INVESTIGATIVE NUCLEAR MEDICINE

Effects of Prostaglandin on Experimental Bone Malignancy and on Scintigrams of Bone and Marrow

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The correlation between prostaglandin E (PgE) and scintigrams of bone (Tc-99m MDP) and bone marrow (Tc-99m SC) was investigated in normal and VX-2-bearing rabbits. PgE in plasma of normal rabbits was 486.2 ± 185.7 pg/ml (n = 86) and the maximum-to-minimum (max/min.) ratio was 1.85 ± 0.26 at 4 wk after tumor implantation. In rabbits with VX-2 transplanted into femoral muscles, PgE was in the normal range unless the tumor invaded bone. PgE did not increase significantly in rabbits when the tumor was transplanted into the marrow cavity. When tumor invaded bone, PgE increased markedly (to 1335 ± 584 pg/ml). Elevation of PgE did not necessarily coincide with the appearance of positive bone scans. PgE in an indomethacin-treated group was not higher than in the untreated group. There was no significant difference between the two groups regarding the time of appearance of abnormal bone scans. However, when the number of transplanted cells in the bone marrow was reduced, the treatment with indomethacin delayed the increase in tracer uptake in the affected bone and resulted in a photon-deficient area. Indomethacin may suppress the local acceleration of calcium metabolism.

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Unquestionably the early detection and early treatment of malignant tumors are factors that enhance survival rate. On the other hand, many malignancies metastasize through the blood stream, and this thwarts efforts to prolong life. Recent advances in nuclear medicine have greatly improved early diagnosis, as is widely recognized. Especially valuable is early diagnosis of bone metastases, and when compared with other methods of examination, such as TCT, radiotracer diagnosis is far superior. Whereas metastasis to bones can be observed in almost all malignant tumors, it is most likely to occur in breast carcinoma and cancer of the prostate. Therefore, the elucidation of the mechanisms involved in bone metastasis in these tumors would greatly contribute to early detection and facilitate early treatment. Recently, prostaglandin (Pg) has been recognized as one of the factors concerned in the bone metastases from breast carcinoma (*1–4*). Briefly, malignant neoplasms produce larger quantities of Pg, and it may play a potential role in the osteolysis due to bone metastases.

For the purpose of establishing a therapeutic plan to follow early diagnosis, we have used VX-2-bearing rabbits as a model in experimental studies of the formation of metastases and have explored the role of prostaglandin E (PgE) in the scintigraphic detection of tumors in bone and bone marrow.

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MATERIALS AND METHODS

Animal model. Albino rabbits weighting 2.5–3.9 kg were used as experimental animals.

Transplantation. For transplants we used the VX-2

tumor, an epidermoid carcinoma derived from Shope virus.

Transplantation to thigh muscles. The tumor was excised from a rabbit bearing VX-2 cancer, and minced. A phosphate buffer containing 2000 U/ml penicillin and 100 μg/ml streptomycin was added. The suspension was then filtered through gauze and the filtrate centrifuged. With cells thus isolated, a 20% cell suspension was prepared, and 0.1-0.5 ml of the cell suspension was transplanted into the femoral muscles.

Transplantation into bone marrow. With an 18-gauge needle, 0.1 ml of 5, 10, and 20% cell suspension from the VX-2 carcinoma was transplanted into the marrow of the iliac bone.

Blood aspiration. Blood specimens were obtained from the heart with a heparinized syringe. Blood was immediately centrifuged for 30 min at 4°C, 1600 *g*, and the plasma so obtained was stored at −20°C. Within 1 wk, Pg was determined by the method described below. Pg assays and scintigrams were run concurrently.

Collection of urine. The urine was collected daily using metabolic cages. Two-milliliter samples of urine were obtained from the total volume, stored at −20°C, and within 3 days Pg content was measured. From this, daily urinary output of Pg was calculated, and this was monitored in each rabbit for 4 wk.

Radioimmunoassay of Pg. This was done with a PgB, RIA kit.* The unlabeled Pg (specimen) competes with H-3-labeled Pg (kit) for binding sites on the Pg antibody (anti-Pg rabbit serum) in the kit. From the ratio of uncombined labeled antigen (F) and labeled antigen-antibody compound (B), Pg is determined. A double-antibody precipitation was used for the separation of B from F.

