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Effect of Defibrination with Batroxobin on Growth and Metastasis of JW Sarcoma in Mice*

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Abstract—*In order to gather information on the possible role of fibrin in tumour and metastasis growth, the development of JW sarcoma (JWS) was studied in BALB/c mice defibrinated with batroxobin. The spontaneous lung metastasis number was significantly reduced (as compared with controls) in mice defibrinated during the initial phase of tumour growth and dissemination, whereas it was unaffected in mice given batroxobin at later stages. Lung colony formation, upon i.v. injection of JWS cells, was also reduced in defibrinated mice. In this experimental model, removal of the host's circulating fibrinogen appeared therefore of benefit only during lodgement of cancer cells.*

INTRODUCTION

THE USE of defibrinating agents such as ancrod and batroxobin has been proposed in the treatment of thrombotic diseases as an alternative to anticoagulant and/or antiaggregating drugs [1–5]. Some authors have also used therapeutic defibrination in experimental cancer treatment [6–9]. This approach was based on the assumption that fibrin formation would play a role in the pathogenesis of tumour growth and metastasis formation [10, 11]. So far, though, no definite conclusions can be drawn on the effect of defibrination on tumour cell growth, because of the variety of models and experimental conditions used.

The present study aimed to assess the effect of a defibrinating enzyme, batroxobin, on primary growth and metastasis of a new tumour spontaneously metastasizing in mice, the JW sarcoma (JWS) [12, 13].

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MATERIALS AND METHODS

Animals

A total number of 300 BALB/c mice (Charles River, Calco, Italy), weighing 20–25 g, were used. The animals were housed in plastic cages in air-conditioned premises with a constant light–dark rhythm; they had free access to food pellets and water.

Tumour

JWS arose as a spontaneous lung tumour in a BALB/c mouse [12], and was subsequently maintained in ascitic form by weekly passages of cells collected from the peritoneal cavity. When cancer cells were implanted intramuscularly (i.m.) or subcutaneously (s.c.) a solid tumour was obtained which metastasized selectively to the lungs. The histological characteristics and growth pattern of this tumour have been described [13].

Spontaneous metastasis

Cells harvested from the peritoneal cavity were washed, resuspended in phosphate buffered saline and injected s.c. in the upper back of the animals at the concentration of 5×10^4 per mouse. In these conditions BALB/c mice had a mean lifespan of 49 ± 2 days. The primary tumour was palpable about 14 days

after implantation and weighed 5–8 g at death of the animals. Lung metastases were visible around day 28 and numbered 15–20 at death of the animals.

Experiment 1. A total number of 120 BALB/c mice were implanted s.c. with 5×10^4 JWS cells and subdivided into groups of 20 animals. One group was taken as control and the other treated with batroxobin (20 U/kg); three different periods of treatment were chosen, i.e., days −4–10; 14–28; 21–35.

Experiment 2. Eighty animals were implanted s.c. with 5×10^4 JWS cells. One group (20 mice) acted as control and the other three were treated with batroxobin at different doses (20, 40 or 160 U/kg). In each experiment, control mice were treated i.p. with isotonic saline. All the animals were sacrificed at day 42 after tumour implantation; primary tumour weight and lung metastasis number, counted by the method of Wexler [14], were recorded.

Lung colonies

JWS cells were injected i.v. at the concentrations of 5×10^3 or 25×10^3 per mouse. In these conditions the mice had a mean lifespan of 25±2 days and 21±2 days, respectively. Lung colonies numbered 8–12 and 22–26 for the two cell inoculi considered. In the experiments reported here, 40 mice per cell inoculum were injected: 20, acting as controls, were treated with isotonic saline and the other 20 with batroxobin (20 U/kg) during the period: day −2 to day 2. Mice injected with the lower cell inoculum were sacrificed at day 24 and the others at day 21. Lung colonies were counted by the method of Wexler [14].

Drug

Batroxobin (Defibrase®, Pentapharm, Basel, Switzerland) was administered i.p. twice a day at the doses of 20, 40 or 160 U/kg body wt, during different periods of tumour development, as above reported. Preliminary experiments indicated that both batroxobin doses used, when administered for 10 days to normal mice, were able to keep the fibrinogen level lower than 40 mg% until 12 hr after the treatment (Fig. 1).

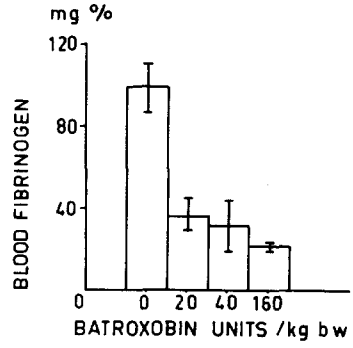

Fig. 1. *Blood fibrinogen levels in normal BALB/c mice treated chronically* (10 days) *with three different doses of batroxobin. Blood was collected by intracardiac puncture* 12 hr *after the last i.p. injection of the drug. Fibrinogen level was measured by the fibrin polymerization time-test as previously described* [17]. *Mean* ±S.E *of* 6–8 *animals per group.*

RESULTS

Table 1 reports the effect of treatment with batroxobin (20 U/kg) on JWS at different stages of s.c. tumour growth. Both primary tumour weight and metastasis number were reduced (the latter significantly) when cells were injected into defibrinated animals and treatment continued during the first phases of primary growth and dissemination. Treatment at more advanced phases did not show any significant beneficial effect. Higher dosages of batroxobin (40, 160 U/kg) administered during the initial period of tumour growth did not reduce primary tumour weight; in contrast, metastasis number was significantly decreased by treatment with 40 U/kg but unchanged by treatment with 160 U/kg (Table 2).

