Eur J Cancer Clin Oncol, Vol. 17, No. 8, pp. 889–892, 1981.
Printed in Great Britain.

0014-2964/81/080889-04$02.00/0

Peptides Cleaved from Fibrinogen by Plasmin Enhance the Progression of L-1 Sarcoma in BALB/c Mice*

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Abstract—*Crude mixtures of peptides derived from fibrinogen (LMW–FDP) were injected into BALB/c mice repeatedly before and after s.c. or i.v. injection of L-1 sarcoma cells. Treatment with LMW-FDP was followed by enhanced growth of the primary tumor. The median duration of survival of tumor-bearing mice was decreased and the number of lung metastases increased. It is concluded that LMW-FDP promote the growth of a malignant tumor. This is due to an effect on the host rather than on the tumor cells.*

INTRODUCTION

NEOPLASTIC diseases are accompanied by thrombotic and haemorrhagic complications. Abnormalities in blood clotting and fibrinolytic systems have been detected in over 95% of patients with advanced malignancies [1]. The most frequent was an increase in circulating fibrin(ogen) degradation products (FDP), which showed a relation to the extent of tumor spread [2, 3]. Accumulation of fibrinogen labelled with radioactive iodine in tumor tissue has been demonstrated in many types of experimental and human malignancies [4]. Hence it is probable that, owing to digestion of fibrin(ogen) deposits by plasmin and tissue proteases, FDP can be generated locally in tumors in higher concentrations than those occurring in systematic venous blood. Low molecular weight peptides cleaved from fibrin (ogen) by plasmin (LMW-FDP) have vasoactive [5] and immunosuppressive properties [6–11]. Since the immune status of the host [12] and the vacularization of the tumor [13] are considered to play a major role in its progression, it may be assumed that LMW–FDP influence the growth and spread of neoplasms.

In this paper we report findings of an enchancing effect of LMW–FDP on the growth of a primary tumor and on metastasis of transplantable L-1 sarcoma in BALB/c mice.

L-1 sarcoma is a tumor that arose spontaneously in the lung of a BALB/c mouse and can be maintained by subcutaneous (s.c.) passages in this strain. It is also called JW sarcoma, since the first report [14] on this transplantable tumor was published by Dr. Janik from the Warsaw Institute of Oncology.

MATERIALS AND METHODS

The tumor was implanted into BALB/c mice by s.c. injection of a suspension of L-1 sarcoma cells derived from 50 to 60 passages. BALB/c male mice, 6–8 weeks old, weighing 19–21 g, were used for the experiments. They were obtained from the breeding unit of the State Institute of Hygiene in Warsaw. LMW-FDP isolated from a digest of human fibrinogen by plasmin [15] contained a mixture of peptides of molecular weights under 3500.

Groups of animals (20 mice each) were treated with LMW-FDP injected either intraperitoneally (i.p.) or at the site of tumor cell implantation and subsequently into the tumor (i.t.) itself. For the LMW-FDP doses and times of administration, see legend to Fig. 1. The influence of LMW-FDP treatment on the

Accepted 6 March 1981.

*This work was supported by grants from the Biochemical and Biophysical Committee of the Polish Academy of Sciences, the Polish National Cancer Program (PR6, Grant 1308/13) and the Swedish Medical Research Council.

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growth of the primary tumor, lung metastases and survival was evaluated. Measurements of mean tumor diameters were made every three days, starting from the 10th day after tumor cell inoculation. The three dimensions (*a*, *b* and *c*) of the tumor were measured with calipers and the tumor volume was calculated in cm^3 according to the formula, $V = 0.5 \cdot a \cdot b \cdot c$. The lungs were examined for metastases by perfusion with Indian ink diluted in fixative [16]. The number of macroscopically visible surface nodules was counted.

The effect of LMW-FDP on metastasis was also investigated in an artificial model, i.e. by lung colony assay consisting in counting of tumor nodules in lungs two weeks after i.v. injection of an L-1 sarcoma cell suspension.

RESULTS

The rate of growth of the primary tumor was markedly higher in animals treated with LMW-FDP than in control groups (Fig. 1). Enhancement of tumor growth was somewhat less pronounced in the group in which LMW-FDP were injected i.t. than in the group treated with peptides by the i.p. route.

The median length of survival of tumor-bearing mice was shortened from a control value of 36 to 31 days under the influence of i.p. injected LMW-FDP, and from 37 to 28 days when peptides were administered locally before tumor cell implantation and subsequently into the tumor itself (Table 1). The mean volumes attained by the primary tumors at the median time of survival were similar in LMW-FDP-treated and control groups (Fig. 1). Earlier development and larger numbers of lung metastases were observed in LMW-FDP-treated groups than in controls (Tables 1).

It was found (Table 2) that the number of tumor nodules in the lungs after injection of tumor cells i.v. depended on the dose of injected cells and was considerably higher in animals treated i.p. with LMW-FDP than in controls injected with PBS. Incubation of L-1 sarcoma cells with LMW-FDP *in vitro* (1000 μg of LMW-FDP per ml, 10^6 cells, 37°C, 30 min) prior to i.v. injection in mice did not change the results of the lung colony assay (Table 2). Hence the enhancement of lung colony formation by LMW-FDP seems to depend rather on their action on the host than on their direct interaction with tumor cells.