The assay of PgE described in the kit requires an extraction procedure, which was done by the method of Jaffe et al. (*5*). Three milliliters of petroleum ether are added to 1 ml of plasma to remove neutral fat. Pg is then extracted with ethyl acetate. After the organic layer is solidified by evaporation, the isogel Tris buffer contained in the kit dissolves the Pg, and this mixture is used as the assay material. To 1 ml of PgE 0.1 ml of 1 *N* sodium hydroxide is added. This solution is immersed in a boiling-water bath for 5 min to convert PgE to PgB, and the pH is adjusted to 7.0-7.4 with acetic acid. The assay is then done with the PgB_1 kit.

When urine is the starting material, it should be acidified in order to extract PgE in the organic solvent. Under this procedure, however, PgE is converted into PgA. Accordingly, before the extraction PgE was converted to PgB by shifting to alkaline pH, and the PgB was then extracted with acidic pH. For this step, 2 ml of urine were dissolved in phosphate buffer (0.05 *M*, pH 7.4) and 1 ml of 1 *N* KOH was added for conversion of PgE to PgB. Then the pH of PgB was adjusted to 3-4 with 1 *N* HCl. PgB was extracted with ethyl acetate and separated using a silica column. Pg assay was done in rabbits carrying VX-2 transplants and in normal rabbits.

Diagnosis of bone tumor. *Scintigraphy.* This was done with the rabbits prone. Bone-marrow scanning with Tc-99m sulfur colloid (Tc-99m SC) was done at 5, 7, 10, 12, and 14 days after transplantation or until the scan became positive, at which time bone scanning with Tc-99m methylene diphosphonate (Tc-99m MDP) was started and followed for up to 3 wk. Both Tc-99m SC and Tc-99m MDP were administered in doses of 2-4 mCi in an ear vein. Using a scintillation camera with high-resolution collimator, the marrow scan was done 10 min after the injection and the bone scan 4-24 hr later.

Bone radiography. This was done with nonscreen films, using 65 Kvp, 300 mA, and 0.3 sec.

Treatment with indomethacin and mitomycin C(MMC). After the intramedullary transplantation of 0.1 ml 10% cell suspension of VX-2, the rabbits were divided into a group receiving 50 mg indomethacin by rectum and a group receiving none. In these groups, bone and bone-marrow scanning, as well as measurement of PgE, were performed. Further, the rabbits receiving the transplant of 0.1 ml 5% cell suspension were subdivided into those receiving 0.2 mg/kg of MMC every other day for 10 days and those receiving indomethacin in the same dose for the same length of time. In these latter groups, bone and bone-marrow scanning and PgE measurement were performed.

Measurement of serum calcium. This was done using tintometry with *o*-cresolphthalein complexone (O-CPC). To 0.02 ml of serum, 2.0 ml of the color reagent were added, and 10 min later the tintometry was done at 570 nm, the values being calculated from the standard curves. The color reagent was prepared as follows: 5.0 ml of 14.8 *M* ethanolamine boric acid buffer solution (pH 11.0), 5.0 ml O-CPC solution, and 2.0 ml of 5 g/dl oxine (8-hydroxyquinoline) were mixed; the mixture was then diluted to 100.0 ml with redistilled water for use.

RESULTS

Recovery of PgE. The recovery yield of the present

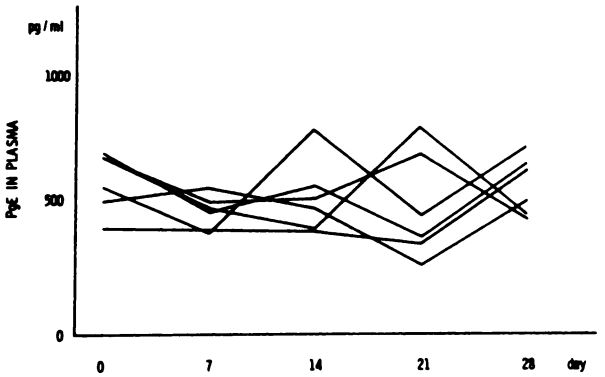

FIG. 1. PgE plasma level versus time in normal rabbit.

