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Development of Novel Angiopep-2 Conjugates for Cancer Treatment

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Introduction

Transporting therapeutics through the blood-brain barrier (BBB) is a demanding/ambitious challenge in treating brain tumors. The BBB is semipermeable; therefore, most active substances are poorly transported through this barrier, decreasing therapeutic effects. Angiopep-2 (TFFYGGSRGKRNNFKTEEY) is a peptide containing 19 amino acids that was shown to be a ligand of the low-density lipoprotein receptor-related protein-1 (LRP1). This peptide can cross the blood-brain barrier *via* receptor-mediated transcytosis and simultaneously target glioblastoma. Thereby, dual targeting may be achieved [1]. Recently, we demonstrated that substituting the three conjugation sites (two lysine side chains and the N-terminus) with drug molecules is not the best choice for developing effective peptide-drug conjugates. The substitution of Lys in position 15 decreased the cellular uptake by U87 glioma cells, while the accessibility of Lys in position 10 was somewhat hindered; therefore, the drug release was inefficient (Figure 1). However, the side chain substitution of this Lys highly increased the cellular uptake [2].

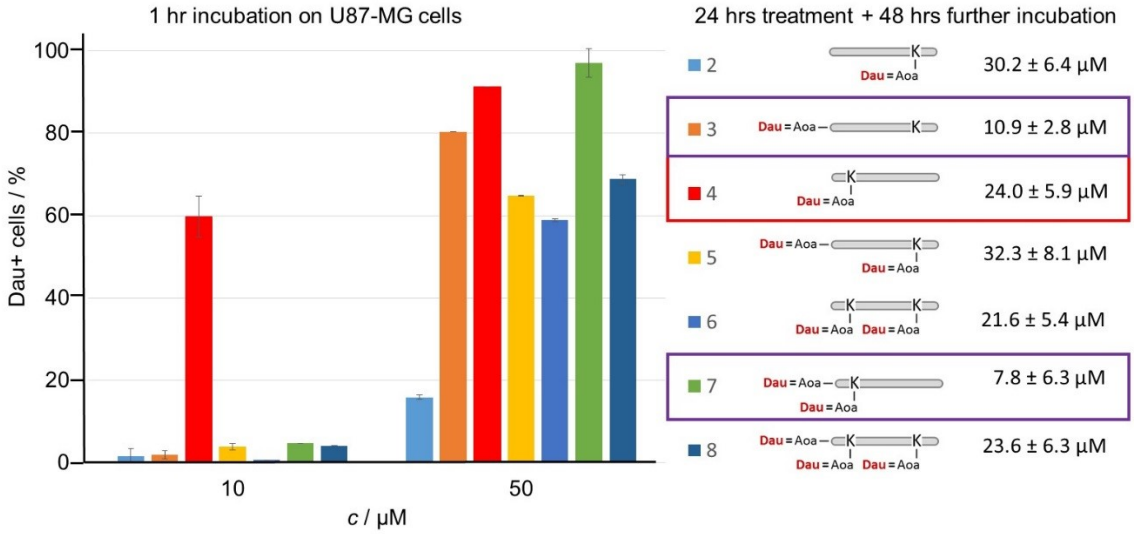

Figure 1. *In vitro* cellular uptake and cytostatic effect of Angiopep-2 – daunomycin conjugates

Thus, in this study, we developed daunomycin-Angiopep-2 conjugate in which the drug was attached to the N-terminus *via* oxime-linkage, and the side chain of Lys in position 10 was substituted with butyric acid (Dau=Aoa-TFFYGGSRGK(Bu)RNNFKTEEY-OH). The modification was used earlier efficiently in order to increase the cellular uptake and the antitumor effect of peptide-drug conjugates [3].

Results and Discussion

The Angiopep-2 peptide was built up on Wang resin by standard SPPS with Fmoc/tBu strategy. At the end of the sequence isopropylidene protected aminooxyacetic acid (Aoa) was attached similarly with Oxima Pure/HOBt coupling reagents. After the peptide cleavage from the resin the isopropylidene group was removed with 1M methoxyamine in 0.2M NH_4OAc buffer at pH 5 followed by purification and ligation with daunomycin (Dau) under the same condition. In the case of the butyrylated derivative the side chain of Lys in position 10 was protected with Mtt group that was removed prior the final cleavage with 2% TFA/DCM solution followed by butyrylation using butyric anhydride. The further reaction steps were the same as in the case of non-butyrylated derivative.

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The cellular uptake and the *in vitro* cytostatic effect of butyrylated conjugate was significantly higher (IC_{50} value was 0.678 ± 0.089 μM) than the non-butyrylated version (1.418 ± 0.153 μM), which confirmed our hypothesis (Figure 2).

