroelectrolytic disorders, hyperglycemia, peptic ulcers and glucosuria restricts the use of dexamethasone in prolonged therapy ¹.

In the last years much interest has been focused on nanoparticles, as a drug delivery systems, due to their possibilities of increasing drug efficacy, reducing toxicity and controlling drug release ². However, few data are available about

the entrapment of dexamethasone in nanoparticles.

Seijo *et al.* ³ reported a dexamethasone entrapment efficiency of 75% in PIBCA nanospheres prepared by the *in situ* polymerization procedure and Song *et al.* ⁴ presented a drug entrapment of 79.6 % in PLGA nanospheres obtained using an emulsification/solvent evaporation technique. In addition, Fessi *et al.* ⁵ using nanodispersion of the preformed poly(DL-lactide) showed a drug entrapment of 40% in nanocapsules. Nevertheless, these works used dexamethasone as a lipophilic drug model and the only parameters evaluated were the entrap-

KEY WORDS: Anti-inflammatory activity; Dexamethasone; Nanoparticles.

PALAVRAS-CHAVE: Atividade antiinflamatória; Dexametasona; Nanopartículas.

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ment efficiency, the particle size ³⁻⁵ and *in vitro* drug release ⁵.

Considering this lack of information, the goal of the present work was to develop and characterize nanoparticles containing dexamethasone, or its acetate derivative, prepared by nanodispersion of different biodegradable polymers and to evaluate the anti-inflammatory activities of the formulations.

MATERIALS AND METHODS

Materials

Poly (ϵ -caprolactone) (PCL, MW: 65 000) and poly (DL-lactide) (PLA, MW: 103 000) were purchased from Sigma-Aldrich Co. (Steinheim, Germany). Tween 80[®] and Span 80[®] were supplied by Delaware (Porto Alegre, Brazil). Dexamethasone and dexamethasone acetate were gift from Hoechst-Roussel (Romainville, France) and Merck S/A (Rio de Janeiro, Brazil), respectively. All other chemicals and solvents used were of pharmaceutical grade. All reagents were used as received.

Male Wistar rats (Biotério Central, UFSM) weighing between 200 e 400 g were used in the *in vivo* anti-inflammatory activity evaluation. The rats were fed a regular diet with no restrictions on the amount of food or water consumed.

Preparation of PLA and PCL Nanoparticles

Nanospheres (NS) were prepared according to the procedure of preformed polymer dispersion ⁶, using poly (DL-lactide) - PLA and poly (ϵ -caprolactone) - PCL, as polymers, and dexamethasone as drug, both in its baseform (D) or as acetate ester (DA). The colloidal suspensions were abbreviated for NS-PLA-D; NS-PCL-D; NS-PLA-DA; NS-PCL-DA, respectively. The final concentration was 0.5 mg/mL. Briefly, 200 mg of polymer (PLA or PCL), 300 mg of Span 80[®] and the drug (D or DA) were first dissolved in 40 mL of acetone. This organic solution was poured, with moderate magnetic stirring, into 80 mL of an aqueous phase containing 300 mg of Tween 80[®]. The resulting mixed phase immediately turned milky with bluish opalescence as a result of the formation of nanoparticles. The acetone was finally removed under reduced pressure and the colloidal suspension concentrated to the desired final volume (20 mL). Empty NS were also prepared (NS-PLA and NS-PCL) as described above. Formulations were made in triplicate.

Determination of Entrapment Efficiency

Free drug was determined in the clear supernatant following separation of nanoparticles from the aqueous medium by a combined ultrafiltration-centrifugation technique (Ultrafree-MC[®] 10,000 MW, Millipore, Bedford, U.S.A.). Total drug was determined after dissolution of nanoparticles in acetonitrile (1 mL of suspension to 25 mL of acetonitrile). The drug content was calculated from the difference between the total and free drug concentrations measured in the nanoparticles suspension (total drug) and the filtrate (nontrapped drug), respectively.

Dexamethasone (D or DA) was assayed by high-performance liquid chromatography ⁷. The system consisted of a Merck Lichrospher[®] RP 18 column (Darmstadt, Germany), an Intralab 5050 pump, an Intralab 5100 detector and an Intralab 4290 integrator. The mobile phase consisted of water/acetonitrile (55:45% v/v). The total sample amount injected was 20 μ L. Dexamethasone (D or DA) was detected by absorption at 254 nm. The linear response range was 3.125-50.000 μ g/mL with a correlation coefficient of 0.9999.

Drug/Polymer Ratio

This parameter was determined by the quotient between the total amount of incorporated drug (mg) and polymer (mg) presented in suspensions.

