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Prostaglandin E Production in Bone Lesion of Transplanted VX-2 Tumor in Rabbits

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ABSTRACT. The correlation between prostaglandin E (PGE) and advance of tumor metastasis was investigated in normal and VX-2 bearing rabbits. PGE in plasma of normal rabbits was 486.2±185.7 pg/ml (n=86). In rabbits with VX-2 dispersed into heart cavity, the circulating PGE levels increased when tumor cells lodged and proliferated in the lymphnode, lung, liver and bone marrow. However, the plasma levels of PGE in the rabbits with bone involvement were significantly higher than those without bone involvement. This data indicates that PGE produced at the bone lesion may mediate the osteoclastic bone resorption and destroy bone. *In vitro* studies revealed that PGE level increased in the culture medium of VX-2 tumor cells, while an appreciable increase was not noted in HeLa S3.

Key words : VX-2 tumor — prostaglandin E — bone lesion

In order to clarify the role of prostaglandin E (PGE) in the development of bone metastasis, our previous study reported[1,2] the correlative changes in the plasma PGE levels with the finding of bone and bone marrow scintigraphy following an induced bone metastasis with VX-2-tumor cells in rabbits. This study was undertaken in attempting to elucidate the change in the plasma PGE levels associated with the growth of metastatic tumors and the role of PGE in the metastatic bone lesions. Also, PGE production by VX-2 and HeLa S3 were investigated *in vitro* study.

MATERIALS AND METHODS

Animals : Albino rabbits weighing from 2.0 to 3.5 kg were used.

Intracardiac implantation of VX-2 : One-tenth ml of 1% VX-2 cell suspension in PBS was injected into the heart cavity of 7 rabbits through 18-gauge syringe needles. Blood sample were obtained at 14 and 21 day and bone scintigraphy (2-4 mCi of ^{99m}Tc-MDP) and bone marrow scintigraphy (2-4 mCi of ^{99m}Tc-sulfur colloid) were performed at 14 and 21 days after implantation. All rabbits were sacrificed and histological surveys were performed at 25th of post implantation day. Plasma PGE level was measured by radioimmunoassay kit, provided by Clinical Assay Inc., which required an extraction procedure.[3]

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Intramedullary implantation of VX-2 : One-tenth ml of 1% VX-2 cell suspension in PBS was injected percutaneously into the iliac bone marrow cavity of 4 rabbits. Marrow and bone scintigraphy were performed to delineate the development of metastasis at 14th, 17th, 20th, and 21st day after the implantation. Serial blood samples were collected for PGE assay.

Culture cells and conditions : The cells used for the experiment consisted of VX-2 cells, HeLa S3, and normal skin fibroblasts. A series of increasing amount of the tumor cells (5×10^4 ml, 10, 20, 30-40, 50, 100×10^4 ml) were incubated in a CO_2 incubator with Eagle's MEM medium containing 10% fetal calf serum for 48 hrs. The incubator medium were not exchanged. Cells were incubated for 48 hrs at 37°C in the 2 ml medium in a plastic petri dish 35 mm in diameter, and the concentration of PGE in the medium was measured by the radioimmunoassay kit.

RESULTS

Plasma PGE values in normal rabbits : PGE levels in plasma of 86 normal rabbits were 486.2±185.7 pg/ml (mean and standard deviation, n=86).

Changes in the plasma PEG levels following the intramedullary transplantation of VX-2 tumor cells (Table 1) : Plasma PEG levels before the transplantation were 556±114 pg/ml. At 17-21 days after the transplantation, plasma PGE levels rapidly increased from the basal levels to the high value of 2433±1489 pg/ml when the bone scintigraphy showed first abnormality at the transplanted site of the iliac bone.

TABLE 1. Bone scintigraphy and PGE level in rabbits following intramedullary VX-2 transplantation

Animal no.	Concentration and volume of VX-2 implanted	PGE level before implantation (pg/ml)	Bone scan	
			day*	PGE level (pg/ml)
1	10% 0.1 ml	620	17	2250
2	10% 0.1 ml	675	20	4500
3	10% 0.1 ml	506	21	956
4	10% 0.1 ml	422	21	2026
		556		2433

* Represents first day when abnormal scan was obtained.

