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TUFTSIN A NATURAL PEPTIDE WITH ANTIVIRAL ACTIVITY — STRUCTURAL BASIS OF ITS ACTION

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Summary. — The influence of tuftsin — the natural phagocytosis stimulating tetrapeptide and its elongated analogues — on the mortality of encephalomyocarditis virus-infected NMRI mice was investigated. It seems that amino acids elongation in the parent peptide chain does not result in significant increase of antiviral activity of the compounds tested.

Key words: peptide; tuftsin; antiviral activity; encephalomyocarditis virus

Introduction

Tuftsin a natural tetrapeptide has been found to exhibit several biological activities connected with immune system function (Najjar and Nisioka, 1970; Najjar and Bump, 1984). Preliminary studies indicate that tuftsin has antitumour activity in vivo (Knyszynski *et al.*, 1983). Recently, it was found that tuftsin analogue with a double linear peptide sequence showed anticancer activity against murine L 1210 leukaemia cells. This effect was more pronounced than that of tuftsin, possibly because of the lower probability of formation of tuftsin inhibitor, a tripeptide (Lys-Pro-Arg) (Najjar *et al.*, 1981; Najjar and Bump, 1984). Based on this, similar tuftsin analogues with elongated peptide chain were prepared. Preliminary biological observations showed that one of such analogues — (Lys^4)-tuftsinyltuftsin, has higher antitumour activity than tuftsin in M-MSV infected mice (Konopińska *et al.*, 1983).

The aim of the present studies was to investigate the effect of tuftsin and its elongated analogues on acute viral infections in mice.

Materials and Methods

Peptides. Tuftsin (Thr-Lys-Pro-Arg), Arg-tuftsin, Pro-Arg-tuftsin, Lys-Pro-Arg-tuftsin, Thr-Lys-Pro-Arg-tuftsin, Thr-Lys-Pro-Lys-tuftsin(Lys^4 — tuftsinyltuftsin), Lys-Pro-Arg (inhibitor). The peptides were synthesized by the classical methods or by the Merrifield method using a polystyrene divinylbenzene copolymer and DCC as the condensing agent (Konopińska *et al.*, 1983). Their homogenicity was checked by thin-layer chromatography (TLC) on silica gel plates and paper electrophoresis at 700 V. The purity of the products was further checked by amino-acid analysis and nitrogen determination.

Table 1. Influence of peptides on mortality of EMC virus-infected mice

Compounds	Schedule					
	A		B		C	
	No. of survivors /total	Survivors %	No. of survivors /total	Survivors %	No. of survivors /total	Survivors %
TUFTSIN (THR-LYS-PRO-ARG)	11/30	36.7(+)[a]	7/30	23.4	12/20	40.0(+)
ARG-TUFTSIN	5/30	16.7	3/30	10.0	11/30	36.7(+)
PRO-ARG-TUFTSIN	4/30	13.4	3/30	10.0	4/30	13.4
LYS-PRO-ARG-TUFTSIN	3/30	10.0	2/30	6.7	3/30	10.0
THR-LYS-PRO-ARG-TUFTSIN	2/30	6.7	3/30	10.0	4/30	13.4
THR-LYS-PRO-LYS-TUFTSIN	12/30	40.0(+)	9/30	10.0	14/30	46.7(+)
LYS-PRO-ARG	3/30	10.0	3/30	10.0	2/30	6.7
CONTROL	3/30	10.0	3/30	10.0	3/30	10.0

[a] significant

Administration schedule: A — four days before infection; B — five days after infection; C — four days before and five days after infection

Virus. Encephalomyocarditis virus (EMC, Columbia strain) was propagated after s.c. infection in NMRI mice. Stock of virus was prepared from brain extracts and stored in −70 °C.

Animals. Male NMRI mice 8—10 week old were used for the experiments. Mice were infected s.c. with 1 LD_{70} of EMC virus (0.5 ml). Groups of mice received i.p. injections of 20 μg of the peptides tested dissolved in 0.5 ml of PBS. The peptides were given according to three various administration schedules: A — one injection with the tested peptides four days before infection, B — one injection with the tested peptides given five days after infection and schedule, C — mice were injected twice with 20 μg of the peptides each time, four days before and five days after infection. Control groups received the same volume of PBS according to the same schedules. Mortality of the animals was monitored daily; the first death was observed on the seventh and the last on the twelveth day post-infection (p.i.) Per cent of survivors were calculated 3 weeks p.i.

For statistical analysis of the results chi-square test was used and p values less than 0.01 were considered significant (66.6% of mortality).

