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Preparation of a Sustained-Release Implant of the Acetylcholinesterase Inhibitor ZT-1 by Hot-Melt Extrusion (HME) and Evaluation in Rats

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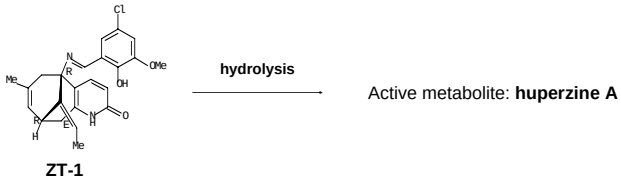
Introduction

ZT-1 is a derivative of huperzine A (Fig. 1), a quinolizidine alkaloid isolated from the club moss *Huperzia serrata* and a potent and selective acetylcholinesterase inhibitor[1]. ZT-1 administration in rats induces an increase in cerebral acetylcholine (ACh)[2], making it an attractive candidate for symptomatic Alzheimer's disease (AD) treatment.

To avoid the drawbacks (lack of compliance, pulsed drug levels) linked to oral administration, an injectable and biodegradable sustained-release implant formulation of ZT-1 was developed, aiming at achieving a prolonged release of the active metabolite over several weeks.

Hot-melt extrusion (HME) is a widely applied processing technique used mainly in plastic industry. This technique is also used in the pharmaceutical industry to prepare granules, sustained-release tablets or transdermal drug delivery systems. HME does not require the use of water nor organic solvents and few processing steps are needed, making it simple, efficient and continuous.

The objective of this work was to develop a HME process to produce ZT-1 implants to be administered monthly and to evaluate the sustained drug release in rats *in vivo*.



ZT-1

Figure 1 Structure of ZT-1

Materials & Methods

Drug product and Implants

ZT-1 was obtained by hemi-synthesis from (-)huperzine A. Several poly(D,L-lactide-co-glycolide) (PLGA) polymers, differing in composition and viscosities, were used to prepare ZT-1 implants.

Biodegradable polymeric implants of ZT-1 were prepared first by mixing ZT-1 and PLGA using a Retsch centrifugal ball Mill. The powder blend was extruded using a single-screw extruder. A cylindrical die of Ø 1.3 mm was used and temperatures were set in between 70^{0}C and 90^{0}C. The biodegradable polymeric strand was cut into implants containing 3.0 mg of ZT-1. Implants were packaged and sterilised by gamma irradiation (25kGy). ZT-1 and huperzine A analysis were performed by HPLC-UV (230 nm).

Animal study

For each formulation, a group of six Sprague-Dawley male rats (360-400 g) was given a single subcutaneous (s.c.) administration of ZT-1 implants (dose: ca. 15 mg/kg of ZT-1) under the neck skin using a puncture needle (Fig. 2). The day before ZT-1 administration, a reference plasma sample was collected. Plasma samples for drug measurement were then collected at 2h, 4h, 8h, 10h, 24 post-dosing and then every 3-4 days from day 4 to day 49 after administration.

Animals were observed for clinical signs throughout the study. At the end of the study, animals were sacrificed and the sites of implantation were recovered for evaluation of local inflammation and implant degradation.

Figure 2 ZT-1 implant and puncture needle used for s.c. administration in rats

Pharmacokinetic evaluation

ZT-1 and its active metabolite huperzine A were simultaneously quantified in plasma following solid phase extraction using a LC/MS/MS method developed and validated at the Laboratoire d'Etude du Métabolisme des Médicaments (CEA-Saclay, France). Because direct determination of ZT-1 in biological matrices is hampered by its rapid degradation into huperzine A, $NaBH_4$ was used during blood collection to circumvent this problem by transforming (hydrogenation) ZT-1 into the stable reduced form ZT-1R.

Time concentration curves were established for each rat and mean pharmacokinetic profiles were calculated.

Results & Discussion

This work showed that PLGA implants containing from 20% up to 70% (w/w) of ZT-1 could be successfully produced by HME (Fig. 3), without using any plasticizer. Key parameters in HME include temperature of the extruder, screw type and speed, drug loading, particle size distribution and flowing properties of the blend.

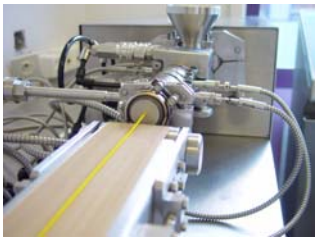


Figure 3: Production of ZT-1 implants by hot-melt extrusion

Stability studies showed that implants could be sterilized by gamma irradiation and stored at 25^{0}C.

Implants produced by HME exhibited controlled drug release after s.c. administration in rats. Huperzine A plasma profiles could be modulated by varying the type of polymer used (composition and viscosity) and drug loading, as can be seen in Figure 4. No burst in drug release was observed. A lag time of maximum 7 days between administration and drug release was observed for some implants. Sustained plasma levels of huperzine A were achieved over several weeks and inter-individual variability was low. No clinical signs were observed throughout the study and no signs of local intolerance were reported.

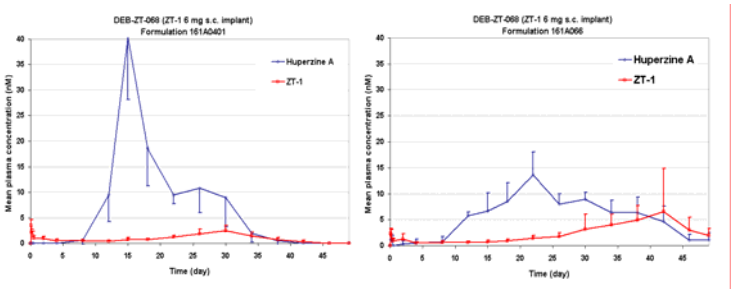


Figure 4 Pharmacokinetic profiles (mean ± SD) of ZT-1 and its active metabolite huperzine A following a single s.c. administration of two implants of ZT-1 3 mg in rats

As compared to oral administration, the ZT-1 implant offers the following perspectives for human use :

- once-a-month dosing
- improved compliance
- implant-controlled progressive increase in huperzine A plasma levels
- no influence of food nor variability in drug absorption
- sustained plasma levels

Conclusion

A biodegradable sustained-release formulation of ZT-1 for monthly dosing was prepared by hot-melt extrusion. Plasma profiles in rats did not show any burst release. Plasma levels and release period could be modified by appropriate choice of PLGA and drug loading. Controlled drug release may be a significant advantage for symptomatic Alzheimer's disease (AD) treatment.

The ZT-1 PLGA implant is currently tested in clinical trials.

References : [1] Giacobini E. Cholinesterases: new roles in brain function and in Alzheimer's disease. Neurochem Res 28(3-4): 515-22 (2003). [2] X. C. Tang. The studies of ZT-1 on cortical acetycholine level in rats: comparison with huperzine A and donepezil. State Key Laboratory of Drug Research, Shangai Institute of Materia Medica (China) (2002).