TABLE 1. CHANGES IN URINARY EXCRETION OF PgE IN NORMAL RABBITS

Animal no.	Weight (kg)	Urine* (ml)	PgE level in urine (ng/day) 0 wk	2 wk later	3 wk later	4 wk later	Max./min. ratio of PgE in urine during period
1	3.3	160 ~ 210	165.4	146.0	112.0	123.8	1.48
2	3.9	220 ~ 485	71.5	80.0	108.0	119.6	1.67
3	3.8	270 ~ 500	107.5	53.6	94.2	84.4	2.00
4	3.6	120 ~ 150	40.3	52.5	—	25.5	2.06
5	3.3	70 ~ 110	31.6	33.3	21.0	42.0	2.00
Mean ± s.d.							1.84 ± 0.25

* Range of daily volume during 4 wk.

method, as observed after adding 1 ng each of PgE_1 and PgE_2 to plasma, proved to be 46.5% (n = 4).

Plasma PgE value in normal rabbits. The plasma PgE level was 486.2 ± 185.7 pg/ml (n = 86) in normal rabbits.

Normal changes of PgE with time.

1. Observations on the max/min ratio of PgE in plasma, followed during a 4-wk period, resulted in a value of 1.85 ± 0.25 (n = 6) (Fig. 1).

2. Daily urinary excretion of PgE showed similar variation: max/min ratio over the 4-wk period was 1.84 ± 0.25 (n = 5, Table 1). Because of this parallel behavior, the following studies were based only on the plasma levels.

Change of PgE after VX-2 transplantation. *Intramuscular transplants* (*Table 2*). One-half milliliter of 20% VX-2 cell suspension was transplanted into the femoral muscles and PgE was done at 2 or 3 wk (Fig. 3). One group of animals showed positive bone-scan findings, and this group had high levels of PgE. There was no rapid rise of PgE in the group giving normal bone scans.

After transplantation of 0.1 ml of 20% cell suspension, the bone scans in the fourth week were normal and the Pg levels were low. In the rabbits with normal scans, however—and whether they received 0.1 or 0.5 ml of tumor suspension—the Pg showed a significant rise along with growth of the tumor. Nonetheless, in the periods between the second and the third weeks—and between the third and fourth weeks, when the tumor became necrotic—no significant rise in the Pg level was observed.

Intramedullary transplants. *Bone and marrow scintigrams and PgE level* (*Table 3*). VX-2, as 0.1 ml of 20% cell suspension, was transplanted into the marrow of the iliac crest in 12 rabbits, and the tumor growth was followed with both bone (Tc-99m MDP) and marrow (Tc-99m SC) scans, along with measurements of PgE levels. Results show that the marrow scan turned positive (lesion showing as a photon deficiency) at an average of 10 days after transplantation, whereas the bone scan turned positive, on average, at the 17th day. At the time when the marrow scan showed a defect, the PgE level, on average, was 295 ± 161 pg/ml, which was somewhat lower than that before transplantation (Table 3). In contrast, at the time when the *bone* scan went positive, mean PgE reached the high level of 1335 ± 584 pg/ml. The standard deviation was large, however (mean max/min ratio = 7.7 ± 5.2), and one cannot say that in *all* rabbits the appearance of an abnormal bone scan coincided with the rise in PgE level.

Scan and PgE findings when fewer tumor cells are injected into marrow (*Table 4*). One-tenth milliliter 10% cell suspension of VX-2 was transplanted into iliac marrow in four rabbits and the bone scans and Pg levels were studied. Plasma PgE levels before the transplantation were 556 ± 114 pg/ml. The bone scan became positive at 17–21 days after the transplant; PgE then showed a high value of 2433 ± 1489 pg/ml, and by 23–24 days it had reached 3019 ± 357 pg/ml. When the

TABLE 2. PgE LEVELS IN RABBITS* FOLLOWING I.M. TRANSPLANTATION OF VX-2 CARCINOMA

20% VX-2 cell suspension (ml)	Bone scan finding	PgE level in plasma (pg/ml) Before transplantation	2 wk later	3 wk later	4 wk later
0.5 ml	positive	449 ± 219	793 ± 221	1467 ± 730	—
0.5 ml	negative	430 ± 153	590 ± 92	454 ± 154	—
0.1 ml	negative	318 ± 136	440 ± 175	577 ± 170	633 ± 357

* n = 6.

