Encapsulation of a BCS Class IV Drug in order to Improve its Absorption

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INTRODUCTION

Developed as anti-obesity drug, Orlistat is a highly lipophilic drug with poor membrane permeability as well as a poor aqueous solubility. After oral administration, the drug stays in the GI tract as it was primarily not designed to cross the intestinal barrier and to act locally. According to the Biopharmaceutic Drug Classification (BCS), Orlistat can be categorized as BCS class IV drugs (low solubility and low permeability). This class of drugs presents significant problems for effective oral delivery (Amidon G.L., 1995). Recently, formulations using lipidic excipients had already shown effectiveness on BCS class II drugs (low solubility and high permeability) (Kovarik J.M., 1994). So, appropriate lipid-based formulations could also improve the solubility of BCS class IV drugs. Nevertheless, their poor membrane permeability may be a major hurdle in increasing their bioavailability. Usually, for BCS class IV drugs, returning to an earlier development step in order to improve the drug candidate solubility and permeability property is preferred (Pouton C.W., 2006).

Orlistat is also a potent inhibitor of Fatty Acid Synthases (FAS), enzymes that break down triglycerides in the intestine. By binding irreversibly these enzymes, triglycerides from the diet are prevented from being hydrolyzed into absorbable free fatty acids. The primary effect of this molecule is a local FAS inhibition within the gastrointestinal tract after an oral dose. Recently, therapeutic effects of FAS inhibitors like Orlistat on many tumors including those of the prostate, breast, colon, ovary and others were highlighted. These anticancerous effects are based on the inhibition of the FAS expressed at significantly higher levels in many human cancers (Little J.L., 2007). As FAS is a critical enzyme involved in the anabolic conversion of dietary triglycerides to fatty acids in mammals, its up-regulation generally correlates with the development, maintenance and enhancement of the malignant phenotype (Kuhajda F.P., 2006).

In this study, formulations using Self MicroEmulsifying Drug Delivery Systems (SMEDDS) to encapsulate Orlistat were investigated to improve its permeability across a Caco-2 cells monolayer as such formulation have been reported to improve the solubility and the intestinal absorption of lipophilic drug (Kovarik J.M., 1994). The lipid-based systems contain Cremophor® ELP, Labrafil® M1944CS and/or PEG 300 (Table 1). In parallel, physical and biological characterizations were conducted on these systems such as size measurement and cytotoxicity assays.

MATERIAL AND METHODS

A solution of transport medium containing 1% DMSO and adjusted to pH 6.0 is used as a control to establish the permeability of Orlistat without any absorption enhancers. The dispersed lipid-based systems compositions are listed on Table 1. The formulation A contains a higher proportion of oily solubilizer and, conversely, the formulation B contains more non-ionic surfactant.

	Formulation A	Formulation B
Non-ionic surfactant	Cremophor® ELP, 3%	Cremophor® ELP, 7.2%
Co-solvent	-	PEG300, 1.8%
Oily phase	Labrafil® M1944CS, 7%	Labrafil® M1944CS, 1%
Aqueous phase	Transport medium, 90%	Transport medium, 90%

Table 1 : Composition of the three microemulsifed systems

Dynamic light scattering measurements of the microemulsified systems were performed. Results are presented as droplet size and polydispersity index.

Caco-2 cells were cultured at 37°C in a humidified air-5% CO_2 atmosphere. In 21-day, cell culture experiment, Caco-2 cells were seeded on 0.4µm pores inserts on the cell culture plates at a density of 6×10^4 cells/insert. Culture medium was replaced every 48 hours for the first 6 days and every 24 hours thereafter. After 21-25 days in culture, Caco-2 cells monolayers were utilized for the transport experiments.

Apical-to-basal permeability of Orlistat was measured in a proton gradient (pH 6.0 for the donor compartment and pH 7.4 for the receiver compartment). The medium containing the drug was introduced into the apical side. Thereafter, aliquots were taken from the basal side at 15, 30, 60, 90 and 120 minutes. The volume of the basal solution was maintained by adding fresh transport medium. All experiments were performed at 37°C and under an orbital agitation of 50 rpm. Every assay was realized in triplicate. Results are presented as apparent permeability.

The concentration of Orlistat in the sample was determined with a reversed-phased HPLC system equipped with a MS-MS-ESI detector. This analytical technique provides a great sensibility to our analyses without the safety and wastes issues inherent to radioactive methods.

Cytotoxicity studies consisted in measuring the lactate dehydrogenase (LDH) leakage, the Transepithelial Electrical Resistance (TEER) and the Lucifer Yellow (LY) apparent permeability. Firstly, LDH leakage indicated cell membrane damages. To determine LDH leakage, cell culture supernatants were collected after the transport experiment. Secondly, TEER experiments consisted in measuring TEER values at different moments before and after the transport study. The first measurement was performed to ensure that TEER values are above 300 $\Omega.cm^2$. The second one was made after the transport study. The Caco-2 cell monolayers were then incubated with culture medium before a final TEER measurement to study any recovery of the cells. Thirdly, Lucifer Yellow (LY) was the dye used to study the paracellular pathway. After the transport experiment and the second TEER monitoring, LY permeability study was performed. LY apparent permeability results provided information about tight junctions' integrity.