Particle Size Determination

The particle size of nanoparticles was estimated by photon correlation spectroscopy using a Coulter Zetasizer (Malvern, UK).

Morphologic Examination

Nanoparticles samples were observed with a transmission electron microscope (Jeol, Jem 1200 Ex-II, Japan) after negative staining with 2% (p/v) aqueous solution of uranyl acetate.

Stability Studies

The formulations were monitored following preparation, at time 0, 1, 2 and 3 months, determining the efficiency of drug entrapment. The suspensions were stored at room temperature and protected from light. The kinetic of drug leakage from polymeric matrix was calculated using the graphic method ⁸.

Anti-Inflammatory activity evaluation

The anti-inflammatory activity was evaluated for the formulation that presented the best re-

sults in the characterization step and stability study. Two methods were used in testing the anti-inflammatory activity in rats: the inhibition of acute edema produced by injection of carrageenin and inhibition of cotton pellet granuloma formation.

Inhibition of acute edema produced by injection of carrageenin

The study was carried out on male Wistar rats (200-300 g, n = 6). Thirty minutes before the intraperitoneal injection of each compound, the basal volume of the hind paws was measured by means of a mercury plethysmometer (Ugo Basile). Afterwards, the rats were injected with one of the following compounds at the dose of 30 µg/Kg⁹: (a) dexamethasone nanoparticles suspension; (b) empties nanoparticles; (c) water; (d) dexamethasone commercial form - Decadron®. Thirty minutes after the treatment, carrageenin (0.05 mL of 1% suspension in saline) was injected intraplantarly into the right hind paw of each rat to induce inflammation and 0.05 mL of saline into the contralateral paw. Paw volumes up to the ankle joints were measured before and at hourly intervals for 6 h following carrageenin administration. The basal volume of each rat paw was taken as 100% and variations from this volume were given as percent difference.

Inhibition of cotton pellet granuloma formation

Cotton pellets weighing 38-42 mg were sterilized by autoclaving and implanted into male Wistar rats (300-400 g, n = 8) under chloroform anesthesia. A small dorsal, mid-line incision was made, and the dermis was separated from the underlying peritoneal wall by an insertion of a trocar. Two pellets were implanted in each rat, one on each side of the incision. The incision was closed with a surgical suture. The following compounds were administered to rats by injection in the tail vein at the dose of 30 µg/Kg⁹: (a) dexamethasone nanoparticles suspension; (b) empties nanoparticles; (d) dexamethasone commercial form - Decadron®. Seven days after, the rats were killed by decapitation. Cotton pellets and the accompanying granulomatous tissue were removed from the rats, placed in a glass Petri dish, air dried at 60 °C for 18 h, and weighed.

Statistical Analysis

Two-way ANOVA compared the physicochemical parameters of the control of formula-

tions and the *in vivo* anti-inflammatory evaluation. *p*-values less than 0.05 and 0.10 were considered as representing a significant difference of the characterization step and anti-inflammatory activity evaluation, respectively.

RESULTS AND DISCUSSION

Physicochemical Characteristics

The main objective of this work was to obtain dexamethasone loaded nanoparticles and to investigate the possibility of increase its anti-inflammatory activity. Therefore, we prepared nanospheres from two different biodegradable polymers (PLA or PCL) and dexamethasone, as its baseform (D) or as acetate ester (DA).

The formulations prepared with dexamethasone acetate (NS-PLA-DA and NS-PCL-DA) were unstable presenting a precipitate just after preparation. These phenomenon could be explained by the low solubility of dexamethasone acetate in water (5.47 µg/mL) and thus, by the diffusion of a certain amount of DA from the nanoparticles to the aqueous medium, followed by its precipitation as free crystals. On the other hand, dexamethasone, which is less hydrophobic (water solubility of 100.88 µg/mL)¹⁰ than the acetate form, and had a hydrogen at C21, increasing the possibility of interactions by hydrogen bonds between drug and the polymers used, allowed the preparation of nanospheres colloidal suspensions.

