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EFFECT OF AZAGLYCINE ON THE INTERNALIZATION OF PENETRATIN DERIVATIVES

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INTRODUCTION

Delivering therapeutic and imaging agents into cells is still challenging. Cell-penetrating peptides (CPPs); short, mainly positively charged peptides, are promising tools to transport biologically active compounds into cells. The firstly discovered and well-known member of CPP family is the Penetratin (R^{43}QIKIWFQNRRMKWKK^{58}) [1–3]. The structure-activity results showed that replacement of Trp at position 48 with Phe revealed lower cellular uptake [4]. Although it is very efficient CPP, many efforts were made to improve its properties [5-8]. Penetratin and its derivatives are potentially useful as an uptake enhancer for intracellular drug delivery [9]. Among the possible structural modifications one is the substitution of one or more amino acids by an aza-amino acid in which C^{α} is replaced by nitrogen results in an aza-peptide **(Figure 1).** This modification may change the structure of the original peptide and thus can increase its biological activity [10].

AIMS

Our aim was to investigate the effect of aza-glycine substitution of tryptophan residues (Trp48, Trp56, or both) on the internalization of Penetratin, and to assess how these modifications influence the cellular uptake and the internalization mechanism, utilizing 5(6)-carboxyfluorescein labeling of the peptide N-terminus for flow cytometry analysis.

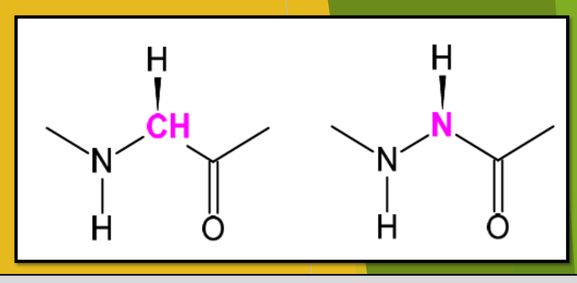

Figure 1. The structure of glycine and aza-glycine moiety

RESULTS

1) Synthesis of Peptides

Peptides were synthesized by SPPS on Rink-Amide MBHA resin using standard Fmoc/^{t}Bu protocol. The azaglycine was introduced using CDI and Fmoc-hydrazide dissolved in DMF and in the presence of 2 eq. of DIEA **(Figure 2).**

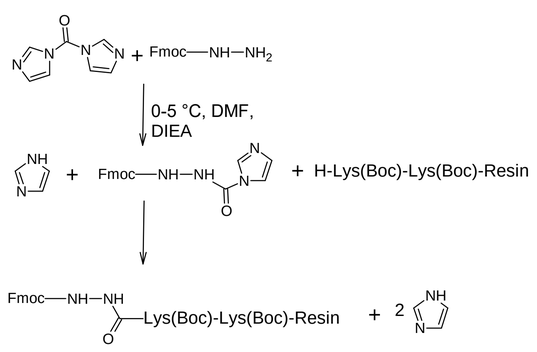

Figure2. The introduction of azaGlycine into the peptide sequences

The crude peptides were purified by RP-HPLC and the peptides were characterized by analytical RP-HPLC and ESI-MS **(Table 1).**

Table 1. Chemical Characterization of Peptides

Sequence	Code	Rt [a]	M_{Calc}	M_{meas} [b]
Cf-RQIKIWFQNRRKWKK	Pen(desMet) [c]	15.1	2472.9	2472.2
Cf-RQIKIWFQNRRK-azaGly-KK	Trp56aGlyPen(desMet)	14.8	2344.7	2344.2
Cf-RQIKI-azaGly-FQNRRKWKK	Trp48aGlyPen(desMet)	13.4	2344.7	2344.1
Cf-RQIKI-azaGly-FQNRRK-azaGly-KK	Trp48,56aGlyPen(desMet)	12	2216.5	2216.2
Cf-RQIKIWFQNRRKGKK	Trp56GlyPen(desMet)	15	2343.7	2343.2
Cf-RQIKIGFQNRRKWKK	Trp48GlyPen(desMet)	13.6	2343.7	2343.2
Cf-RQIKIGFQNRRKGKK	Trp48,56GlyPen(desMet)	13.2	2214.5	2214.1

[a] The analytical chromatogram was obtained using Hypersil Hypurity C18 column (4.6 mm × 150 mm, 5 µm, 190 Å). Linear gradient elution (0 min 0% B, 2 min 0% B, 22 min 90% B) was used at 1 mL/min flow rate. [b] ESI-MS on a Bruker Amazon SL (Bremen, Germany). [c] Pen(desMet) refers to a penetratin derivative which does not have Met in its sequence.

2) Cellular Uptake of Peptides

A-431 human skin squamous cancer cells were treated with a solution of peptides at 5 µM concentration at 37 °C for 90 min. The cellular uptake was measured by flow cytometry **(Figure 3).**

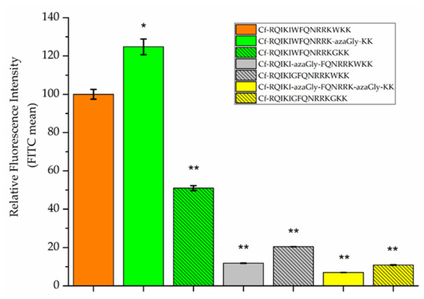

Figure 3. Cellular uptake of the labelled penetratin derivatives into A-431 cells.