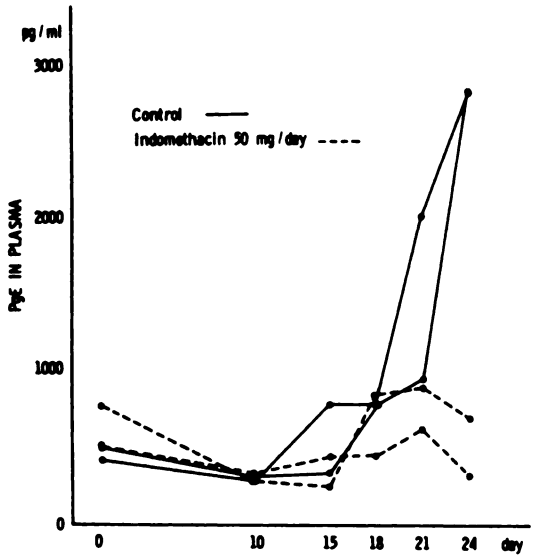

FIG. 2. Effect of chemotherapy on PgE level in VX-2 rabbits (iliac crest).

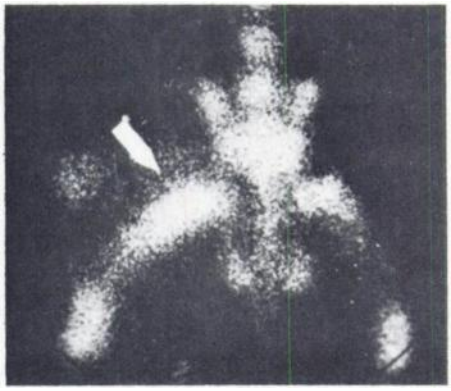

FIG. 3. Bone scan of rabbit 3 wk after implantation of 0.5 ml of 20% VX-2 cell suspension in femoral muscle. Note increased activity in proximal region of femur (arrow). PgE markedly increased, 2400 pg/ml.

number of transplant cells is halved, then, the development of an abnormal bone scan may be slightly delayed, and at that time the PgE level showed its first rise.

Effects of indomethacin and MMC on the Pg level and scintigraphy. *Indomethacin and Pg* (*Fig. 2*). Rabbits were divided into two groups, one receiving 50 mg of indomethacin every day and the other receiving none. Both groups had intramedullary transplantation of 0.1 ml 10% VX-2 cell suspension, and bone and marrow scans were studied as well as PgE levels.

In the group without indomethacin administration (intramedullary transplants); rabbits No. 15 and 16 showed a rise of PgE as the bone scan became positive, whereas the group receiving indomethacin gave no rise. In this group, the scan became positive at 21 days, compared with 21 and 24 days in the group receiving indomethacin. The difference between the two was not significant.

Mitomycin C and Pg (*as compared with indomethacin group*) (*Table 5*). After intramedullary transplantation of 0.1 ml 5% VX-2 cell suspension, rabbits were divided into an indomethacin group (n = 5) and an MMC group (n = 5), with the PgE levels and bone scans followed in each. In the MMC group, findings on bone scans were positive on Days 17, 17, 21, 21, and 21, when

TABLE 3. BONE AND BONE-MARROW SCINTIGRAPHIC FINDINGS, PgE LEVEL, AND MAX/MIN RATIO IN RABBITS FOLLOWING INTRAMEDULLARY TRANSPLANTATION OF VX-2 CELL SUSPENSIONS

