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RGD-CONTAINING PEPTIDES OF VP1 OF FOOT-AND-MOUTH DISEASE VIRUS (FMDV) PREVENT VIRUS INFECTION IN VITRO

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Summary. - RGD-containing peptides from the immunodominant region of VP1 between amino acids 135 - 160 from foot-and-mouth disease virus (FMDV) type O_1 Kaufbeuren (O_1K) prevented virus adsorption to piglet kidnex (PK) cells. The highly conserved amino acid RGD sequence (Arg.-Gly.-Asp.) was a prerequisite of this effect. To prevent infection with 100 - 200 $TCID_{50}$ in 10^6 PK cells, 20 - 250 μg of each peptide should have been added.

Key words: foot-and-mouth disease virus; synthetic peptides; amino acid sequence RGD; in vitro blocking of virus infection

FMD viruses are aphtoviruses belonging to the family *Picornaviridae.* These spheric viruses with a diameter of 25 - 30 nm consist of a single-stranded positive-sense RNA and 60 copies of each four structural polypeptides VP1 - VP4, the protein VP4 not being located on the virus surface. VP1 with a molecular weight of about 24 000 kD is the most exposed protein. Since 1982 chemically synthesized peptides of the main epitope of VP1 are known to be able to induce virus-neutralizing antibodies in laboratory animals, cattle and pigs (Bittle *et al.*, 1982; Pfaff *et al.*, 1982; Strohmeier *et al.*, 1982) and to protect guinea pigs and partially also cattle, pigs, and sheep from FMDV-infection (Di Marchi *et al.*, 1986; Francis *et al.*, 1987; Liebermann *et al.*, 1987; Surovoy *et al.*, 1987).

Surovoy *et al.* (1988) first reported of successful blocking of FMDV-infection in pig kidney cell cultures by such peptides. Their results showed that the cell recognition site for FMDV must include the RGD sequence (Arg-Gly-Asp). Similar results were described by Fox *et al.* (1989) and Liebermann *et al.* (1989). As shown by Pierschbacher and Rouslahti (1984) this sequenc is also necessary for interaction of fibronection and other proteins with corresponding cell receptors. Here we present results obtained in PK cell cultures with peptides of different length in the VP1 range (135 - 160) of FMDV O_1K. They contribute to a better understanding of the initial steps of virus-cell-interactions and may antiviral drugs.

Table 1. Inhibition of FMDV-infection in vitro by synthetic peptides of VP1

Peptide	Virus O_{1L}	Dose: 100-200$TCID_{50}$/0.5 ml A_{5R}	C_{1T}
VP1 (135-160) Tyr^{161}/O_{1K} H-R-Y-N-R-N-A-V-P-N-L-R-G-D-L-Q-V-L-A-Q-K-V-A-R-T-L-P-Y	+ (500)	-	-
VP1 (141-160) Tyr^{161}/O_{1K}	+ (50)	+ (25)	+ (25)
VP1 Ac(141-160) Tyr^{161}×3HF/O_{1K}	+ (50)	-	-
VP1 (145-160)/O_{1K}	+ (100)	+(200)	+(200)
VP1 (144-159)/O_{1K}	+ (200)	+ (50)	+(200)
VP1 (145-152)/O_{1K} H-R-G-D-L-Q-V-L-A-OH-2HOAc	+ (100)	-	+(200)
O_1-virus inact., UF	+ (16)	-	-
12 S of O_1	+ (5)	-	-
O_1-virus inact., PEG	+ (49)	+ (49)	-
A_5-virus inact., PEG		+ (28)	+ (28)
VP1 (146-156) A_{22} G-D-L-E-P-L-A-A-R-V-A	-(1000)	-	-
H-R-G-D-S-OH×2TFA	-(1000)	-	-
H-R-F-D-OH×2HOAc	- (500)	-	-
Di-Ac etyl-Splenopentin Ac-R-K (Ac) -EV-Y-OH	-(1000)	-	-

100% protection of PK-cell culture (monolayer) at minimal peptide concentration (μg/ml) for 0.5 ml/microflask

Peptides were synthesized using the classical method (Dölling *et al.* 1985). When inoculated in doses of up to 500 μg/0.5 ml into 3 - 4 culture flasks with about 10^6 cells and following an incubation period of 1 hr at 37 °C the supernatant was removed and the cell cultures were infected with 0.5 ml of virus suspension containing the FMDV-types 0_1-Lausanne, A_5-Riems, or C_1-Teterow in a titre of 100 - 200 $TCID_{50}$/0.5 ml. The results are shown in Table 1. Peptide concentration marked with "+" is the minimum one which prevented virus infection.

After having tested the longer peptides we also examined the shorter ones concerning their virus neutralizing activity. Di-acetyl-splenopentin (a gift from Dr. Forner, Berlin, F. R. G.) served as negative control. For comparison, we furthermore replaced the peptides by the virus inactivated with binary ethyleneimine and concentrated by ultrafiltration (UF) or by precipitation with PEG. The Table shows that octa- and longer peptides containing the RGD-sequence can inhibit FMDV infection independent of the virus type applied. In contrast, the peptide RGDS was obviously too short, or serine abolished the effect or the concentration of the peptide was still too low.

The peptide aa 146 - 156 of VP1 from FMDV A_{22} (a gift from Dr. Wladimir Rybakow, U. S. S. R.) in a concentration of 1000 μg/ml was not able to prevent

O_{1L}-virus infection, possibly due to the fact that the sequence begins with glycin. In comparison, the inactivated virus and the 12 S-component inhibited virus multiplication at relative low concentrations.

Surovoy *et al.* (1988) could prove the peptide aa 141 - 152 and aa 145 - 152 of VP1 from FMDV O_{1K} at a minimum concentration of 30 and 60 μg/ml, respectively, was able to protect PK cells from an O_{1K} and also from an A_{22} virus infection. The sequence RGELQVLA analogous to the peptide 145 - 152, in which D is replaced by E, was inactive similarly as the sequence 141 - 148. Consequently, the C-terminal part following the RGD sequence seems essential for inhibition of FMDV infection.

We could also confirm these results with other peptides and FMD viruses. The blocking effect against A_5-Riems and C_1-Teterow is an additional argument for a common receptor of different FMDV subtypes. On this occasion the RGD sequence holds a key position in the recognition sequence for the cell receptors. At present we are engaged in experiments for determining the minimum length og the RGD sequence containing the peptide able to block virus infection in PK cells.

Surprisingly, Fox *et al.* (1989) using BHK cells and FMDV O_1BFS 1860 have found recently that the sequences RGD and RGDL reduced the virus attachment by 39 - 63% but only when very high peptide concentrations (15 mM) were applied. Concerning the $A_{10}61$ virus reduction consisted in 65% by RGD and in 73% by RGDL. However, VP1 (141 - 160) did not inhibit virus attachment, probably because other factors influence the virus adsorption as well.

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