The apparent permeability and cytotoxicity data are presented as means ±SD. The Student's test was used to evaluate the statistical significance of mean values.

RESULTS AND DISCUSSION

As shown on Figure 1, the transport experiments across the Caco-2 cells monolayers gave a greater apparent permeability of Orlistat with the lipid based systems than with the drug particle incorporated in the transport medium without any absorption enhancers. While the formulation A

showed the lowest permeability across the *in vitro* intestinal barrier, the formulation B presented the highest one. So, the use of a higher proportion of surfactant could increase the Orlistat permeability, whereas the use of a higher amount of oil showed less interesting results, even if the oil could improve the drug solubility. These observations suggested that Orlistat solubilization pattern could require a higher amount of surfactants which could be responsible for the improved permeability.

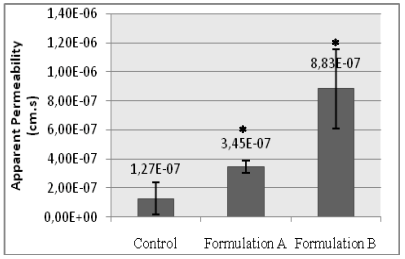

Figure 1: Orlistat apparent permeability results. Bars represent mean values ± SD, n=3. Asterisks indicate significance from the experiment with the control ($p<0.05$)

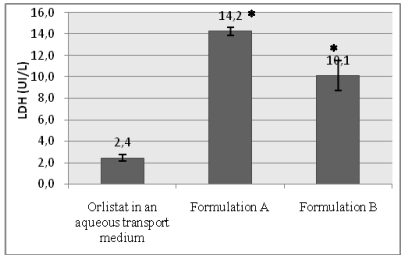

Figure 2: LDH leakage results. Bars represent mean values ± SD, n=3. Asterisks indicate significance from the experiment with the control solution of Orlistat ($p<0.05$)

Concerning the cytotoxicity studies, both formulations increased significantly the LDH leakage (Figure 2) and the LY permeability (Figure 3) in comparison with the aqueous solution of Orlistat ($p<0.05$). Nevertheless, they did not show the same results. Indeed, the formulation A presented the greatest increase of LDH leakage and LY permeability meaning that the paracellular pathway and the cell membrane integrity were damaged. In spite of these harms and a higher proportion of oil, Orlistat permeability across the Caco-2 cells monolayer did not improve in comparison to the formulation B. But in both cases, tight junctions were affected. According to the literature, lipidic absorption enhancers, as sodium caprate, can change the morphology of the cytoskeleton and alter the structure of the tight junctions (Anderberg E.K., 1993). These changes were highlighted by using LY as a paracellular permeability marker.

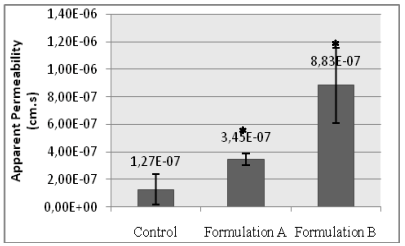

Figure 3: LY apparent permeability. Bars represent mean values ± SD, n=3. Asterisks indicate significance from the experiment with the control solution of Orlistat ($p<0.05$)

	Formulation A	Formulation B
Diameter (nm)	77	18
Polydispersity index	0.18	0.29

Table 2: Particles size of the formulations A and B

Table 2 represents the particles size of the two studied formulations. Droplet size measurements showed that the formulation A presented the highest droplet size. Conversely, the formulation B presented smaller droplet sizes. By decreasing its size, the surface of contact increases providing higher absorption potential. This emphasizes that the reduction of particles size could significantly improve the systemic absorption of poorly soluble drugs. The use of non-ionic surfactants to emulsify Labrafil® M1944CS is a good alternate for drug encapsulation.

CONCLUSIONS

The lipidic encapsulation systems presented in this study enable the solubilization and permeability of Orlistat, a BCS class IV drug with anticancerous properties and initially designed to not cross the intestinal barrier. This study also showed that a higher proportion of surfactant and especially the use of Cremophor® ELP contribute to this enhancement.

Developing such systems has an obvious interest in the anticancerous therapy and also in improving patient compliance by providing a medication administered by oral route. The effectiveness of Orlistat as an anticancerous drug has already been assessed in association with Trastuzumab indicated in the breast cancer (Menendez J.A., 2005). Additional studies on the association of this monoclonal antibody with the encapsulated form of Orlistat that we have developed on this study should be undertaken to prove and confirm the interest of the lipid-based systems.

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