Animal no.	Concentration and volume of VX-2 implanted	PgE level before implantation (pg/ml)	Bone-marrow scan		Bone scan		Max/min ratio of PgE during observation
			day*	PgE level (pg/ml)	day*	PgE level (pg/ml)	
1	20% 0.1 ml	675	10	292	15	1013	3.5
2	20% 0.1 ml	731	10	253	18	900	3.6
3	20% 0.1 ml	253	10	338	15	984	4.9
4	20% 0.1 ml	225	10	158	18	618	3.9
5	20% 0.1 ml	225	11	506	21	1294	5.8
6	20% 0.1 ml	405	10	225	17	2137	17.8
7	20% 0.1 ml	394	10	253	17	1688	18.8
8	20% 0.1 ml	450	10	169	17	2137	11.6
9	20% 0.1 ml	236	14	225	21	563	2.5
10	20% 0.1 ml	563	10	150	14	1181	13.9
11†	20% 0.1 ml	505	7	675	17	1280	2.5
12	20% 0.1 ml	169	—	—	16	2220	13.0
Mean ± s.d.		403 ± 188	10.1 ± 1.7	295 ± 161	17.2 ± 2.2	1335 ± 584	7.7 ± 5.2

* Represents first day when abnormal scan was obtained.

† See Fig. 4.

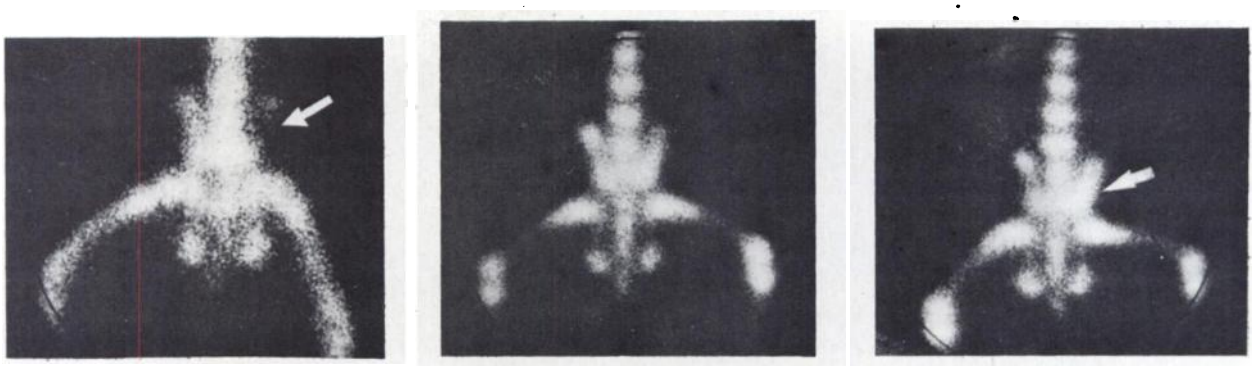

FIG. 4. Case No. 11 in Table 3. Bone marrow scan 7 days after intramedullary implantation of VX-2 (left). Note decreased activity in right ilium (site of implantation) (arrow). Bone scan 8 days after implantation (center). No apparent abnormality. Bone scan 17 days after implantation (right). Increased activity is in ilium (arrow).

PgE levels were 1406, 1280, 1460, 1181, and 1406 pg/ml, respectively. In the indomethacin group, in contrast, no rise of PgE was observed: its levels were 444 ± 372 pg/ml at Day 17, 470 ± 212 at Day 21, and 483 ± 95 at Day 24. Four out of five rabbits revealed defects on the bone scan, but the times of appearance were 25, 25, 25, 25, and 22 days. These times differed significantly ($p < 0.05$) from those in the MMC group. Bone breakage was observed by radiograph at 25 days after transplantation in both MMC and indomethacin groups.

Serum calcium levels in the VX-2 group. *Serum calcium levels.* These were followed for 4 wk after transplantation of 0.5 ml of 20% VX-2 cell suspension into femoral muscle (n = 6). The levels were 7.29 ± 0.18 mEq/l before the transplantation, while at 2, 3, and 4 wk after transplantation they were 6.96 ± 0.60, 6.48 ± 0.39, and 6.55 ± 0.39 mEq/l, respectively, all being within the normal range.

