In Vitro Evaluation and Permeability Assessment of Liquid and Solid SMEDDS for the Lymphatic Targeting of the Cyclic LyP-1 Peptide

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Purpose
The purpose of the present work was to evaluate the cytotoxicity and bioactivity of designed liquid and solid SMEDDS formulations of the anticancer peptide cyclic LyP-1 and compare the transport of the formulations across the Caco-2 cell monolayer.

Methods
Optimized formulations which were formulated with Labrasol®, Gelucire® 44/14, Labrafil®, D-α-tocopheryl polyethylene glycol succinate (TPGS) and Tween 80 as surfactants; Peceol™ and Maisine™ as the oil phase and polyethylene glycol 300 and propylene glycol as cosolvents were solidified by lyophilization or spray-drying after characterization. The cytotoxicity and bioactivity of the formulations were evaluated by the MTT assay in Caco-2 human colorectal cancer cell line and MDA-MB-231 breast cancer cell line. Permeability studies were carried out with Caco-2 cell monolayer as a model of intestinal barrier and the samples collected from the basolateral compartment were evaluated with a novel LC-MS/MS method which was developed for the cyclic LyP-1 peptide (Triple Quad LC/MS, Agilent Technologies, USA).

Results
Optimum liquid SMEDDS formulations, which were selected after stability assessment for two weeks, were solidified with mannitol (20 %) or Aerosil (0.5 %). Cell viability after the application of optimized blank liquid formulations which were diluted in the 1:200-1:64000 range were found to be 69 % as the lowest value after 24 hours. No statistical difference was found between the dilutions of U9-F21 liquid formulation (1:200-1:64000) after 24 hours (p > 0.05) and the lowest cell viability was found as 89 %. For the optimized solid formulations in five different compositions were found to be statistically the same with the positive control group (p>0.05) except lyophilized U9-F23 and U12-F17 formulations after 24 hours. There was no significant difference between the lyophilized formulations and positive control group after 48 hours (p>0.01), which shows the safety of the formulations in the gastrointestinal track. No statistically significant difference was found between the cell viability of the positive control group and the solid U9-F21 formulations, which contained the linear and cyclic peptide (p>0.05) in MDA-MD-231 cell line after 24 hours. The bioactivity of U9-F21 formulation, which was applied as 1:10 and 1:16000 dilutions in solid SMEDDS were found to be significantly different from the positive control group after 24 and 48 hours (p<0.05) in MDA-MD-231 cell line showing that the bioactivity was the result of the solidified formulations containing the peptide. The apparent permeability coefficients (Papp, cm/s) of the peptide in different liquid and solid formulations were found to be higher than the peptide alone, specifically for the U9-F21 (p= 0.00005) and U9-F23 (p= 0.00115) formulations.

Conclusion
Optimized liquid and solid SMEDDS formulations were evaluated utilizing the Caco-2 and MDA-MB-231 cell lines for cytotoxicity and bioactivity, respectively. The transport of the anticancer peptide, LyP-1, across the Caco-2 cell monolayer in liquid and solid formulations was compared for further studies. Acknowledgment: This project was supported by TÜBİTAK (The Scientific and Technological Research Council of Turkey), grant number: SBAG 1002-113SS69.