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Due to their clinical importance, the development of therapeutic proteins has accelerated immensely over the past years. However, the expression of highly glycosylated recombinant therapeutic proteins like for example blood coagulation factors or serum proteins, has remained a challenging task.

C1-Inhibitor (C1-Inh) holds 7 N- and 8 O-glycosylation sites. Plasma derived C1-Inh (Berinert) as well as recombinant C1-Inh from transgenic rabbits (Ruconest) are approved for the treatment of acute attacks in patients with hereditary angioedema. However, the recombinant product shows a dramatically reduced serum half-life in pharmacokinetic studies in comparison to the plasma derived counterparts.

We have developed the CAP-Go protein expression platform to confer optimal glycosylation to complex glycoproteins like C1-Inh. The CAP-Go.1 cell line has been modified to facilitate expression of proteins with fully sialylated N-glycans. Recombinant proteins like human alpha-antitrypsin or human placental alkaline phosphatase produced with CAP-Go.1 show a significantly prolonged serum half-life in rats. However, expression of rhC1-Inh in CAP-Go.1 cells has no positive impact on the pharmacokinetic profile.

Expression of rhC1-Inh in CAP-Go.2 cells, which in addition addresses the O-linked glycosylation patterns, results in a significantly increased serum half-life compared to its counterpart and is actually indistinguishable from the plasma-purified protein. C1-Inh expressed in CAP-Go.1 or CAP-Go.2 cells show a similar reduction of terminal galactose on N-glycans, but their O-glycans differ. O-glycan analysis shows that rhC1-Inh expressed by CAP-Go.2 cells contains only core1 O-glycan structures, highly comparable to plasma-derived Berinert. Our results indicate that in addition to N-glycosylation, also the structure and composition of O-linked glycans plays a crucial role for pharmacokinetic properties of glycoproteins.

In conclusion, rhC1-Inh expressed from CAP-Go.2 cells, which have been optimized for the expression of N- and O-glycosylated proteins, display glycan patterns closely similar to plasma-derived C1-Inh. The resulting molecule has a significantly prolonged serum half-life as compared to C1-Inh generated on a conventional human cell line.

Our new recombinant molecule matches serum-derived C1-Inh in all aspects analyzed: specific activity, serum half-life, and glycosylation pattern and offers the advantage of being producible at large scale on a safe platform.