Intramedullary transplantation and serum calcium. As mentioned previously, the rabbits receiving 0.1 ml 10% VX-2 cell suspension were divided into a group receiving indomethacin and a group without, and the serum calcium level was studied at the same time as the measurement of PgE. The serum calciums before indomethacin administration were 7.02 and 7.20 mEq/l. Twenty-five days after transplantation (at the time when positive bone-scan findings appeared) the serum calcium levels were 6.20 and 6.67 mEq/l. These differences are minor, and even in the group given indomethacin the levels were 7.25 and 7.20 mEq/l before transplantation and 6.83 and 7.48 mEq/l after it, all being within the normal range.

DISCUSSION

The introduction of RIA (radioimmunoassay) for the in vitro determination of minute quantities of Pg has made possible the handling of many problems and has greatly contributed toward the elucidation of Pg's complex, pathophysiological actions (*6–8*). Nevertheless, there are still many problems in the RIA of Pg (*9–12*). For example, there is no specific antibody, and the cross-reactions between Pgs demand extraction procedures. Moreover, instability of Pg results readily in interconversions during the extraction procedures. Even if the material is kept at −20° for a long time, Pg increases by conversion so that the assay must be done within a short time. In the present study we used the Pg

TABLE 4. BONE SCINTIGRAPHY, PgE LEVEL, AND MAXIMUM PgE LEVEL DURING OBSERVATION PERIOD 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 and value when PgE value reached maximum	
			day*	PgE level (pg/ml)	day	max. PgE (pg/ml)
13†	10% 0.1 ml	620	17	2250	23	3431
14	10% 0.1 ml	675	20	4500	—	—
15	10% 0.1 ml	506	21	956	24	2813
16	10% 0.1 ml	422	21	2026	24	2813
		556		2433		3019

* Represents first day when abnormal scan was obtained.
† See Fig. 5.

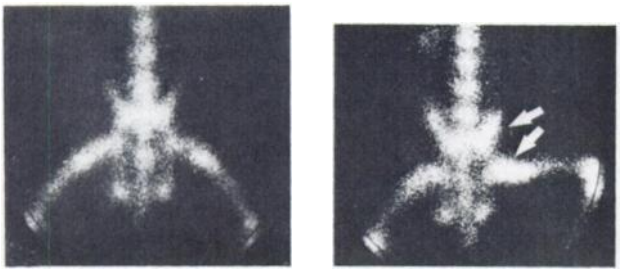

FIG. 5. Case No. 13 in Table 4. Bone marrow scan 5 days after intramedullary implantation of VX-2 (left). Scan does not reveal any abnormality. Bone scan 17 days after implantation (right). Areas of increased activity are noted (arrows). PgE was 2250 pg/ml.

kit to explore the role played by plasma PgE in the development of metastases, while also using gamma imaging to follow tumor growth in bone and bone marrow. In the study of recovery of PgE by this method, 1 ng each of PgE_1 and PgE_2 was added to the plasma and measurements were made. The result was 930 ± 82 pg/ml, which is lower than the actual level of 2 ng. This we believe is due to two factors: (a) partial conversion of PgE to PgB by alkaline treatment, and (b) partial destruction of PgE during the procedure. Therefore, the recovery yield was 46.5% in this case. Moreover, since we used PgE_1 and PgE_2 in the diluted dose of 1 mg, there might have been a deviation. Nonetheless, since we made a standard curve for each PgE measurement and found a repeatable curve, we consider the antibody to be stable.

In addition, considering that Pg has localized effects and its role in the lung is well recognized, the importance of estimating Pg in urine is logical. According to our results, urine and plasma PgE levels were identical in rabbits followed for up to 4 wk. Consequently the PgE levels obtained by this method were suitable for the study. In the report of Sugiyama et al. (*13*) the ratio of gas chromatography to mass spectrometry proved to be reliable, so it can be used sufficiently well to measure the in vivo changes of Pg.

