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Solid lipid nanoparticles for Anle 138b administration in Parkinson disease: towards an industrial scale up with nutraceutical formulations

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INTRODUCTION & AIM

Anle 138b, a novel drug for geriatric application in Parkinson disease (PD), suffers with low water solubility and, hence, low bioavailability (1-2). Therefore, the research addressed towards novel water soluble products as vehicle for Anle 138b still represents a challenge for the neurological disorder of PD. The current study focused on some formulations consisting of solid lipid nanoparticles (SLNs) administering Anle 138b combined with nutraceutical components and evaluated in vitro for their stability under storage in different conditions (3).

METHODS

Materials : Gelucire® 50/13 was received as a gift by Gattefossè (Milan, Italy). Anle 138b was purchased by DBA (Milan, Italy). DMG-GOLD, Maitake extract and marigold extract were provided by Erbenobili (Corato, Italy).

Preparation of Anle 138b SLNs : Anle 138b SLNs were prepared according to the melt emulsification method previously reported (4) with slight modifications. Briefly, 60 mg of Gelucire® 50/13 were melted at 70°C, and in a separate vial, 1.37 mL of a diluted Acetic solution (0.01 %, w/v) containing the surfactant (Tween 85®, 30 mg) was heated at 70°C. 0.5 mg of Anle 138b were predissolved in 30 mg of Tween 85® and then added to the melted lipid at 70°C to ensure high mixing with the same melted Gelucire® 50/13. Afterwords, the aqueous phase was poured into the lipophilic mixture at 70°C. Homogenizing such mixture at 12,300 rpm for 3 min with an UltraTurrax model T25 apparatus an emulsion was obtained. By cooling at room temperature of the nanosuspension the resulting SLNs were subjected to centrifugation at 13,200 × g, 45 min and the obtained pellet was used for subsequent studies while the supernatant was discarded.

Storage of Anle 138b SLNs in the presence of nutraceuticals: Freshly prepared suspensions of the pellets of Anle 138b SLNs were mixed in 1:1 (v:v) with each one of the following nutraceuticals: DMG-GOLD, Maitake extract and marigold extract and the resulting liquid was stored at 4°C. At appropriate time intervals, two aliquots of the liquid were taken for the following analysis. One aliquot was centrifuged and, after discarding the pellet, the supernatant was assayed for Anle 138b content. The other aliquot was mixed in a kuvette with 0.5 mL double distilled water for particle size analysis according to Malvern Zetasizer Apparatus (ZEN 3600, Malvern, UK).

Quantitative determination of Anle 138b : The quantitative determination of Anle 138b in Gelucire® 50/13 based SLNs was carried out by spectrophotometric analysis using a calibration curve obtained dissolving the drug in DMSO (concentration range 5–0.02 µg/mL, R2 > 0.999. LOQ = 0.02 µg/mL, LOD = 0.015 µg/mL). The measurements were performed at the wavelength of 300 nm by using a Perkin-Elmer Lambda Bio 20 UV–Vis spectrophotometer (Perkin-Elmer, Milan, Italy). After lyophilization, SLNs underwent enzymatic digestion by esterases to determine Anle 138b content (5). The study was performed in triplicate.

Cytobiocompatibility of SLN-Anle 138b + DMG-GOLD in differentiated SH-SY5Y cells: For cytocompatibility assessment, differentiated as previously described (6) SH-SY5Y cells were treated with SLN-Anle 138b + DMG-GOLD, SLN-Anle138b and Anle138b + DMG-GOLD at four different concentrations of Anle 138b for 24 h (or untreated as control) and then MTT test was performed. Each experiment was performed in triplicate.

RESULTS & DISCUSSION

Anle 138b SLNs particle size storage stability in Erbenobili marigold extract: after 1 week of storage in a viscous liquid such as marigold extract at 4°C, SLNs reduce their Anle 138b content by 40% (Fig. 1a), while their diameter increases over time, until they form aggregates at 2 months (Fig. 1b).

