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Pessaries with progesterone-loaded nanostructured lipid carriers (NLC) for prolonged release

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PURPOSE

Progesterone is responsible for the embryo implantation and for the maintenance of pregnancy and is commonly administered vaginally, avoiding the hepatic first pass. However, vaginal progesterone dosage forms require repeated administrations to ensure maintenance of therapeutic levels [1]. Thus, it is desirable to use alternative systems for the prolonged release of progesterone. Among these, the nanostructured lipid carriers (NLC) have been widely studied, due to their advantages to improve the bioavailability of drugs. In addition, pessaries remain the vaginal forms of choice, given the ease of application and the low cost of production [2]. Accordingly, the objective of this work was to develop pessaries for the prolonged vaginal delivery of progesterone. The studies began with the preparation and characterization of progesterone-loaded NLC (NLC_PRG), followed by the evaluation of their cytotoxicity. Finally, NLC_PRG were incorporated into pessaries and *in vitro* drug release studies were carried out.

METHODS

1. Preparation and characterization of progesterone-loaded NLC

Table 1 shows the composition of the tested NLC formulations, which were prepared from the method previously employed by Silva *et al.* [3] (Figure 1).

Table 1: Composition (%, w/w) of the tested NLC formulations: progesterone-loaded NLC (NLC_PRG) and placebo NLC (NLC_placebo).

Composition	(%, w/w)	
	NLC_PRG	NLC_placebo
Compritol® 888 ATO	5.70	6.00
Miglyol® 812	3.80	4.00
Progesterone (PRG)	0.50	-
Tween® 80	3.00	3.00
Cetrimide®	0.50	0.50
Ultrapure water	86.50	86.50

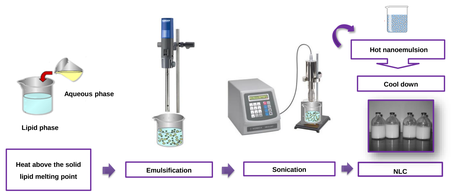

Figure 1: Schematic representation of the NLC preparation.

The particle size of the NLC formulations was evaluated by laser diffractometry, on the production day and after 1 month of storage (Mastersizer 3000, Malvern).

The non-encapsulated progesterone was separated by filtration/centrifugation, using an Ultracel-50K (Amicon®) centrifugal filter device. The amount of free drug was estimated by spectrophotometry UV-Vis (Spectrophotometer V-650 JASCO). The analyses were performed in triplicates, on the production day, and the encapsulation efficiency (EE) was obtained indirectly, through to the following equation [4]:

EE (%) = [(total amount of drug – free drug) / total amount of drug] x 100.

2. Biocompatibility studies

The cytotoxicity of the NLC-PRG and NLC placebo formulations (0-100 µg/mL) was evaluated by the neutral red (NR) uptake, resazurin (REZ) reduction and sulforhodamine B (SRB) assays, 24h after exposure to human keratinocytes (HaCaT cells) (Figure 2).

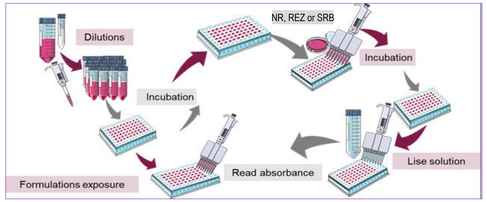

Figure 2: *In vitro* studies in human keratinocytes (HaCa T cells), evaluating the cytotoxicity of the NLC formulations by the neutral red (NR) uptake, resazurin (REZ) reduction and sulforhodamine B (SRB) assays.

3. Preparation of pessaries

Pessaries containing 70% of Witepsol® H32 and 30% of NLC_PRG were prepared by the fusion method and characterized according to the European Pharmacopoeia (Figure 3) [2].

Figure 3: Macroscopic appearance of the pessaries containing NLC_PRG.

