Inclusion of α-bisabolol into PLGA nanoparticles enhances its pro-apoptotic activity in human tumoral pancreatic cells.

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α-Bisabolol (figure 1), a sesquiterpene alcohol present in essential oils derived from a variety of plants, is known to have a pro-apoptotic activity against several human cancer cell lines [1,2] but its poor aqueous solubility limits the *in vitro* and *in vivo* tests of this action. Here we report the production and characterization of α-bisabolol loaded nanoparticles prepared using poly(lactic-co-glycolic acid) (PLGA). The human therapeutic use of PLGA has been approved by the US Food and Drug Administration (USFDA) thanks to its biocompatibility. This copolymer is one of the most successfully used compounds in nanomedicine applications because it is hydrolyzed in the body to produce the common metabolites lactic and glycolic acid [3].

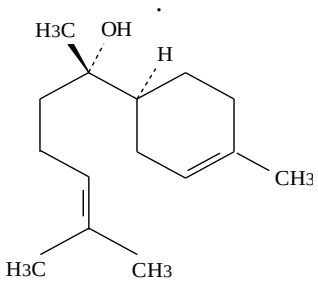

Figure 1 Structure of α-Bisabolol

RESULTS AND DISCUSSION

Nanoparticle preparation and characterization PLGA nanoparticles were prepared using an emulsion solvent evaporation method. Several parameters including polymer/drug ratios, the emulsifier and stabilizer volume (poly vinyl alcohol, PVA) and the organic phase solvent were optimized. PLGA (50:50) and α-bisabolol were dissolved in methylene chloride then drop wise mixed to 0.5% aqueous PVA during sonication in an ice bath. The emulsion was stirred continuously to evaporate the organic solvent then washed by centrifugation with PBS and freeze-dried [4].

The particle size, zeta-potential and polydispersity index (PDI) were determined and showed a unimodal particle size distribution with a mean diameter of 160–180 nm, a charge of -3mV and a PDI of less than 0.1. The nanoparticle shape and size were also examined by Atomic Force Microscopy (AFM).

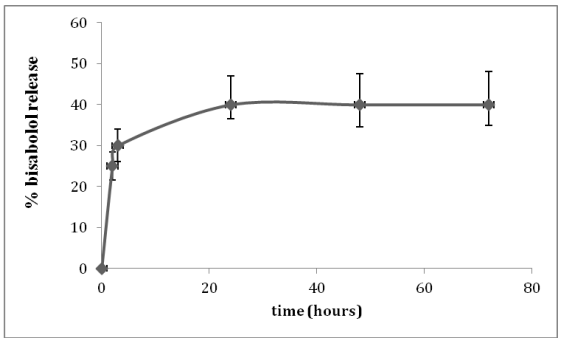

Figure 2: *in vitro* release profile of bisabolol from PLGA nanoparticles. All the measurements were done in triplicate and expressed as arithmetic mean±standard error on the mean (S.E.M.).

α-Bisabolol loaded PLGA nanoparticles exhibt a higher cytotoxicity in cancer cells compared to free bisabolol dissolved in EtOH. The in vitro effect on cell viability of α-bisabolol loaded PLGA nanoparticles was tested by countess automated cell counter (Invitrogen) on a highly malignant human pancreatic cell line (MiaPaca) and compared with the effect of α-bisabolol dissolved in EtOH. The results showed that the loaded nanoparticles induced cytotoxicity in a dose and time dependent manner and that the particles without encapsulated α-bisabolol did not affect cell viability.
All the cells were killed after 24 hours by 90μM nanoparticle treatment and the same effect was obtained with a much higher dose of free α-bisabolol. Replication experiments are in progress.

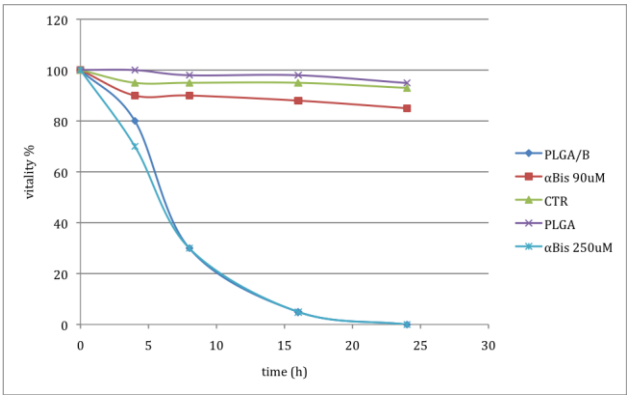

Figure 2 Cytotoxic activity of α-bisabolol towards human cancer cells. Number of living cells after treatment was tested by countess automated cell counter (Invitrogen) (preliminary data).

α-Bisabolol loaded PLGA nanoparticles induce apoptosis. We have previously reported that a-bisabolol can induce apoptosis in several malignant tumoral cell lines through the mitochondrial pathway [1,2] and thus we decided to investigate if this mechanism was also present in the α-bisabolol loaded PLGA nanoparticles.
In order to evaluate apoptosis induction, the activation of caspase-3 and PARP cleavage were analysed by Western Blot.
Western blot analysis showed that 90μM and 45μM α-bisabolol loaded into nanoparticles induced caspase-3 and PARP cleavage in 3-5 hours. The same effect could be obtained after treatment with the free α-bisabolol molecule at a dose equal to 250 μM.
These data show that the loaded nanoparticles preserve the pro-apoptotic effect of α-bisabolol allowing the use of lower doses of the sesquiterpene to obtain the same effect of the free alcohol.
In conclusion, the enhanced aqueous solubility and higher pro-apoptotic efficacy of α-bisabolol loaded PLGA nanoparticles advocates their potential therapeutic use in cancer treatment.

References

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