Table 1 shows pH, particle size, entrapment efficiency (%) and drug/polymer ratio, just after preparation, of the formulations containing dexamethasone. All formulations were acidic and particle sizes are in the submicrometric range, with no significant differences (*p*<0,05) in relation to their mean diameter size. The TEM morphologic observation of NS-PLA-D, NS-PLA, NS-PCL-D and NS-PCL suspensions (Figure 1) demonstrated uniform and rounded particles for all formulations. Also, the presence of drug nanocrystals around the nanoparticles were not detected. These crystallization phenomenon could be occurred due to the low solubility of dexamethasone in water. The visualization of indomethacin crystals around nanoparticles by TEM is reported by Calvo *et al.*¹¹

Regarding to the entrapment efficiency, similar results were measured for both formulations (76.30 ± 2.10% for NS-PLA-D and 77.11 ± 1.36% for NS-PCL-D). Despite the highest hydrophobicity of PCL, the polymer has not significantly influenced on this parameter. The drug/polymer ratio was 3.65 ± 0.12 for NS-PLA-D and 3.83 ±

Formulation	pH	Particle size (nm)	Entrapment efficiency (%)	Drug/polymer ratio
NS-PLA-D	4.91 ± 0.17 ^b	285 ± 50 ^a	76.30 ± 2.10 ^b	3.65 ± 0.12 ^c
NS-PLA-B	5.28 ± 0.07 ^c	230 ± 46 ^a	–	–
NS-PCL-D	6.42 ± 0.08 ^a	385 ± 70 ^a	77.11 ± 1.36 ^b	3.83 ± 0.05 ^c
NS-PCL-B	5.66 ± 0.05 ^d	280 ± 37 ^a	–	–

Table 1. pH, particle size, entrapment efficiency and drug/polymer ratio of formulations after preparation.

^a The data showed are the mean ± standard deviation (n = 3). Means with the same letter are not significantly different (ANOVA, F test).

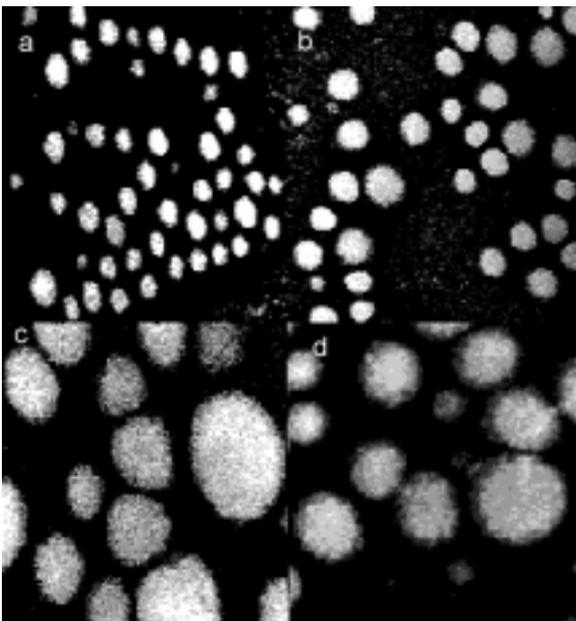


Figure 1. TEM of (a, top left) dexamethasone loaded PLA nanoparticles (NS-PLA-D), (b, top right) PLA nanospheres without drug (NS-PLA), (c, bottom left) dexamethasone loaded PCL nanospheres (NS-PCL-D) and (d, bottom right) PCL nanospheres without drug (NS-PCL).

0.05 for NS-PCL-D. These values were in agreement with those reported by Seijo *et al.*³ for polyisobutylcyanoacrylate nanoparticles containing dexamethasone prepared by interfacial polymerization (entrapment efficiency: 75.00 ± 10.00% and drug/polymer ratio: 3.75 w/w) and by Song *et al.*⁴ for poly(lactic-co-glycolic acid) nanoparticles obtained using as emulsification/solvent evaporation technique (entrapment efficiency: 79.60%). On the other hand, these results are better than that demonstrated by Fessi *et al.*⁵ for PLA nanocapsules containing dexamethasone prepared by interfacial polymer deposition (entrapment efficiency:40%).

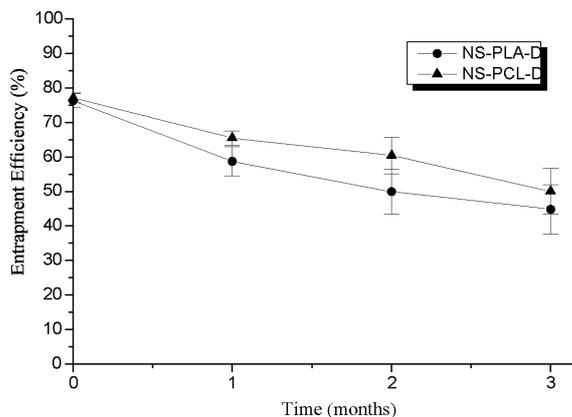


Figure 2. Percentage of the initial load of dexamethasone found as a function of storage time in nanospheres prepared at different polymers (NS-PLA-D or NS-PCL-D) and dexamethasone.