Table 1. *Effect of batroxobin* (20 U/kg body wt) *on JWS development. Animals were sacrified at* day 42 *after tumour implantation. Mean* ±S.E. *of values obtained from* 20 *animals per group.*

	Treatment period (days)	Tumour weight (g)	(% of control)	Lung metastases (*N*)	(% of control)
Saline	−4–10	7.84±0.97	73	25.0±2.3	68
Batroxobin		5.75±0.97		17.1±2.6*	
Saline	14–28	8.79±0.25	88	23.6±1.2	88
Batroxobin		7.73±0.74		20.9±2.4	
Saline	21–35	7.98±0.77	80	17.0±6.4	113
Batroxobin		6.36±0.27		19.3±1.9	

*$P<0.05$ at Student's *t*-test.

Table 2. Effect of batroxobin administered at different doses during the period day −4 *to* day 10 *on JW sarcoma development. Animals were sacrificed at* day 42 *after tumour implantation. Mean* ± S.E. *of values obtained from* 20 *animals per group.*

	Tumour weight (g)	(% of control)	Lung metastases (*N*)	(% of control)
Saline	5.1 ± 0.5		20.2 ± 3	
Batroxobin (20 U/kg)	4.7 ± 0.7	92	11.0 ± 1.5*	54
Batroxobin (40 *U*kg*)	5.0 ± 1	98	11.1 ± 2*	54
Batroxobin (160 U/kg)	5.5 ± 1	107	22.0 ± 5	108

*$P < 0.05$ at Dunnett test.

Table 3 shows the effect on the number of lung nodules of i.v. injection of two JWS cell doses in defibrinated and control mice. The number of lung nodules formed was related to the inoculum size. Only in mice given the smaller amount of cells was the defibrinating treatment able to significantly reduce the pulmonary colonies number.

Table 3. Effect of batroxobin treatment (from day −2 *to* day 2) *on the number of lung colonies developed in mice given two different concentrations of JWS cells i.v. Animals given the lower inoculum size were killed at* day 24, *the other group at* day 21. *Means* ± S.E. *of* 20 *animals per group.*

	Cell dose (per mouse) 5×10^3	25×10^3
Saline	10.2 ± 1.4	24.1 ± 1.0
Batroxobin (20 U/kg)	6.0 ± 1.1*	20.0 ± 2.0

*$P < 0.05$ at Student's *t*-test.

DISCUSSION

Snake venom enzymes have been reported to have antitumour effects in artificial experimental systems, such as those deriving from i.v. tumour cell injection [15] and in allogeneic tumours [6] but did not in spontaneously metastasizing and/or syngeneic forms [8, 9, 16]. We have reported here that in JW sarcoma not only lung colony formation, but also spontaneous metastasis growth in syngeneic mice were significantly decreased by batroxobin treatment. This is to the best of our knowledge the first report of beneficial effect of a defibrinating snake venom on a spontaneously metastasizing syngeneic tumour. Indeed, in another spontaneous metastasis model, the Lewis lung carcinoma of C57Bl mice, treatment with batroxobin even enhanced metastasis growth [9, 17]. Such an effect was observed when the animals were kept defibrinated for a very prolonged period of time, i.e., the whole tumour development period. With shorter treatments, a slight reduction of metastasis number was observed [9]. In JWS defibrination was applied at various stages of tumour development. A decrease in metastasis number was only observed when the tumour cells were injected into defibrinated animals and defibrination continued for the first 10 days after tumour implantations. No significant effects were observed in animals treated during two more advanced stages of tumour growth. In view of these results it was felt of interest to investigate whether the beneficial effect observed with a low batroxobin dose (20 U/kg) during the first period of tumour growth could be enhanced by using higher doses of the enzyme. Metastasis number was indeed decreased also with 40 U/kg but it was unchanged with a still higher dose (160 U/kg). Such an effect did not seem to be closely related to the degree of hypofibrinogenemia induced, which was very similar with the three doses used. It should be realized that complete defibrination in laboratory animals is hardly achieved even with much higher doses of batroxobin than in humans; thus, the effects observed should, at least partially, be ascribed to the changes in blood flow connected with lowering viscosity [5, 18]. It is also conceivable that other properties, such as the capacity to interact with the host's immune system, could be of importance in determining the observed biphasic effect with

higher doses of the enzyme. It may be relevant to mention that in some preliminary experiments batroxobin preparations have displayed an immunodepressant effect both *in vitro* and *in vivo* in mice [19].

A reduction in metastasis number was also observed in the lung colony system when JWS cells were injected i.v. into defibrinated mice. In recent experiments, trapping of ^{51}Cr-labelled cells in the lungs was significantly decreased by previous defibrination of the animals [20]. Moreover, defibrinated animals were protected from sudden death induced by i.v. injection of huge amounts of JWS cells. The latter phenomena were also observed when cells were injected into mice anticoagulated with either warfarin or heparin (Chmielewska *et al.*, unpublished observations). This allows to speculate that prevention of fibrin formation, in whichever way it is achieved, may impair lodging in the lungs of i.v. injected cancer cells. In agreement with such an hypothesis is the observation, reported in this paper, that a reduction in spontaneous metastasis number was only achieved in mice defibrinated during the early stages of primary growth, when most cells are supposed to detach from the tumour and circulate before lodging.

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