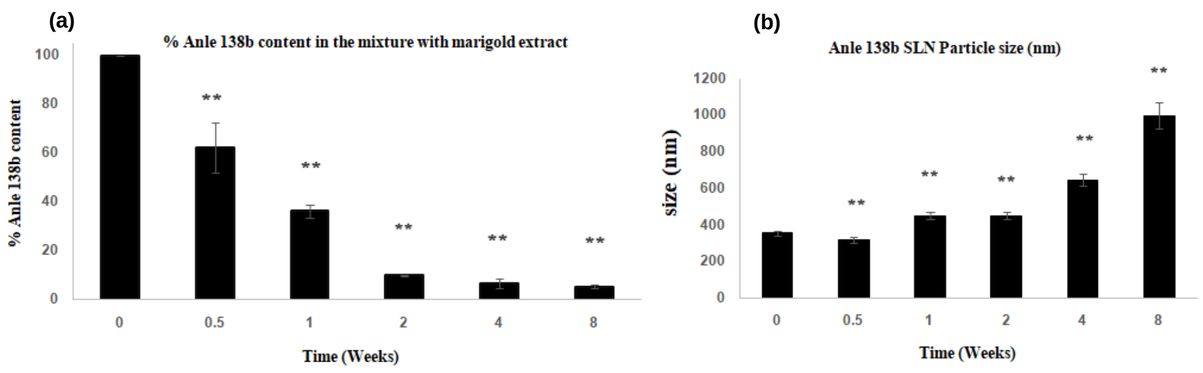

Figure 1. Storage stability of suspensions of Anle 138b mixed with marigold extract at 4°C. Evaluation of Anle content (a) and particle size (b) over the time. Values were compared by one-way ANOVA following Bonferroni's post hoc test. ** p < 0.001.

Anle 138b SLNs particle size storage stability in Erbenobili Maitake extract: After 4 weeks of storage in Maitake extract at 4°C, SLNs reduce their Anle 138b content by 50% (Fig. 2a). Agglomeration occurred since few days at 4°C, but the precipitate is redispersable after manual shaking up to1 month. After 1 month, flocculation is not easy to be antagonized and, hence, to re-obtain native SLNs.

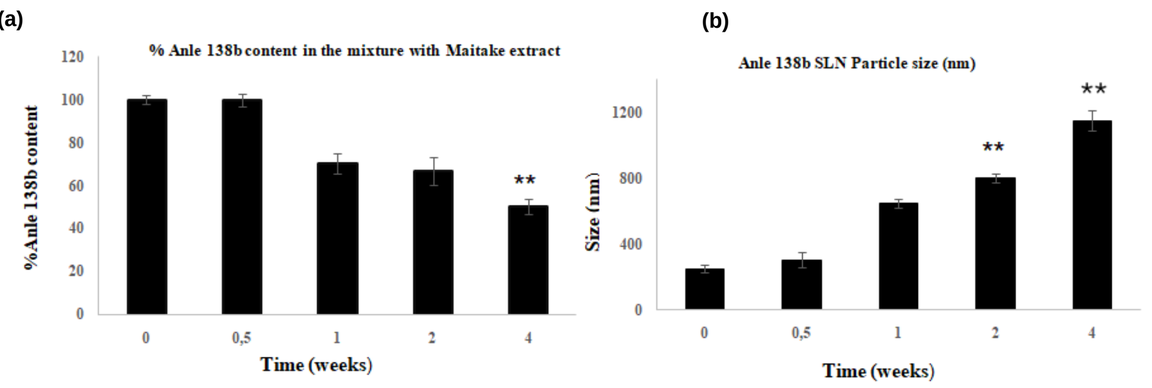

Figure 2. Storage stability of suspensions of Anle 138b mixed with Maitake extract at 4°C. Evaluation of Anle content (a) and particles size (b) over the time. Values were compared by one-way ANOVA following Bonferroni's post hoc test. ** p < 0.001.

Anle 138b SLNs particle size storage stability in Erbenobili DMG-GOLD extract: When SLNs were stored in DMG-GOLD (whose composition is depicted in Fig. 3a) at 4°C, although SLNs underwent a drop in Anle content of up to 40% after 1 week, they maintained the same diameter. Therefore, the suspension made of SLN and DMG-GOLD was preferentially subjected to studies of cytocompatibility with differentiated SH-SY5Y cells (Fig. 3b,c).

