Potent inhibition of CYP3A4 by the endomorphin-2 analogues

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Introduction

Endomorphins (endomorphin-1, EM-1, Tyr-Pro-Trp-Phe-NH₂, endomorphin-2, EM-2, Tyr-Pro-Phe-Phe-NH₂) are endogenous peptides, which are very potent and highly selective μ -opioid receptor agonists. Moreover, they possess a potent analgesic activity comparable to that of morphine but are devoided of its' undesirable effects [1, 2]. However, their use as therapeutic agents is limited because of their pharmacokinetic features, such as stability and permeability *via* blood-brain barrier (BBB). In our previous developments, modifications in the structure of endomorphine-2 have shown an improvement in stability and permeability through the membranes [3]. In the current study, the goal was to investigate another pharmacokinetic feature, namely the risk of drug interactions at the level of cytochrome enzymes. CYP3A4 is the most important drug metabolizing enzyme in humans. It is highly expressed in liver and gastrointestinal mucosa and is involved in the metabolism of more than 50% of the used drugs. Therefore, the risk of drug interactions is greatest in the drugs modifying the activity of CYP3A4.

Materials and Methods

Peptide synthesis and analysis

Peptides in this study were synthesized by manual solid-phase procedures using techniques for Fmoc-protected amino acids on Wang or MBHA Rink-Amide peptide resins respectively. 20% Piperidine in DMF was used for deprotection of Fmoc-groups and DIC and HBTU were employed as a coupling agent. Simultaneous deprotection and cleavage from the resin was accomplished by treatment with TFA/TIS/water (95:2.5:2.5) for 3 h at room temperature. Crude peptides were purified by preparative TLC and their purity was checked by analytical HPLC.

Measurement of CYP3A4 activity *in vitro*

The test compound was mixed with a master pre-mix comprising CYP450 BACULOSOMES[®] reagent and regeneration system, which contained glucose-6-phosphate and glucose-6-phosphate dehydrogenase. The mixture was incubated at room temperature for 20 min. Following incubation, CYP enzyme-specific substrate (Vivid DBOMF for CYP3A4) and NADP⁺ were added and the mixture was incubated at room temperature for 30 min. CYP activity was evaluated by measuring the fluorescence of the fluorescent metabolite generated from CYP3A4 enzyme-specific substrate. The fluorescence was measured using a microplate reader BioTek Synergy.

Results and Discussion

For the purpose of our study, we used four endomorphin-2 analogues, two of them were modified at third position - Phe(pF) (1, Tyr-Pro-Phe(pF)-Phe-1,2-ethylenediamine) and conjugated at fourth position with 1,2-ethylenediamine (2, Tyr-Pro-Phe-Phe-1,2-ethylenediamine). The other two, was conjugated at first position with deoxycholic acid and modified at third position - Phe(pF) (3, Deoxycholic-Tyr-Pro-Phe(pF)-Phe-OH) and Phe (pCl) (4, Deoxycholic-Tyr-Pro-Phe(pCl)-Phe-OH) (Figure 1).

Figure 1: Modifications in chemical structure of endomorphin-2

The four endomorphin-2 analogues we used, showed potent and concentration-dependent inhibition of CYP3A4 in concentrations of 6,25 to 100 μM . The observed effect of two of them (1 and 2) was more distinct. The other two analogues (3 and 4), have shown slightly less pronounced inhibition of CYP3A4 (Figure 2). For comparison was used ketoconazole, classical inhibitor of CYP3A4, in concentration of 10 μM (90 % inhibition of CYP3A4).

Figure 2: Inhibition of CYP3A4 by the endomorphin-2 analogues

After obtaining the results and analyzing them, we calculated the following IC_{50} values for each analogue (Table 1).

Table 1: IC₅₀ values of EM-2 analogues

<i>EM-2 analogues</i>	<i>IC₅₀ values with 95% CI</i>
<i>Tyr-Pro-Phe(pF)-Phe-1,2-ethylenediamine</i>	15,00 μ M (9,89-22,73) p<0,05
<i>Tyr-Pro-Phe-Phe-1,2-ethylenediamine</i>	16,42 μ M (10,28-26,25) p<0,05
<i>Deoxycholic-Tyr-Pro-Phe(pF)-Phe-OH</i>	31,37 μ M (20,02-54,07) p<0,05
<i>Deoxycholic-Tyr-Pro-Phe(pCl)-Phe-OH</i>	29,04 μ M (21,33-32,96) p<0,05

Conclusion

All four endomorphine-2 analogues showed potent inhibition of CYP3A4 isoenzyme, which should be considered if they are used in clinical practice for possible drug-drug interactions.

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References

1. Zadina, JE, Nilges, MR, Morgenweck, J, Zhang, X, Hackler, L, Fasold, MB *Neuropharmacology*, 2016, 105, 215-227.
2. Georgiev, K., Dzimbova, T., Iliev, I., Datcheva, R., Pajpanova, T. *Comptes rendus de l'Academie bulgare des Sciences*. 2014, 67, 1281-1286.
3. Georgiev, K., Dzimbova, T., Iliev, I., Datcheva, R., Pajpanova, T. In: Naydenova E, Danalev D, Pajpanova T (eds) *Peptides 2014. Proceedings of the 33rd European Peptide Symposium*, Sofia, Bulgaria, 156-157 ISBN 978-619-90427-1-7.