Stability Studies

NS-PLA-D and NS-PCL-D suspensions presented a similar significant decline of entrapment efficiency during storage time (Figure 2), indicating drug leakage (surface desorption and/or diffusion to the aqueous medium). However, no aggregation was observed.

The NS-PLA-D presented a dexamethasone leakage (desorption and/or diffusion) following a second order kinetics ($r = 0.9905$), whereas the NS-PCL-D suspension showed drug leakage following a zero order kinetics ($r = -0.99009$). These kinetics difference could be attributed to the drug incorporation pattern, where in the PLA nanospheres the drug is more incorporated into polymeric matrix, than in the PCL nanospheres in which the drug is more adsorbed onto the particle surface.

Anti-Inflammatory Activity

Due to the best results observed in the physicochemical characterization, NS-PCL-D formulation was chosen to undergo the pharmacological studies. Figure 3 shows the increase in

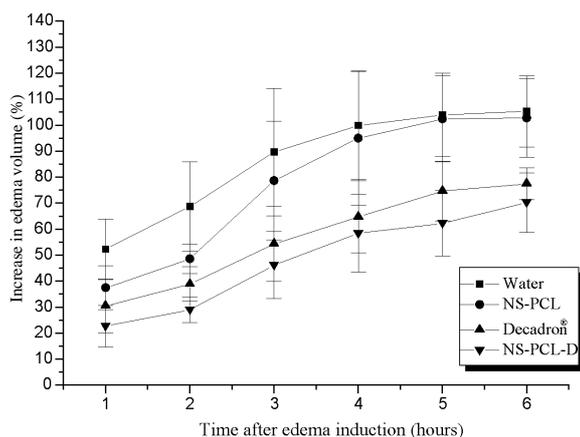


Figure 3. Increase in edema volume (%) in anti-inflammatory activity evaluation of nanospheres containing dexamethasone (NS-PCL-D), empties nanospheres (NS-PCL), free dexamethasone (Decadron®) and water using the method of acute edema inhibition produced by carrageenin injection (6 rats per group) (ANOVA, F Test).

edema volume (%) using the method of acute edema inhibition produced by carrageenin injection, as a function of time. The evaluation of the anti-inflammatory activities was performed by the comparison of NS-PCL-D with a dexamethasone commercial injection product (Decadron®) used as reference. When dexamethasone was associated with NS and its anti-inflammatory activity evaluated by inhibition carrageenin-edema a significant reduction of edema ($p < 0.10$) was measured in comparison to the commercial product. On the other hand, the anti-inflammatory activity evaluated by the inhibition of cotton pellet granuloma formation (Figure 4) did not show statistically significant difference between the products containing dexamethasone (NS-PCL-D and Decadron®), however a tendency of bigger activity of NS can be observed.

CONCLUSIONS

This paper describes the preparation feasibility of colloidal systems, PLA and PCL nanoparticles containing dexamethasone. The drug form (dexamethasone or dexamethasone acetate) has influence in drug entrapment efficiency. However, all formulations presented a decline in this entrapment efficiency on aging. The importance of the dexamethasone nanoparticles was further evidenced by the fact they significantly improve the pharmacological activity of drug in comparison to a commercial formulation. Nevertheless, dexamethasone nanoparticles suspensions are

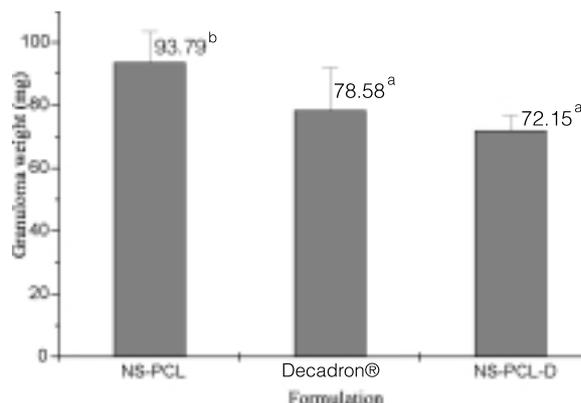


Figure 4. Granuloma weight, in mg, in anti-inflammatory activity evaluation of nanospheres containing dexamethasone (NS-PCL-D), empties nanospheres (NS-PCL) and free dexamethasone (Decadron®), using the method of inhibition of cotton pellet granuloma formation in rats (8 rats per group). Means with the same letter are not significantly different (ANOVA, F test).

poorly stables. Work is in progress to optimize the stability of the preparation by means of spray-drying.

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