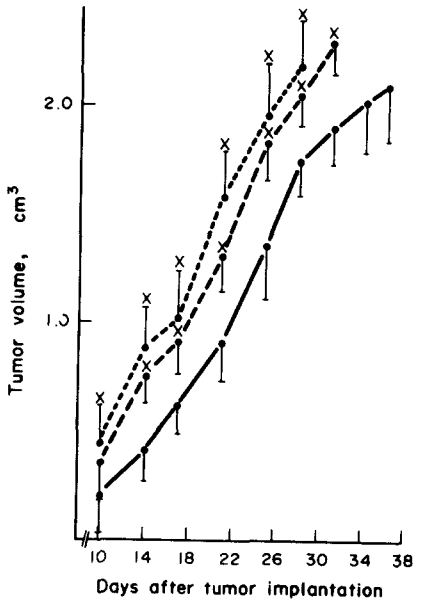

Fig. 1. Enhancement of L-1 sarcoma growth by LMW-FDP. A suspension of L-1 sarcoma cells (5×10^4 viable cells in 0.2 ml PBS-phosphate buffered saline, pH 7.2, 0.15 M) was injected s.c. into BALB/c mice divided into four groups (20 animals per group). The first group was injected i.p. with four 200 μg doses of LMW-FDP in 0.1 ml PBS prior to sarcoma cell implantation, and subsequently at three-day intervals (----). In the second group the same dose of LMW-FDP in 0.05 ml of PBS was administered s.c. four times at the site of tumor cell implantation prior to sarcoma cell injection and subsequently intratumorally (i.t.) at three-day intervals up to the time of spontaneous death or killing (--). Control groups received i.p. or i.t. injections of PBS alone (–). The results are expressed as a mean value ± S.D. and were analysed statistically by Student's t-test.x = $P < 0.05$ vs rats given PBS alone.

Since the results for the two for the two control groups were overlapping, only those obtained in mice injected i.t. with PBS are included in the figure.

DISCUSSION

Biological activities of human LMW-FDP used in this study are not species-specific. These peptides inhibit to a similar degree the proliferation *in vitro* of human blood lymphocytes, rat lymph node cells and mice spleen cells [7–11]. This inhibitory effect of LMW-FDP *in vitro* was not associated either with cytotoxicity or with a decrease in cell culture viability [7–11]. Under *in vivo* conditions LMW-FDP of human origin supress humoral [6], as well as cell-mediated, immune response in mice [17], and increase the microvascular permeability in rats [5].

L-1 sarcoma is an immunogenic tumor [18] with a poorly developed vascular net [19]. L-1 sarcoma cells show potent plasminogen activator activity [20]. Concomitant with advancement of growth and spread of the tumor, progressive changes in fibrinolysis and in fibrinogen metabolism have been observed. These changes consisted in a fall in plasma fibrinolytic activity, an increase in concen-

Table 1. Median length of survival and mean numbers (± S.D.) of lung metastases in mice treated with LMW-FDP

Treatment	Median time of survival, days	Mean (± S.D.) number of lung metastases	
		On 16th day	On the day corresponding to median time of survival
PBS i.p.	36	0	11.7 ± 3.1
LMW-FDP i.p.	31	1.5 ± 1.0	22.3 ± 4.5
PBS i.t.	37	0	10.9 ± 3.6
LMW-FDP i.t.	28	0.5 ± 0.5	21.8 ± 4.1

Four groups of BALB/c mice (25 animals each), treated as described in Fig. 1, were used for the experiment. Five animals from each group were killed on the 16th day after tumor implantation and lung metastases were counted. Survival was checked daily, starting on day 15 after tumor implantation, and 10 animals from each group were killed on the day of median survival time and their lung metastases were counted.

Table 2. Effect of LMW-FDP in vivo *and* in vitro *on the mean numbers (± S.D.) of tumor nodules in BALB/c mice injected i.v. with sarcoma L-1 cells*

Treatment \ Number of L-1 sarcoma cells injected i.v.	0.5×10^5	1.0×10^5	2.0×10^5
PBS i.p. *in vivo*	0	1.9 ± 1.3	3.4 ± 1.1
LMW-FDP i.p. *in vivo*	3.1 ± 2.0	7.8 ± 2.6	12.5 ± 3.4
Tumor cells treated *in vitro* with LMW-FDP	0	1.3 ± 1.4	4.0 ± 2.6

The cell suspension for i.v. injection was prepared by mincing the tumor tissue with scissors and then teasing it over a wire mesh. Cells were washed to remove cell debris and suspended in PBS. The final dilution consisted of 2.5×10^5, 5×10^5 and 10^6 viable cells per ml. Groups of animals (5 mice per group) were injected with 0.2 ml of suspension and the number of lung nodules was counted 14 days later as described in Table 1.

In another experiment (lower part of Table) sarcoma L-1 cells were incubated with LMW-FDP *in vitro* (1000 μg of LMW-FDP per ml, 10^6 cells, 37°C, 30 min) prior to i.v. injection in mice.

tration and survival time of fibrinogen in the circulating plasma, and a slight rise in content of FDP in serum [19].

Our preliminary results show that LMW-FDP enhance the progression of L-1 sarcoma and shorten the survival of BALB/c mice bearing this tumor. Tumor-promoting properties of LMW-FDP are presumably the main reason for shortening of the life span, since the volumes of primary tumors were similar in LMW-FDP treated and control animals and the numbers of lung metastases considerably higher in the former group, at the median time of survival of tumor bearing mice.

It has been suggested that fibrin deposited at the periphery of malignant tumors may favour their progression by such mechanisms as formation of the lattice supporting the cell growth, protection against the defence mechanisms of the host, and supplying of nutrients and/of growth stimulatory agents [4, 18, 19, 21, 22]. The presented results confirm the hypothesis that low molecular weight peptides derived from fibrin(ogen) can promote the growth of a malignant tumor. The lowered fibrinolytic activity in the circulating plasma in patients and animals with malignant tumors [20, 21, 23, 24] may favour the persistence of fibrin deposits in the tumor and local liberation of FDP under the influence of tumor and tissue plasminogen activators, as well as other proteases.

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