Suppression of Friend Virus-induced Leukaemia in Mice by Tuftsin

By MAREK WLEKLIK, 1, 2 STUART B. LEVY, 3 MIROSŁAW ŁUCZAK 2 and VICTOR A. NAJJAR 1

1 Division of Protein Chemistry, Tufts University School of Medicine, Boston, Massachusetts 02111, U.S.A., 2 Department of Virology, Institute of Biostructure, Medical Academy of Warsaw, Chałubińskiego 5, 02004 Warsaw, Poland and 3 Cancer Research Center and Department of Medicine, Tufts University School of Medicine, Boston, Massachusetts 02111, U.S.A.

(Accepted 27 May 1986)

SUMMARY

A significant decrease in mortality was observed when 25 µg of the tetrapeptide tuftsin was given to DBA/2J mice 5 days before infection with Friend leukaemia virus (FLV). The same effect was observed when tuftsin was given 5 days before and twice a week for 3 weeks after FLV infection. No effect was observed when the same amount of tuftsin was given 1 day before infection. A 5 µg dose of tuftsin given 5 days before and twice-weekly for 3 weeks had no effect on leukaemia induced by FLV infection. These findings showed that time and dosage were critical to the protective effect of tuftsin against virus-induced leukaemia.

In spite of marked improvements in cancer chemotherapy, there remain many unresolved problems. Some important anticancer agents have been developed through rationally based analogue synthesis and delineation of structure–activity relationships (Goldin et al., 1981). However, the clinical use of many such compounds has been often limited because of their high toxicity.

There is also a group of compounds of natural origin with anticancer and antiviral activity but having variable side-effects usually different from those observed in the case of chemotherapy. Tuftsin is one of these compounds.

Tuftsin, isolated and synthesized for the first time in the laboratory of Najjar (Najjar & Nishioka, 1970; Nishioka et al., 1973), is a tetrapeptide (Thr–Lys–Pro–Arg) with significant biological activity (Najjar, 1983). Its anticancer activity has been shown both in vitro and in vivo (Catane et al., 1983; Nishioka et al., 1983) and the latest clinical trials have suggested its possible use in human therapy (Catane et al., 1983, 1986).

Tuftsin stimulates all known functions of phagocytic cells (Najjar & Bump, 1984), significantly increasing the cytotoxicity of macrophages towards target cells (Nishioka et al., 1983; Catane et al., 1983). Because of the importance of macrophages in resistance to tumours (Schultz et al., 1977), compounds such as tuftsin may be especially useful in cancer immunotherapy alone or as an adjunct to chemotherapy.

The Friend leukaemia virus complex (here called FLV) induces rapid erythroleukaemia which is characterized by massive splenomegaly, erythroblastosisis and early death (Levy et al., 1976). Infection of susceptible mice with FLV results in marked immunological deficiencies (Friedman, 1974), and spleen macrophages were shown to be responsible for the loss of resident natural killer (NK) cell activity in infected (Garaci et al., 1981; Moody et al., 1984). This study examined the effect of tuftsin on the course of lethal FLV infection in mice.

The FLV preparation was a 20% filtered spleen extract from FLV-infected DBA/2J mice (Levy et al., 1976). Freshly passaged virus was titrated on 6- to 8-week-old DBA/2J mice and 1 LD45-50 was used in experiments. The mice were injected intraperitoneally (i.p.) with 0.1 ml of virus diluted in phosphate-buffered saline (PBS).
Tuftsin was synthesized as previously described (Chaudhuri & Najjar, 1979) and obtained in pure form as judged by high-pressure liquid chromatography. It was then dissolved in PBS to an appropriate concentration, sterilized by passage through a Millipore filter (0.22 μm), divided into several vials and kept at -20 °C until used. Each sample was thawed only once. Tuftsin was given i.p. in 0.1 ml volumes. Control groups received the same volume of PBS. Student's t-test was used to determine the statistically significant difference ($P \leq 0.05$) between control and tuftsin-treated animals.

Tuftsin (25 μg) injected only once, 5 days before FLV infection, caused about 50% decrease in mortality (Fig. 1). A lower dose (5 μg) had no effect on the course of the infection. Either dose (5 or 25 μg) given 1 day before infection was ineffective (Fig. 2).
In an attempt to enhance the tuftsin effect, we injected mice 5 days before and twice a week for 3 weeks after infection (data not shown). A significant decrease in mortality of FLV-infected mice was found in the group of mice that received 25 μg of tuftsin before and after infection. However, the decrease was the same as in the experiments when tuftsin was given only once 5 days before FLV infection. A dose of 5 μg/mouse given repeatedly did not modify the course of the disease. At autopsy we found that tuftsin-treated mice died of typical FLV-induced erythroleukaemia (spleen enlargement and massive peritonical haemorrhage).

Similar results have been obtained in experiments with the closely related Rauscher murine leukaemia virus (Knyszyński et al., 1983). In those experiments, tuftsin given 4 or 7 days before infection significantly prolonged the survival of infected mice.

The mechanism of the tuftsin-induced protection from FLV disease may be related to several of its reported effects such as activation of macrophages (Najjar & Bump, 1984). After specific binding to polymorphonuclear cells, the peptide stimulates enhancement of immunogenic and tumouricidal effects of these cells (Ergaz et al., 1985). The other effect of tuftsin concerns the increase in cytotoxicity of different cell populations such as cytotoxic T-lymphocytes (Phillips et al., 1981) and spleen cells (Catane et al., 1983), and stimulation of the tumouricidal activity of macrophages, NK cells and granulocytes (Phillips et al., 1981; Nishioka et al., 1981). Considering the pathogenesis of FLV infection, it cannot be excluded that tuftsin-induced cytotoxicity (especially of NK cells) may also play a decisive role in modification of FLV infection.

We have recently shown that tuftsin stimulates the production of tumour necrosis factor (TNF) (Wleklik et al., 1985), which is known to be responsible for the necrosis of various tumours (Maranaska et al., 1984). This may be of special interest as Suyama et al. (1985) have shown that TNF is cytotoxic towards FLV-transformed erythroleukaemic cells.

The results of our present experiments indicate that tuftsin administration can decrease the mortality of FLV-infected mice. The observed effect was dependent on the time as well as on the dose of the peptide.

This work was supported by Public Health Service Grant AI09116, March of Dimes Birth Defects Foundation Grant 1-556, American Cancer Society Grant RDP-32E, by The Arnold D. Imperatore Research Scholarship of the National Association and by Polish grant (PB 04.02).

REFERENCES


Short communication


(Received 3 December 1985)