- Trp56aGlyPen(desMet) peptide had higher internalization (124.8%) than Pen(desMet) (100%).
- The internalization of Trp56aGlyPen(desMet) peptide was 2.4-fold higher than that of Trp56GlyPen(desMet) peptide (50.0%).
- Substitution of the Trp residue near the N-terminus (Trp48aGlyPen(desMet) and Trp48GlyPen(desMet)) or in both positions (Trp48,56aGlyPen(desMet) and Trp48,56GlyPen(desMet)) resulted in markedly decreased internalization (it was between 6% and 20.5%).

3) Mechanism of the Internalisation

To study the mechanism of internalisation, cells were pretreated with the solution of inhibitors (EIPA (500 µM) as macropinocytosis, CPZ (300 µM) as clathrin-mediated endocytosis; mBCD (36 mM) as caveolae/lipid raft-mediated endocytosis and COL (200 mM) as microtubular inhibitor) for 30 min, followed by the treatment of the most promising peptides solution at 5 µM for 90 min. To inhibit all endocytosis routes, NaN_3 (500 µM) and DOG (250mM) was used. **(Figure 4)**.

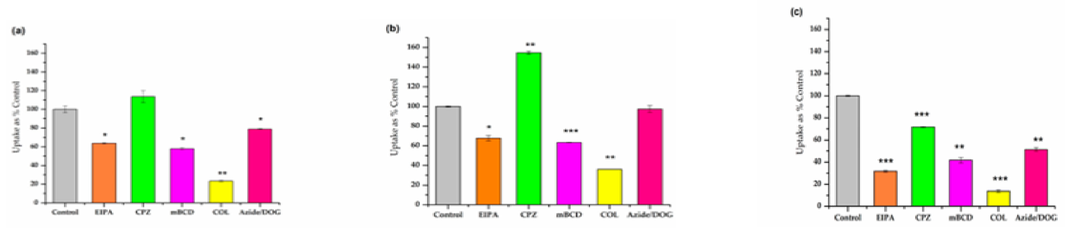

Figure 4. Effect of endocytosis inhibitors on the internalization of peptides (a) Pen(desMet), (b) Trp56aGlyPen(des), and (c) Trp56GlyPen(des).

- The EIPA, mβCD, and COL had the same effect on the cellular uptake in case of all peptides.They significantly inhibited their uptake by 14% - 68%.
- The Gly substituted derivative was more sensitive to these inhibitors, showing reductions of 32%, 42%, and 14% respectively.
- CPZ had varied effects: no influence on internalization of Pen(-Met), decreased cellular uptake of Trp56GlyPen and increased internalization of Trp56aGlyPen derivative.
- Pretreatment with NaN_3 and DOG resulted in significant decrease in uptake of Trp56GlyPen(50%), moderate decrease in uptake of Pen(-Met) (79%) and no effect on the uptake of Trp56aGlyPen (97%).

CONCLUSIONS

- The substitution of Trp48 dramatically reduced the internalization, showing the importance of Trp48 in cellular uptake.
- Trp56 (Trp56aGlyPen(desMet)) is the most active CPPs.
- The Trp56GlyPen(desMet) and Trp56aGlyPen(desMet) peptides demonstrated altered internalization pathways, suggesting that the difference in their backbone - natural or aza-amino acid - significantly influence their cellular uptake mechanisms.
- Our results suggest that aza-amino acid insertion may be a useful modification to alter the internalization of a CPP.

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ABBREVIATIONS

CDI: 1,1′-carbonyldiimidazole; DIEA: N,N-diisopropylethylamine; EIPA: 5-(N-Ethyl- N-isopropyl)amiloride; CPZ: chlorpromazine; mBCD: methyl-β-cyclodextrin; Col: colchicine; DOG: 2-Deoxyglucose.

REFERENCES

[1] Derossi, D. et al. Trends Cell Biol. **1998**, 8, 84–87.
[2] Bánoczi, Z. et al. Bioconjug. Chem. **2007**, 18, 130–137.
[3] Watson, G.M. et al. ACS Omega **2017**, 2, 670–677.
[4] Dom, G. et al. Nucleic Acids Res. **2003**, 31, 556–561.
[5] Khafagy, E. et al. *Int. J. Pharm.* **2009**, *381* (1), 49–55.
[6] Keum, T. et al *Int. J. Nanomedicine*. **2022**, *17*, 697–710.
[7] Garibotto, F. et al. *J. Med. Chem.* **2011**, *46* (1), 370–377.
[8] Thorén, P. E. G. et al. *Biochem. Biophys. Res. Commun.* **2003**, *307* (1), 100–107.
[9] Liu, C. et al. Mol. Pharm. **2014**, *11*, 1218–1227.
[10] Tarchoun, K. et al. Futur. Pharmacol. **2022**, *2*, 293–305.