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Development of Daunomycin-Peptide Antitumor Conjugates Containing Neurotensin-Based Homing Moiety

Levente Endre Dókus[1], Eszter Lajkó[3], Orsolya Láng[3], László Kőhidai[3], Gábor Mező[1,2]

[1]*HUN-REN-ELTE Research Group of Peptide Chemistry, Budapest, Hungary.*
[2]*Eötvös Loránd University, Faculty of Science, Institute of Chemistry, Budapest, Hungary*
[3]*Department of Genetics, Cell- and Immunobiology, Semmelweis University, Budapest, Hungary.*

Introduction

Pancreatic ductal adenocarcinoma (PDAC) is one of the most dangerous cancerous diseases of the pancreas leading to high mortality [1]. Targeted tumor therapy could be a good tool to treat PDAC efficiently. From the literature it is known that PDAC cells overexpress the neurotensin-1 (NTSr1) receptor. This protein binds to neurotensin, a peptide hormone consisting 13 amino acids, as its ligand therefore, it can be a potential target for neurotensin-based homing peptide drug conjugates. The biological activity of neurotensin is attributed to its C-terminal part: ^{8}RRPYIL^{13} [2]. Our aim was to develop new peptide - drug conjugates containing daunomycin as an anticancer agent attached to the homing peptides *via* an oxime bond [3].

Results and Discussion

In the first round six different daunomycin - peptide conjugates were initially developed according to the literature background. In the case of three conjugates, enzyme labile spacer (GFLG) [4] was built into the sequences between the homing peptide and the aminooxy moiety used for oxime ligation. This spacer is cleavable by the lysosomal enzyme cathepsin B, making it suitable for enhancing the intracellular degradation and the release of the active metabolite.

The aminooxyacetyl-group containing homing peptide parts were synthetized by SPPS using Fmoc/^{t}Bu technique on Wang-resin. The payload (Dau) was coupled in solution phase (0.2 M NH_4OAc solution (pH=5)) to the cargo *via* oxime bond (Figure 1). The cytotoxic effect of the conjugates were characterized on PANC-1 (a PDAC type) cell line by an impedimetric technique, xCELLigence System.

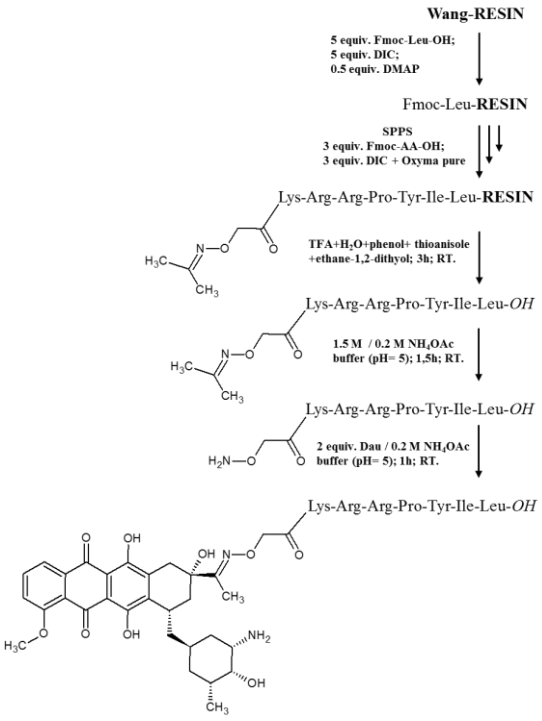

Fig. 1. Outline of the synthesis of conjugates

The results shown that from the first six constructions three conjugates (**2**,**5**,**6**) have a significant cytotoxic effect at 10^{-5} M concentration after 72 hours treatment, but the other three derivatives did not induce antitumor effect on PANC-1 cells (Table 1). The cellular uptake of these conjugates and their metabolism by lysosomal enzymes were also investigated (Figure 2 and 3).

These results were applied for design and prepare new non-natural amino acid (*e.g. tert*-leucine) containing derivatives to increase their enzyme stability [5]. Their antitumor effect (Table 1), internalization ability and lysosomal degradation were also investigated. (Figure 2 and 3)

In the next step the mentioned enzyme labile spacer was built in to the N-terminus of the two structures which resulted highest cellular uptake (**11** and **12)**. Finally, these two structures with best internalization ability (**7**, **8**) were modified with additional non-natural amino acids and leaving of the *tert*-Leu. In the position[8] of this two structures Arg(Me), *h*-Lys, *h*-Arg, D-Lys, D-Arg or in one case L-Arg were built in resulted nine new conjugates. The antitumor activity were also characterized on PANC-1 cell line in 10^{-5} M and $3.3x10^{-5}$M concentrations using 72 or 96 hours incubation. Finally, the IC_{50} values of the three most efficient non-natural amino-acid containing derivatives were characterized (Table 1).