Results and Discussion

Tuftsin and some of its elongated analogues exerted protective effect in EMC virus infected mice. This activity depended on the structure of the peptides and the administration schedule (Tab. 1). Tuftsin showed significant effect either when given before infection (schedule A) or before and after infection (schedule C). The peptide given in a single injection after infection showed no significant effect. Similar results were obtained for tuftsin analogues. None but two of the tested compounds given after infection showed antiviral activity. Thr-Lys-Pro-Lys-tuftsin demonstrated even higher activity than tuftsin in both experiments (schedules A and C) confirming our earlier results with M-MSV infected mice (Konopińska *et al.*, 1983).

Significant activity was observed also for Arg-tuftsin given twice — before and after EMC virus infection (schedule C). These results are in accordance with the studies of the effect of the peptides on phagocytosis in vitro (Constantopoulos and Najjar, 1973; Konopińska *et al.*, 1983). Only Arg-tuftsin possessed approximately 30% of tuftsin activity whereas the remaining elongated analogues did not stimulate phagocytosis.

A somewhat similar results were obtained for two other viruses tested. These were R-MuLV (Rauscher Murine Leukaemia Virus) and FLV (Friend Leukaemia Virus), both type C retroviruses. Tuftsin prolonged the lifespan of R-MuLV-infected mice when it was administered 4 or 7 days before virus injection but not when it was given 1 day or 1 hr before or 2 days after virus inoculation (Knyszynski, 1983). A significant decrease in mortality was observed when 25 μg of the tetrapeptide was given 5 days before infection with FLV or 5 days before and twice a week for 3 weeks after FLV infection (Wleklik *et al.*, 1986). No effect was observed when the same amount of tuftsin was given 1 day before infection.

The mechanism of tuftsin-induced protection against infection with EMC virus seems to be related to the immunostimulating activity of the tetrapeptide. Florentin *et al.* (1978) presented evidence for stimulation of antibody formation following tuftsin injection to mice. On the other hand, it was shown that neutralizing antibody is a critical factor in determining the

duration of the viraemia and in affecting the degree of involvement of target organs and ultimate survival of the EMC virus-infected host (Galasso *et al.*, 1979).

In the present experiments with EMC virus the modification of tuftsin structure did not result in enhanced antiviral activity. However, it cannot be excluded, that compounds with higher activity can be obtained by broad range of structural modifications.

References

Constantopoulos, A., and Najjar, V. A. (1973): The requirements for membrane silic acid in the stimulation of phagocytosis by the natural tetrapeptide, tuftsin. *J. Biol. Chem.* **248**, 3819—3822.

Florentin, I., Bruley-Rosset, M., Kiger, N., Imbach, J. L., Winternitz, F., and Mathe, G. (1978): In vivo immunostimulation by tuftsin. *Cancer Immunol. Immunother.* **5**, 211—216.

Galasso, G. J., Merigan, T. C., and Buchanan, R. A. (1979): Humoral Immunity, pp. 52—55. In *Antiviral Agents and Viral Diseases of Man*, Raven Press, New York.

Knyszynski, A., Gottlieb, P., and Fridkin, M. (1983): Inhibition by tuftsin of Rauscher virus leukaemia development in mice. *J. natn. Canc. Inst.* **71**, 87—90.

Konopińska, D., Luczak, M., Wleklik, M., Gumulka, W., and Kazanowska, B. (1983): Elongated tuftsin analogues — synthesis and biological investigation. *Ann. N.Y. Acad. Sci.* **419**, 35—43.

Najjar, V. A., and Nishioka, K. (1970): Tuftsin — a physiological phagocytosis stimulating peptide. *Nature (Lond.)* **228**, 672—673.

Najjar, V. A., and Konopinska, D., Chaudhuri, M. K., Schmidt, D., and Linehan, L. (1981): Tuftsin, a natural activator of phagocytosis functions including tumoricidal activity. *Mol. Cell. Biochem*, **41**, 3—12.

Najjar, V. A., and Bump, N. J. (1984): Tuftsin (Thr-Lys-Pro-Arg). A stimulator of all known functions of macrophage, pp. 229—242. In R. L. Fenichel and M. A. Chirigos (Eds): *Immune Modulation Agents and their Mechanism*, Marcel and Dekker, Inc., New York and Basel.

Wleklik, M., Levy, S. B., Luczak, M., and Najjar, V. A. (1986): Suppression of Friend virus-induced leukaemia in mice by tuftsin. *J. gen. Virol.* **67**, 2001—2004.