Changes in the plasma PGE levels following intracardiac transplantation (Table 2) : Histologically, metastatic foci were found in various organs, but none

TABLE 2. Change of PGE level in case of transplantation of VX-2 cells in the heart

10% VX-2 cell suspension (ml)	PGE level (pg/ml) before implantation	PGE level (pg/ml) after implantation		Gross metastatic sites*					
		14 day	21 day	Lymph node	Lung	Liver	Kidney	B.M.	Bone
0.1 (n=7)	510±150	1056±315	1535±696	7/7	7/7	2/7	5/7	4/5	0/5

* Autopsy was done 25 days later after implantation

had the evidence of bone metastasis neither by autopsy nor by bone scintigraphy. The plasma PGE levels increased after the intracardiac transplantation gradually as days went by. The value, however, was not as high as seen in the rabbits transplanted into the bone marrow and with positive bone scintigrams (Table 3).

TABLE 3. Comparison of PGE level between marrow and intracardiac transplantation

	Days after transplantation	PGE level (pg/ml)
Bone marrow transplantation	17–21	956–4500 (*2433*)
Intracardiac transplantation	14–21	675–2813 (*1535*)

In vitro study (Table 4) : When VX-2 tumor cells were incubated for 48 hrs at 37°C, significant amounts of PGE were produced in the culture medium and the amounts of PGE produced were paralleled to the numbers of VX-2 tumor cells incubated initially. On the other hand, no significant production of PGE was observed in either medium of HeLa S3 or human fibroblasts.

TABLE 4. PGE production by VX-2, HeLa S3 and human fibroblast in culture

No. of cells in medium ($\times 10^4$/ml)	PGE ng/medium 1 ml			
	VX-2	HeLa S3	Human fibroblast	Medium only
				0.10±0.01 (4)
5	4.5±0 (1)	———	0.97±0.42 (5)	
10	8.9±4.7 (4)	0.13±0.06 (2)	———	
20	20.8±2.4 (2)	———	1.70±0.35 (4)	
30–40	28.8±3.5 (5)	0.16±0.06 (3)		
50	———	0.14±0.03 (4)		
100	———	0.15±0.04 (4)		

DISCUSSION

From the cell culture study, it is evident that the VX-2 tumor cells do produce and excrete PGE into the culture medium.

In vivo study showed that the circulating PGE levels rapidly increased when the VX-2 tumor cells were dispersed into the circulation resulting in the multiple metastasis in various organs. This may indicate that PGE is also produced by the proliferated VX-2 tumor cells in the metastatic sites.

Interestingly enough, however, plasma PGE levels were significantly higher in the rabbits with osseous involvement than those without osseous involvement.

From these data, it is speculated that in the bone tissue, the production of PGE by tumor cells is accelerated or that the tumor cells cause the production of PGE by the normal bone tissue.

Of course, there is a possibility to be ruled out that the growth of the tumor cells is much faster in the osseous lesion than in the other non-osseous lesions. That possibility is, however, not likely since the osseous tumors were not appeared to be bigger than those of the non-osseous lesions, although we did not measure the precise weight of osseous or non-osseous metastatic tumors.

The local production of PGE at the bone lesion is a fascinating idea

that the bone destruction is not a direct effect of metastatic tumor but rather is mediated through the stimulation of osteoclasts which destroy bone.

Although the details of osteoclastic resorption of bone are still debated, several bone resorbing factors associated which malignant tumors are considered, such as vitamin D like sterol,[4,5] PTH-like substances,[6,7] OAF[8,9] and PGE.[10–13] The present study demonstrated that in VX-2 tumor cells, which produce PGE at even it the basal level, PGE production is particularly enhanced in the bone lesion, and that PGE may play a role in destroying bone, mediating the stimulation of osteoclastic activity. It is still not clear whether the increased production of PGE in the bone lesion is associated only with VX-2 tumor or it is associated with other malignant tumors. Further studies would be necessary to investigate the exact role of PGE in the mechanism of osteoclastic bone resorption in the malignant tumors.

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