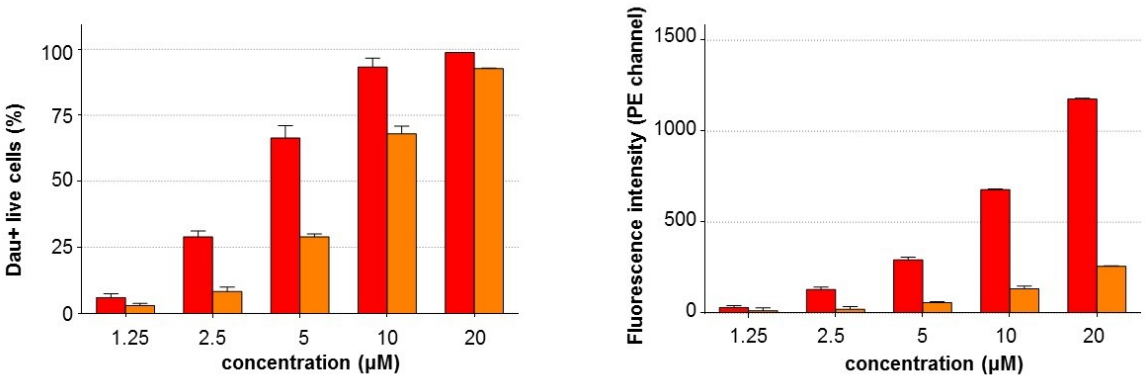

Fig. 2. Cellular uptake of Angiopep-2 – daunomycin conjugates (red: butyrylated; orange: non-butyrylated)

The short-chain acidic acid-containing conjugate showed a bit higher but insignificant (only 2%) passage through the BBB model [4] without LRP1. The conjugates were not cytotoxic on the endothelial cells.

According to these results, the conjugates were also investigated *in vivo* on U87MG *s.c.* tumor model. Dau had no tumor growth inhibition effect in the MTD concentration (1mg/kg) on this type of tumor model. However, the conjugates showed slight but not significant inhibition (10-15%) using a dose of 10 mg Dau content of conjugate/kg. The butyrylated conjugates elevated the tumor doubling time (11%) a bit and showed lower toxicity than the free Dau (Figure 3). Therefore, the butyrylated Angiopep-2 - drug conjugates might be good candidates for targeted tumor therapy, but more potent drug molecules should be used.

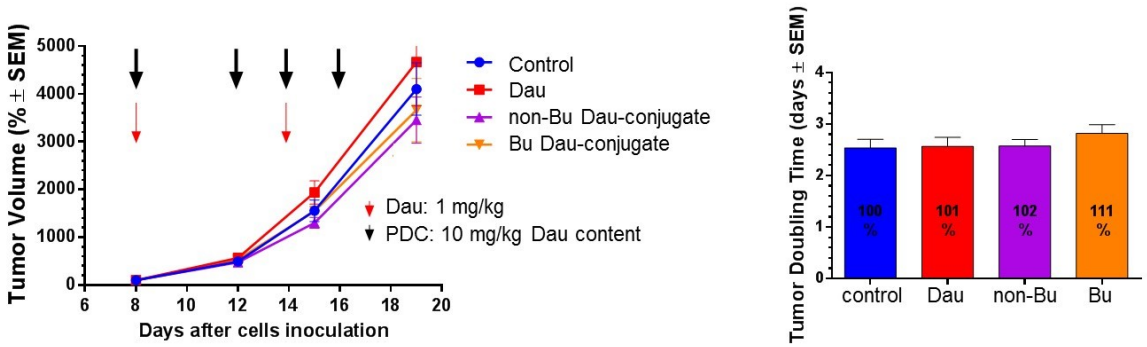

Fig 3. Tumor growth inhibition (black arrows: treatments with conjugates, red arrows: treatments with Dau) and tumor doubling time

Acknowledgments

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References

1. Demeule, M., et al. *J. Pharmacol. Exp. Ther.* **324**, 1064-1072 (2008), doi: 10.1124/jpet.107.131318
2. Pethő, L., et al. *Int. J. Mol. Sci*. **24**, 3106 (2023), doi: 10.3390/ijms24043106.
3. Hegedüs, R., et al. *Eur. J. Med. Chem.* **56**, 155-165 (2012), doi: 10.1016/j.ejmech.2012.08.014
4. Baranyai, Zs., et al. *J. Med. Chem*. **64**, 2982-3005 (2021), doi: 10.1021/acs.jmedchem.0c01399