Table 1. Cytotoxic effect of the conjugates

	Sequence	Viability %		Sequence	Viability %
1	*Dau=Aoa*-RRPYIL-*OH*	> **50**	10	*Dau=Aoa*-KRKPW^{t}LL-*OH*	102.6 ± 18.7
2	*Dau=Aoa*-KRRPYIL-*OH*	4.7 ± 1.8	11	*Dau=Aoa*-GFLGKRRPY^{t}LL-*OH*	116.4 ± 3.1
3	*Dau=Aoa*-KPRRPYIL-*OH*	> **50**	12	*Dau=Aoa*-GFLGKRRPW^{t}LL-*OH*	116.6 ± 6.7
4	*Dau=Aoa*-GFLGRRPYIL-*OH*	> **50**			
5	*Dau=Aoa*-GFLGKRRPYIL-*OH*	13.0 ± 1.5			
6	*Dau=Aoa*-GFLGKPRRPYIL-*OH*	11.0 ± 1.7			
7	*Dau=Aoa*-KRRPY^{t}LL-*OH*	116.4 ± 3.1			
8	*Dau=Aoa*-KRRPW^{t}LL-*OH*	116.6 ± 6.2			
9	*Dau=Aoa*-KRKPY^{t}LL-*OH*	110.0 ± 6.8			

	Sequence	IC_{50} (μM)	
		72 h	96 h
13	*Dau=Aoa*-K-*h*Arg-RPYIL-*OH*	27.8 ± 5.9	23.1 ± 14.2
14	*Dau=Aoa*-K-*h*Lys-RPWIL-*OH*	20.9 ± 2.3	13.1 ± 0.9
15	*Dau=Aoa-K*-Arg(Me)-RPYIL-*OH*	22.9 ± 2.5	16.7 ± 2.4
16	*Dau=Aoa*-K-D-Lys-RPWIL-*OH*	20.9 ± 2.2	20.3 ± 6.5

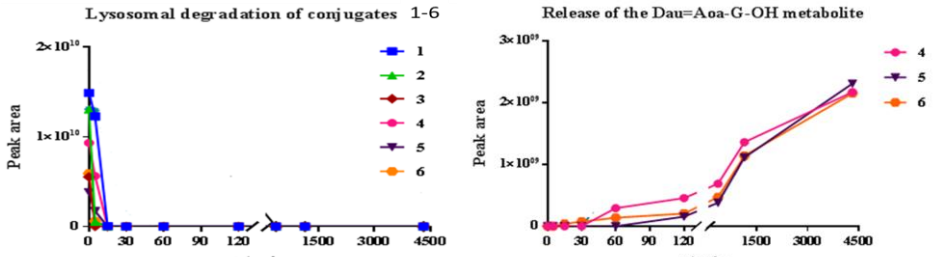

Fig. 2. Time dependent degradation of conjugates by lysosomal enzymes

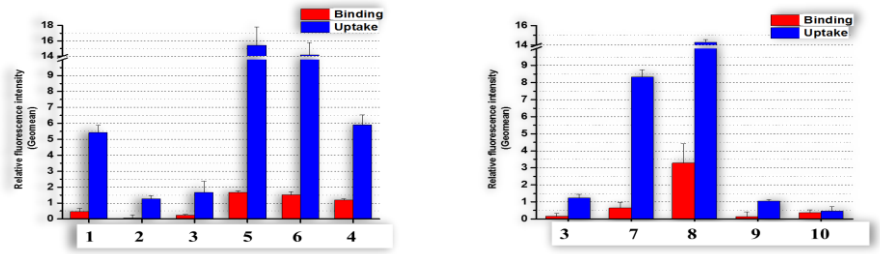

Fig. 3. Cellular binding and uptake of the conjugates

As discussion the results showed that three conjugates (**2, 5** and **6**) have significant cytotoxic effect at 10^{-5} M concentration (the cell (PANC-1) viability was lower than 15%). All of the investigated conjugates were fast degraded by lysosomal enzymes (within 30 minutes) but in case of GFLG containing constructions the presence of the smallest (active) metabolite in huge amount can be observed only after 60 minutes reaction time. (Figure 2) The presence of ^{t}Leu in the 12 position increases the cellular uptake (Figure 3), but these conjugates did not present any antitumor effect (Table 1). The presence of the enzyme labile spacer GFLG does not increase significantly the effectivity in case of different homing moieties.The incorporation of non-native amino acids into the position 8 of homing peptide results high cytotoxicity in case of **13**, **14**, **15**, and **16** conjugates, but only in higher (33 μM) concentration.

These conjugates might be good candidates for further development (other drugs and/or linker). In conclusion, the set of the developed neurotensin derivative – drug conjugates provide evidences to select appropriate molecules for further therapeutic experiments or as diagnostic tools.

Acknowledgments

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