The advance of nuclear medicine has considerably contributed to the diagnosis of cancers, not only by RIA but also by imaging. Especially the diagnosis of metastatic bone tumors by bone scintigraphy greatly facilitates an early diagnosis as compared with bone radiography, which requires 30–50% decalcification. Skeletal metastasis is recognized in all malignant tumors, but it is also known that breast cancer has a high frequency of bone metastasis, and the detection by bone scintigraphy is very useful for this diagnosis (*14–16*). However, the mechanism of development of bone metastases remains unclear. Recently it has been proposed that a tumor may contain osteolytic substances, and Pg as one of such substances has come to attract attention. On the other hand, noting that bone metastasis in most cases occurs through the arterial blood flow, Ito et al. (*17*) stressed the importance of bone-marrow scintigraphy in early diagnosis, as compared with scintigraphy, radiography, and pathological findings. Therefore, a systematic study

TABLE 5. PgE LEVELS AND BONE SCAN FINDINGS IN RABBITS RECEIVING INDOMETHACIN* OR MMC† ADMINISTRATION AFTER INTRAMEDULLARY TRANSPLANTATION OF VX-2 CELL SUSPENSION

Concentration and volume of VX-2 implanted	Indomethacin or MMC (dose)		PgE level in plasma (pg/ml) Before implantation	14 days later	17 days later	21 days later	24 days later	Day when positive bone scan was obtained
5% 0.1 ml	MMC 0.2 mg/kg every other day		480	619	1406	850		17
5% 0.1 ml	MMC 0.2 mg/kg every other day		405	505	1280	1294		17
5% 0.1 ml	MMC 0.2 mg/kg every other day		675	360	585	1460		21
5% 0.1 ml	MMC 0.2 mg/kg every other day		505	360	505	1181		21
5% 0.1 ml	MMC 0.2 mg/kg every other day		505	338	647	1406		21
		Mean	514	436	885	1238		
		±	±	±	±	±		19.4 ± 2.2
		s.d.	99	122	424	242		
	indomethacin	Mean	371		444	470	483	
5% 0.1 ml	50 mg/day	±	±		±	±	±	24.4 ± 1.3
		s.d.	179		372	212	95	

* Five rabbits/indomethacin group.

† Five rabbits/mitomycin C group.

of the relationships of the bone and marrow scintigraphy, combined with therapeutic techniques and Pg plasma levels, would be useful in elucidating the mechanism of development of bone metastases.

In our present experiment the scintigrams showed infiltration of the tumor into the bone after transplantation into the femoral muscles. With normal-looking bone scans, PgE tended to increase along with tumor growth, but no rapid elevation of PgE was observable in the animals with positive bone scans. When the transplantation of VX-2 carcinoma was intramedullary, as long as the marrow scan was positive, PgE remained at a low level, but it increased approximately in step with positive findings in the bone scans. This proved to be the same even if the number of the cells injected was halved and the peak level was not reached until the bone scan showed positive findings.

From these results, it is assumed that an increase of PgE can be observed along with development of the tumor, but its marked increase can be observed only when the tumor has invaded the bone. It is also important to take into consideration the report (*18*) that even mechanical stress stimulates the surrounding tissues to liberate Pg, and that the proliferation of osteoclasts results in osteolysis.

For the purpose of establishing a therapeutic plan, we studied scintigrams and PgE levels in rabbits treated with indomethacin. In these animals the PgE level was low, but no significant difference was observed at the onset of scan findings. When compared with the control group, most cases of the bone scans were not read as positives and appeared only as a defect. It is important to recognize that transplantation was done into a limited, small space of the bone marrow, that the carcinoma can readily develop and bring about the bone changes, and that a bone scan can detect these changes very sensitively. Therefore, it is necessary to conduct experiments on hematogenous metastasis with the VX-2 carcinoma in order to study the relation of the bone and marrow scintigrams, as well as the changes during the treatment. We are currently conducting experiments along these lines.

In the MMC rabbits, PgE is not inhibited: It rises to a high level occurring somewhat before the appearance of positive findings on a bone scan. Clearly, then, consideration should be given to the relation of Pg to osteolysis and to the nature and action of the therapeutic agent. In the present experiment the use of anticancer agent alone did not adequately affect bone metastasis. We will need to study changes in the treatment, such as using an anticancer agent with indomethacin, or radiation therapy combined with indomethacin.

