Peptide conjugates of transportan10 with antimicrobial and antifungal antibiotics

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Introduction

Conjugates, in which two molecules differing by chemical and physical properties are connected, are a promising group of compounds. Conjugates consisting of a cell penetrating peptide (CPP) and a molecule with therapeutic properties are among most frequently studied compounds. CPPs can deliver to eukaryotic and prokaryotic cells a vast range of different biologically active compounds, such as proteins, nucleic acids, oligonucleotides, liposomes, nanoparticles, peptides, PNA, and low-molecular chemotherapeutic agents (e.g. antibiotics). Transportan (TP), reported by Langel group, is a chimeric peptide composed of the first 12 amino acid residues of neuropeptide galanin and 14 amino acid residues-long wasp venom peptide, mastoparan, connected *via* a lysine residue. A short variant, named TP10, with deletion of the N-terminal hexapeptide, retains the efficient cell penetration property of the parent compound with significantly less potential side effects [1].

In this paper we report synthesis and biological studies of peptide conjugates composed of TP10 and antibiotics: levofloxacin, ciprofloxacin and fluconazole. Three different linkers (methylene carbonyl, amide and disulfide bridge) were used to connect both components. TP10 may not only help to transport the antibiotic across the microbial cell membranes, but having intrinsic antimicrobial activity, it can potentiate or even change the molecular mechanism of action of the conjugated antibiotic. In order to determine the cellular uptake of TP10 and its conjugate with ciprofloxacin, their fluorescently-labeled analogues were also synthesized (TP10(F)-NH₂ and CIP-TP10(F)-NH₂). The chemical formulas of antibiotics and synthesized compounds are shown in Fig. 1.

Figure 1: Chemical structures of antibiotics, TP10 and peptide conjugates.

Results and Discussion

All conjugates were obtained using solid phase synthesis and standard 9-fluorenylmethoxycarbonyl (Fmoc) chemistry. In CIP-TP10-NH₂ and LVX-TP10-NH₂ amide bond was formed between carboxyl group of CIP or LVX and peptide α -amino group. In CIP-CH₂-CO-TP10-NH₂, methylene carbonyl linker was applied to connect antibiotic through its amine to the N-terminus of TP10. Intermolecular disulfide bridge, spanning both components in CIP-SS-TP10-NH₂ and LVX-Cys-S-S-Cys-TP10-NH₂, was formed using Lomant's reagent – dithio-bis(succinimidyl propionate) or between two Cys residues.

FLC-CH₂-CO-TP10-NH₂ was synthesized *via* modified Huisgen 1,3-dipolar cycloaddition, known also as click reaction. This required synthesis of the FLC precursor (1 in Fig. 2.) containing alkyne group, which was obtained by the method described by Pore *et al.* [2], and then a click reaction of 2-(2,4-difluorophenyl)-1-(1H-1,2,4-triazol-1-yl)pent-4-yn-2-ol with a peptide containing azidoacetyl moiety attached to α -amino group was

performed. In order to determine the cellular uptake, fluorescently-labeled CIP-CH₂-CO-TP10(F)-NH₂ and TP10(F)-NH₂ were also synthesized. In both compounds, 5(6)-carboxyfluorescein was attached to the ε-amino group of peptide's Lys that replaced Leu in position 12.

Figure 2: Synthesis of FLC-CH₂-CO-TP10-NH₂.

