

SUMMARY Conjugation of ligands to antisense oligonucleotides is a promising approach for enhancing their effects on gene expression. In this study 2'-O-lysylaminoethyl group was linked to the uridine base, which replaces one, two or three thymine bases thus modifies the oligonucleotides. This exchange of bases was tested for improvement of silencing target protein expression. Effectivity of modifications in silencing target protein expression was examined with the alicaforsen sequence (DNA) and siRNA. Alicaforsen, currently in clinical trial 3, is a phosphorothioate targeting ICAM-1, which was the model used to evaluate the influence of modifications. The same target was chosen for siRNA to compare the efficiency of DNA and siRNA substances. For the first time, down-regulation of ICAM-1 was shown on the blood brain barrier cell line ECV304. Unmodified/modified antisense oligonucleotides and siRNA sequences were transfected into ECV304 cells with the help of a transfection agent lipofectamine 2000. After 24 hours of transfection cells were disrupted by a chemical lysis. Protein concentrations were determined by Bradford protein assay. ICAM-1 inhibition was assessed with western blot. The inhibitory effect of ICAM-1 was normalized to the corresponding actin and untreated cells. ICAM-1 protein levels were quantified by a densitometry of autoradiograms with a densitometer and a software program Quantity One. All experiments were conducted at least in triplicates. 2'-O-lysylaminoethyl modification improved the cellular uptake of oligonucleotides and siRNA, and their better stability against degradation by nucleases. Both modified DNA and siRNA sequences have shown inhibition of the target protein ICAM-1 by 50%. The increasing number of modifications (one up to three) influenced the effect of gene expression. Sequences with two and three modifications had higher efficiency than the unmodified oligonucleotides or siRNAs. The most efficient concentration for gene silencing was 200 nM by oligonucleotides and 50 nM by siRNA. 2'-O-lysylaminoethyluridine could be shown to be a beneficiary modification of DNA and siRNA used for gene silencing studies. All tested sequences, especially with two and three modifications are promising for treatment of CNS diseases. The results of this thesis were published in: Winkler J, Gilbert M., Kocourková A, Stessl M, and Noe CR: 2'-O-lysylaminoethyl oligonucleotides: modifications for antisense and siRNA. *ChemMedChem* **3**(1):102-110, 2008.