Bennett et al. (*1, 3*) showed that breast carcinoma synthesized PgE and PgF in vitro, suggesting a possible treatment of bone metastasis from breast carcinoma by anti-prostaglandin agents. Powles et al. (*2*) recognized an osteolytic activity both in culture studies of mouse skull containing Ca-45 and in human breast carcinoma tissue. They also demonstrated that aspirin inhibits release of Ca-45. In animal experiments they demonstrated (*4*) that Walker carcinoma in vitro had an osteolytic activity that could be inhibited by aspirin. They stated that after transplantation of Walker carcinoma into the abdominal aorta of the rat, tumors developed in the soft tissues and bones of the lower limbs, but when aspirin or indomethacin was administered, the tumors developed in the soft tissues but not in the bones. Strausser et al. (*19*) experimented with the Maloney sarcoma virus (MSV) and stated that as the tumor developed, PgE also increased and vice versa, that treatment with indomethacin inhibited the growth of tumor, and that bone changes previously found in the untreated cases could no longer be observed. Histologically, in the untreated mice the osteolytic activity increased on the side of the bone in contact with the tumor, but this did not occur in the mice treated with indomethacin. With a melanoma there was no elevation of PgE. Galasko (*20*) injected VX-2 cell suspension into the thigh muscles of the rabbit and observed destruction of the femur; at the site osteoclasts proliferated, whereas away from the tumor site their numbers decreased. It is stated that as PgE_2 is released from VX-2 carcinoma and bone resorption occurs, in the initial stage there is a possibility that PgE_2 has increased the osteoclasts and that actually both osteoclasts and bone destruction are decreased by indomethacin. Further, it is stated that when the treatment is given after the transplantation to the bone, osteoclasts decrease and the bone destruction can no longer be observed by radiograph (*21*).

On the other hand, hypercalcemia in malignant tumors most often shows metastatic bone destruction, and there are many reports (*22–26*) pointing out the relation of Pg to hypercalcemia.

Tashjian et al. (*22*) state that some malignant tumors are often accompanied by hypercalcemia, irrespective of bone metastasis, and they point to PgE_2 as one of the causative factors. It is said that in experimental fibrosarcoma ($HSDM_1$) there occurs in the culture medium a factor that stimulates bone resorption, and that this factor is PgE_2, which induces elevation of serum calcium. Moreover, as the VX-2 carcinoma develops in the rabbit, serum calcium both increase (*23*). It is suggested that the tumor contains PgE_2 with its bone-resorbing ability; thus the administration of indomethacin decreases PgE_2, inhibits bone absorption, and also lowers the serum calcium level. Robertson et al. (*24*) reported that clinically the patients with solid tumor showing a high level of serum calcium have a high serum PgE and a low parathyroid hormone level (PTH). Demers et al. (*25*) consider that hypercalcemia accompanying tumor is related to PgE. Seyberth et al. (*26*) measured urinary metabolites of PgE and found that individuals with a

high serum calcium had a higher urinary output of PgE metabolites, and vice versa. In hematologic neoplasms, however, even if the serum calcium level was high, the urinary metabolite output was low. They therefore attempted the treatment of the patients with hypercalcemia accompanying the solid tumor by using indomethacin.

In our experiments we did not observe hypercalcemia due to the transplantation of VX-2 carcinoma cells, but we observed the rise of PgE, and indomethacin suppressed the local acceleration of the calcium metabolism. This delayed the onset of abnormal scan findings, and when they did occur they did so as photon deficiencies. Since the bone scan reflects calcium turnover, if the acceleration of metabolic bone destruction is suppressed, the scan would be unlikely to show positive findings.

Intramedullary transplantation of the VX-2 cell suspension was conducted to form the bone tumor and the change in Pg was observed, but it was found that a rise of Pg did not necessarily occur in all rabbits. This means that, aside from Pg, there are other factors (e.g., PTH and cyclic AMP) involved in the osteolysis. Future work should include study of the factors stimulating Pg secretion in culture cells.

FOOTNOTES

* Clinical Assay Inc., Cambridge, MA.

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