Aloctin A, An Active Substance Of Aloe Arborescens Miller As An Immunomodulator

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Aloe has been used as a folk medicine for centuries all over the world. Among the components of Aloe, the low-molecular weight components have been well studied and used as purgatives. In the last few decades, the clinical application of Aloe extract, probably the components of high molecular weight, in skin injury and burns, as well as an anti-inflammatory, has been reported. Aloctin A (Alo A) is a highly purified glycoprotein with molecular weight of 1.8×10^4 from the leaves of Aloe arborescens and exhibits various biological activities, such as mitogenic activity for T lymphocytes, binding reactivity for human a2-macroglobulin and activation of component 3 of complement system via the alternative pathway[1].

In this article, I would like to describe the antitumor activity of Alo A using methylcholanthrene-induced nurine fibrosarcoma (Meth A)[2] and lymphocytic leukemia (P388) (unpublished data) in syngeneic mouse systems.

One million of Meth A cells were implanted into the peritoneal cavity of BALB/c mice. Alo A were administered i.p. at an appropriate concentration in saline, once daily for 5 days, starting 24 hours after tumor implantation. Antitumor activity was evaluated by total cell packed cell volume ratio (Alo A-treated mice/control mice) calculated from collected whole ascites obtained from mice anesthetized with ether. A representative experiment is shown in table 1. Alo A obviously inhibited the growth of the tumor cells and administration at a dose of 10 mg/kg/day, for 5 days, remarkably inhibited it ($p<0.001$). It was important to determine whether this activity was due to cytotoxicity of Alo A for tumor cells or host-mediated effects of Alo A, since Alo A was administered i.p. Therefore, the effect of Alo A on the growth in vitro of Meth A and the other cell lines was examined by ^{3}H-thymidine uptake. Alo A had almost no inhibitory effect on the growth of tumor cell lines tested including Meth A up to a concentration of 200 ug/ml, the highest concentration tested (table 2). This result suggests that Alo A is not directly cytotoxic to tumor cells.

One million of P388 cells were implanted intraperitoneally in CDF1 mice. Alo A was administered i.p. at an appropriate concentration in saline, once daily on the 1st and 5th days after tumor implantation. Antitumor activity was evaluated by survival time. The antitumor activity of Alo A is also obvious in this system (table 3).

The mechanisms of antitumor activity of Alo A seemed to be host-mediated. We have reported a couple of immunomodulatory activities, such as elevation of natural killer cell activity, augmentation of cytotoxicity of peritoneal exudate cells and generation of lymphokine-activated killer cells. We consider that Alo A is a promising candidate as an immunomodulator.

Table 1
The anti-tumor activity of Aloctin A against sarcoma Meth A (ascites form) in BALB/c mice

Treatment	Dose (mg/kg/day x days)	Average TPCV[a] (ml)	T/C Ratio (%)	Complete Inhibition
None		0.61		0/10
Aloctin A	10 x 5	0.05	7.7*	4/6
Aloctin A	.2 x 5	0.37	60.4	1/6
Aloctin A	0.4 x 5	0.41	66.7	1/6

Antitumor test, 5-week-old BALB/c mice were used for this test. The tumor used was methylcholanthrene-induced fibrosarcoma (Meth A) maintained in the ascites form, 1×10^6 washed cells of Meth A were implanted i.p. into the mouse. Aloctin A as injected i.p. once daily for 5 days, starting 24 h after tumor implantation. Antitumor activity was evaluated by the total packed cell volume ratio (T/C %) on the 7th day.

[a]Total packed cell volume, *$p<0.001$, Significantly different from control.

Table 2
Cytotoxicity of Aloctin A in vitro

Concentration of Aloctin A (ug/ml)	^{3}H-TdR Incorporation (cpm)[a]				
	Meth A	EL 4	P815	BW5147	YAC
None	42890	68351	39865	14989	7120
0.02	43195	87497	67506	24247	8345
0.2	40512	90111	74904	24468	7648

2.0	39250	87798	68245	28597	7618
20.0	45924	80864	63572	19300	6835
200.0	45537	66979	49111	11250	818

Cytotoxicity test: Various concentrations of Aloctin A in 00ul of cell suspension, each containing 5 x 10^3 cells. The mixture was incubated at 37 ^oC for 28 h in a humidified atmosphere of 5% CO_2 and 95% air. After 24h, 1 uCi/well of ^3H-thymidine was added. After a further 4 h of incubation, radioactivity incorporated into DNA was determined.

[a]Mean cpm of 3 wells.

Table 3
The anti-tumor activity of Aloctin A against P388 in CDF_1 mice

Treatment	Dose (mg/kg/day)	No. Of Mice	Survival Time Range	Survival Time M. S. T.	T/C %
				days	
Control		10	8-9	8.50	
Aloctin A	10	6	9-11	10.33**	121.6
Aloctin A	5	5	9-11	9.60*	112.9
Aloctin A	1	6	8-10	9.17	107.8
Aloctin A	0.2	6	8-10	8.67	102.0

M. S. T., Median Survival Time, T/C % = M. S. T. of treated group / M. S. T. of Control x 100.
Evaluation of anti-tumor activity; T/C % in life-span *, **,
Significantly different from Control * P<0.01, ** P<0.001.

References

[1] **Suzuki et al.** (1979) *J. Biochem.* 85: 163.
[2] **Imanishi et al.** (1981) *Experientia* 37: 1186.