All conjugates and their parent compounds were investigated for fungicidal activity against four fungal strains: *C. albicans* ATCC 10231, *C. albicans* SC 5314, *C. krusei* DSM 6128, and *C. glabrata* DSM 11226, and antibacterial activity against five bacterial strains, three Gram-positive: *S. aureus* ATCC 25923, *S. aureus* ATCC 29213, *S. epidermidis* ATCC 12228, and two Gram-negative (*E. coli* ATCC 25922, *P. aeruginosa* ATCC 27853). TP10-NH₂, levofloxacin and ciprofloxacin did not exhibit antifungal activity at concentrations up to 100 µg/ml; however, two conjugates with ciprofloxacin CIP-TP10-NH₂ and CIP-SS-TP10-NH₂ were active against both *C. albicans* strains (MIC₅₀ values determined for CIP-TP10-NH₂ were 62 and 30 µg/mL, respectively and for CIP-SS-TP10-NH₂ MIC₅₀ were 24 and 34 µg/mL, respectively). Unfortunately, FLC-CH₂-CO-TP10-NH₂ was not active towards tested fungal strains. TP10 did not display antibacterial activity at concentrations up to 200 µg/ml, whereas its two conjugates with ciprofloxacin CIP-TP10-NH₂ and CIP-SS-TP10-NH₂ and one with levofloxacin LVX-Cys-S-S-Cys-TP10-NH₂ inhibited growth of all studied bacteria (Table 1). Interestingly, fluconazole conjugate was also found active towards bacteria. To assess the cytotoxic effect of TP10, ciprofloxacin and its two conjugates, we estimated IC₅₀ values (using MTT assay) for two human cell lines: liver cancer cells (HepG2) and cell line of embryonic kidney (HEK 293), as well as a cell line isolated from kidney of 3-4 weeks old male pig (LLC-PK1). The conjugates were slightly more cytotoxic than their constituents; however, they induced a relatively low cytotoxic effect in comparison with antibiotics widely used in clinic. Microscopic observations showed that TP10(F)-NH₂ as well as its conjugate CIP-CH₂-CO-TP10(F)-NH₂ penetrate well into *C. albicans* SC 5314, and *C. albicans* ATCC 10231.

Table 1: MIC values determined for selected bacterial strains

Compound	MIC [µg/ml]									
	Gram (+)						Gram (-)			
	<i>S. aureus</i> ATCC 25923		<i>S. aureus</i> ATCC 29213		<i>S. epidermidis</i> ATCC 12228		<i>E. coli</i> ATCC 25922		<i>P. aeruginosa</i> ATCC 27853	
	MIC ₉₀	MIC ₅₀	MIC ₉₀	MIC ₅₀	MIC ₉₀	MIC ₅₀	MIC ₉₀	MIC ₅₀	MIC ₉₀	MIC ₅₀
Ciprofloxacin (CIP)	<0.3	<0.3	<0.3	<0.3	0.9	0.2	<0.3	<0.3	<0.3	<0.3
Levofloxacin (LVX)	<0.3	<0.3	<0.3	<0.3	2.2	1.5	<0.3	<0.3	1.5	1.1
Fluconazole (FLC)	>250	>250	>250	>250	>250	>250	>250	>250	>250	>250
TP10	>300	257	>300	231	>300	215	246	121	>300	284
CIP-CH ₂ CO-TP10	295	132	290	198	215	102	15	9	256	115
CIP-TP10	4	4	2	2	4	4	4	4	8	8
CIP-SS-TP10	1.1	0.7	1.3	0.8	1.6	0.2	<0.3	<0.3	1.1	0.8
LVX-TP10	>>300	199	>>300	289	274	211	74	28	164	140
LVX-Cys-SS-Cys-TP10	62	62	16	16	8	8	8	8	62	31
FLC-TP10	16	16	8	8	8	8	8	8	62	62

In conclusion, we obtained six new conjugates composed of antibiotic (ciprofloxacin, levofloxacin or fluconazole) and TP10-NH₂. Two ciprofloxacin conjugates CIP-TP10-NH₂ and CIP-SS-TP10-NH₂ exhibited fungicidal activity against *C. albicans* 10231 and *C. albicans* SC 5314, even though their constituents were inactive. These conjugates displayed also antibacterial activity, whereas TP10-NH₂ did not inhibit bacterial growth. It is worth noting that the activity of conjugate CIP-SS-TP10-NH₂ was the same as of ciprofloxacin.

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References

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