4. *In vitro* drug release studies

The *in vitro* drug release profile of progesterone from the pessaries was assessed during 24h, through the dialysis bag diffusion technique. The release medium used to ensure sink conditions was water with 0.5% of sodium lauryl sulfate. At predetermined time intervals, samples were collected and the amount of drug released was measured by spectrophotometry UV-Vis. The release profiles of pessaries containing NLC_PRG and pessaries of free progesterone were compared.

RESULTS

On the production day, 90% of the NLC_PRG had sizes ≤ 315.60±0.01 nm, 50% had sizes ≤ 74.60±0.00 nm and 10% had sizes ≤ 21.82±0.00 nm. After 1 month of storage (Figure 4), 90% of NLC_PRG with sizes ≤ 219.20±0.01 nm, 50% with sizes ≤ 67.60±0.00 nm and 10% with sizes ≤ 21.94±0.00 nm, which are all acceptable values for vaginal delivery [2].

The EE of progesterone in the NLC was 96.42±0.00%, showing the effectiveness of the system for drug incorporation.

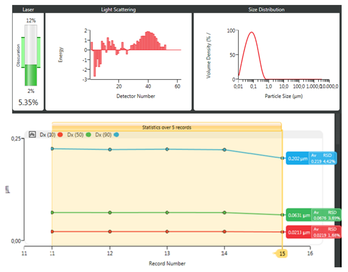

Figure 4: Particle of the progesterone-loaded NLC (NLC_PRG) after 30 days of storage at 5.0±0.5ºC.

Through the NR uptake, REZ reduction and SRB assays (Figure 5) was observed a lack of cytotoxicity of the NLC_PRG and NLC_placebo for formulation's concentrations < 10 µg/mL, and a concentration-dependent cytotoxic effect for concentrations ≥ 25 µg/mL. Also noteworthy is the absence of significant differences between the two tested formulations.

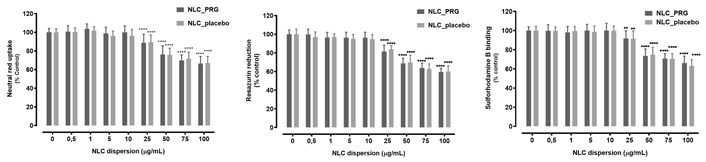

Figure 5: Cytotoxicity of the NLC_PRG and NLC_placebo evaluated by the NR uptake, REZ reduction and SRB assays, 24h after exposure. Results are expressed as Mean+SD from 5 independent experiences, performed in triplicate. Statistical comparisons were made using Two-way ANOVA, followed by the Tukey's multiple comparisons test (for each formulation, for comparisons between concentrations) or by the Sidak's multiple comparisons test (at each concentration, for comparisons between formulations) (**p < 0.01; ***p < 0.0001 vs. 0 µg/mL, for each formulation). In all cases, p values < 0.05 were considered significant.

From the *in vitro* drug release studies (Figure 6) it was observed that, after 24h 72.30±0.02% of progesterone was released from the pessary of NLC_PRG and 97.89±0.03% was released from the pessary of free progesterone. The drug release from both pessaries was similar in the first 30 minutes and a slower release was observed from the NLC_PRG pessary from 30 minutes up to 24h.

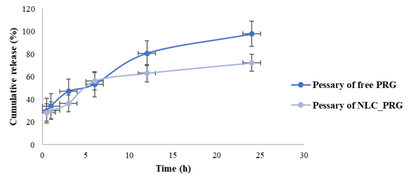

Figure 6: Cumulative progesterone (PRG) release profiles of the NLC_PRG pessary and the free PRG pessary (mean values ± SD, n=3).

CONCLUSION

The findings of these studies suggest the suitability of pessaries containing NLC_PRG for sustained drug release, which is a promising alternative for the vaginal use of progesterone. Nonetheless, more studies are required to confirm this application.

REFERENCES

[1] Warren M (2018). Climacteric 21, 355-357. [2] Correia A *et al.* (2020). Eur J Pharm Sci 153, 105475. [3] Silva AC, *et al.* Colloids Surf B Biointerfaces. 2011, 86, 158 – 165. [4] A.C. Silva *et al.* Int J Pharm 436 (2012) 798– 805.

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