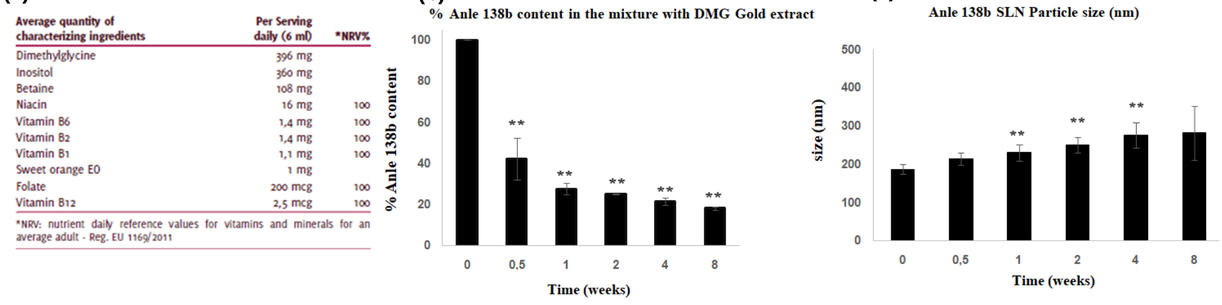

Figure 3. Storage stability of suspensions of Anle 138b mixed with DMG-GOLD extract at 4°C. Composition of DMG-GOLD (a), evaluation of Anle content (b) and particle size (c) over the time. Values were compared by one-way ANOVA following Bonferroni's post hoc test. ** p < 0.001.

Cytotoxicity on SH-SY5Y cells: Generally, all the formulations were well tolerated. At the lower doses of 1 and 10 µM, the formulations containing encapsulated Anle-138b are not cytotoxic compared to the formulations with the drug in its free form. However, at higher concentrations of 50 and 100 µM, cell viability decreases by approximately 40% with SLN-Anle 138b + DMG-GOLD and SLN-Anle 138b, while it decreases by about 50% with free Anle-138b. Notably, at the concentration of 100 µM, the SLN-Anle 138b + DMG-GOLD formulation is more toxic than SLN-Anle 138b. The results suggest that the encapsulation of Anle-138b within SLNs improves its cytocompatibility at lower doses (1 and 10 µM), as the encapsulated formulations (SLN-Anle 138b + DMG-GOLD d and SLN-Anle 138b) are less cytotoxic than the free drug (Fig. 4a-d).

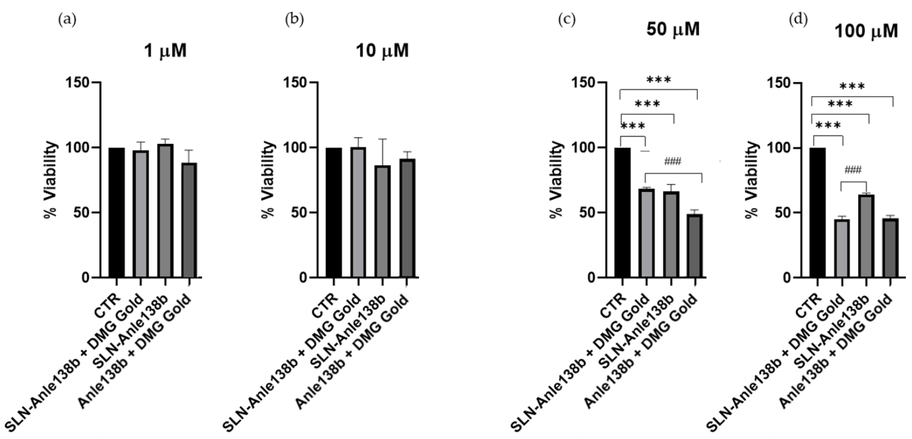

Figure 4. Cytotoxic effects of SLN-Anle 138b + DMG Gold, SLN-Anle 138b, Anle 138b + DMG-GOLD in differentiated SH-SY5Y cells. Viability of differentiated SH-SY5Y cells was evaluated by MTT assay after treatment with different concentrations (a) 1, (b) 10, (c) 50 and (d) 100 µM of Anle 138b for 24 h. The data are means ± SD of three different experiments and are presented as percent of untreated cells (CTR, 100% of vitality). Values were compared by one-way ANOVA following Dunnet test. *** p < 0.0001 vs CTR; ### p < 0.0001 vs SLN-Anle 138b + DMG-GOLD.

CONCLUSION

Candidate industrial products combining nutraceuticals and SLN-Anle 138b were evaluated for the future administration in PD patients, indicating the potential of a multicomponent preparation for non- invasive treatments. Further in vivo studies are to be designed to confirm the successful combination herein shown of SLN-Anle 138b-DMG-GOLD.

CONFLICT OF INTEREST

The authors declare no conflicts of interest

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