

Enhancement of Caco-2 cell permeability of a T-cell activation inhibitor via cyclization

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Introduction

Previously, a linear peptide with a sequence of GLRILLKLV, also known as the core peptide (CP) based on the α -chain of the T-cell receptor, was shown to inhibit T-cell activation in both *in vitro* and *in vivo* studies [1,2,3]. Among these studies, Manolios et al demonstrated that CP was extremely effective in the treatment of T-cell mediated autoimmune diseases such as adjuvant induced arthritis [1], allergic neuritis [1] and diabetes in mice models [1]. Another study by Gollner have shown that the emulsified CP (conjugated with palmitic acid) cream could reduce inflammation when applied to the skin of mice with contact sensitivity [3]. Furthermore, when DNA encoding CP was injected into the skin before the application of allergen to sensitized animals, marked local suppression of inflammatory was observed [3]. These studies suggested that the CP could potentially be a drug target for the treatment of various T-cell mediated autoimmune diseases.

In this study, a cyclic derivative of CP which possesses enhanced activity over CP was synthesized and its permeability through the Caco-2 intestinal cells was examined and compared with different derivatives of CP that have been conjugated to lipid and/or carbohydrate for enhancement of permeability. The results were used to determine the likelihood of developing this peptide into an oral formulation.

Results and Discussion

Peptides were successfully separated from the sample matrix by a C-18 analytical column and quantified using a gradient program with 306 nm as the detection wavelength. The retention time for the cyclic derivative was about 7.8 minutes (Figure 1).

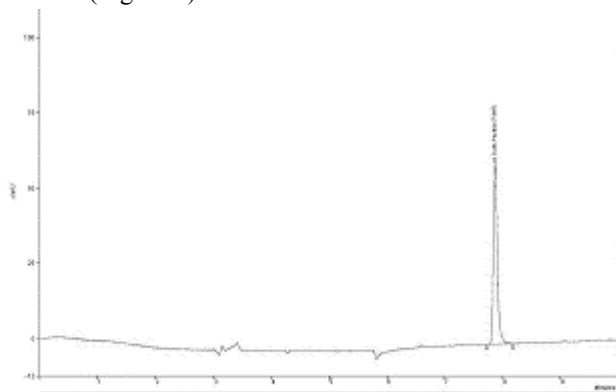


Figure 1. Analytical HPLC of cyclic derivative of CP

The concentrations of the standards containing the cyclic peptide were 1.25, 2.5, 5, 10, 25 and 50 μ M and the linear coefficient of the calibration curve was greater than 0.999 (Figure 2).

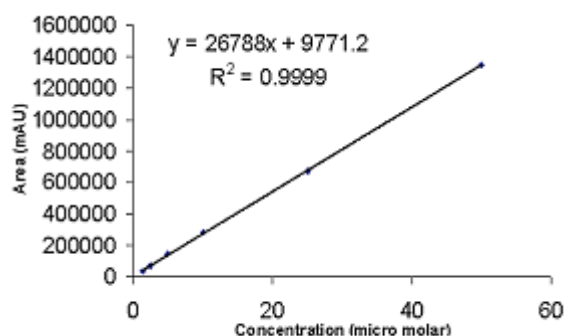


Figure 2. Calibration curve of cyclic derivative

The Caco-2 cell permeability of various CP derivatives was measured and shown in Table 1. The permeability of the cyclic derivative was observed to be at least 1000-fold higher than that of the linear CP. Moreover, this value was significantly greater than all the permeability values of the lipid and carbohydrate CP conjugates ($p < 0.001$). This demonstrated that the method of cyclization was more effective in enhancing intestinal cell permeability than the linear lipid and/or carbohydrate conjugation. To explain this observation, the additional lipid and carbohydrate components increased the size of the peptide up to a point

Table 1. Caco-2 cell permeability of cyclic CP derivative and its linear derivatives

Peptide	P_{app} (cm/s)
Cyclic derivative (200 μ M)	$7.49 \pm 0.52 \times 10^{-6} \dagger$
Cyclic derivative (300 μ M)	$9.69 \pm 2.47 \times 10^{-6} \dagger$
CP	$0.42 \pm 0.14 \times 10^{-7}$
Lipid-CP	$1.10 \pm 0.23 \times 10^{-7}$
Carbo-CP	$0.02 \pm 0.013 \times 10^{-7}$
Carbo-Lipid-CP	$0.13 \pm 0.017 \times 10^{-7}$

(Lipid= lipoamino acid with 12 carbons,
Carbo = carbohydrate conjugation. n = 3 or 4.)
 \dagger Statistically significant difference from CP and its conjugates ($p < 0.001$)

where it hindered the peptide penetration across the cell membrane, whereas the cyclization did not require any additional component. Demonstrating that the size and the tertiary shape of the peptide are the important determining factors for membrane permeability as suggested by Palm [4].

According to the FDA's biopharmaceutics classification system [5], the permeability of the cyclic CP derivative could be classified as a high permeability drug. As drugs with high permeability generally correlate to high oral absorption, this supports the further development of the cyclic CP derivative into an oral formulation.

There are a number of possible explanations behind the dramatic increase in cell permeability by peptide cyclization. In general, it is accepted that the cyclization could increase its cell permeability by reducing its charges and hence increasing its lipophilicity. This has been demonstrated by various research groups such as by Okumu et al⁶ who have shown similar results in investigating the cyclization of a hexapeptide (Ac-Trp-Ala-Gly-Gly-X-Ala-NH₂). Okumu et al [5] proposed that cyclization increases the apparent cell permeability mainly due to the reduction of the molecular radii and the restriction of the conformation flexibility of the peptide. Another explanation could be that the cyclization removes the *N* and *C* terminus of the peptide and therefore increases the resistance against enzymatic degradation at the terminus and hence allowing the intact peptide to diffuse through the cell monolayer [7,8].

Even though the permeability of the cyclic CP derivative was much higher than the linear CP, one of the problems with this peptide is its poor water solubility as the CP sequence mostly consists of hydrophobic residues. An ideal drug candidate for oral absorption should both have high apparent permeability and adequate aqueous solubility. The lipophilicity of the peptide will need to be fine tuned further by chemical modifications to optimize both permeability and solubility properties.

This preliminary study has demonstrated that the cyclization method was effective in enhancing intestinal cell permeability. Future work would need to be carried out to explore the stability of the cyclic derivative with intestinal enzymes to confirm the hypothesis of terminus protection; to measure the oral bioavailability by conducting intravenous and oral pharmacokinetic studies in animals; chemical modifications such as conjugation of lipid and/or carbohydrate to the cyclic CP derivative to further enhance its permeability.

Acknowledgments

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