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Effects of Treatments for Experimental Bone Tumor on Prostaglandin E Level and Bone Scintigrams

Nobuaki OTSUKA, Yasuhiko ITO, Masaya YONEDA, Akira MURANAKA, Soichi NISHISHITA and Rikushi MORITA

Division of Nuclear Medicine
Department of Radiology
Kawasaki Medical School, Kurashiki 701-01, Japan
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ABSTRACT. The role of Prostaglandin E (PgE) level was studied experimentally as follows: 1) intrahepatic implantation of VX-2, 2) intravenous injection of VX-2, 3) effect of treatments on intramedullary implanted VX-2. The levels of PgE in intrahepatic and intravenous transplantation were not higher than that of intramedullary transplantation. Mitomycin C (MMC) did not reduce the PgE level and appearance time of bone scan abnormality was the same as that of untreated animals. A combination of indomethacin and MMC caused a delay in appearance time of bone scan abnormalities.

Key words : experiment bone tumor — prostaglandin E — bone scintigraphy — bone marrow scintigraphy

Prostaglandin E (PgE) is considered to play an important role in bone metastasis. Using nuclear medicine procedures, Otsuka et al.[1] reported that PgE level increased when intramedullary implanted VX-2 developed invasion of bone cortex. These experiments were undertaken in order to elucidate the changes of PgE in extraosseous development of tumor. Also, the therapeutic effects of indomethacin, Mitomycin C and their combination were investigated using experimental bone tumors.

MATERIALS AND METHODS

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

Intrahepatic implantation of VX-2 : One % or 10% of VX-2 cell suspension (0.1 ml each) was injected in the liver. The development of tumor in the liver was studied by liver scintigraphy.

Intravenous implantation of VX-2 : The cell numbers implanted were the same as those of intrahepatic implantation.

Intramedullary implantation of VX-2 : One-tenth ml of a 5% VX-2 suspension was injected in the iliac bone marrow cavity. Marrow and bone scintigraphy were performed to detect development of metastasis.

Radioimmunoassay of PgE : 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

大塚信昭，伊藤安彦，米田正也，村中　明，西下創一，森田陸司

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.[2)]

Treatments of indomethacin and MMC : Using VX-2-bearing rabbits (5% VX-2 cell suspension, 0.1 ml), 50 mg of indomethacin per day and/or 0.2 mg/kg of MMC every other day was given. The effect was followed up by bone- and bone marrow scanning.

RESULTS

Plasma PgE value in normal rabbits :

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

Normal changes of PgE with time :

Maximum to minimum ratio of PgE level in plasma of normal rabbits followed up to 4 weeks was 1.85±0.25 (n=6) (Fig. 1).

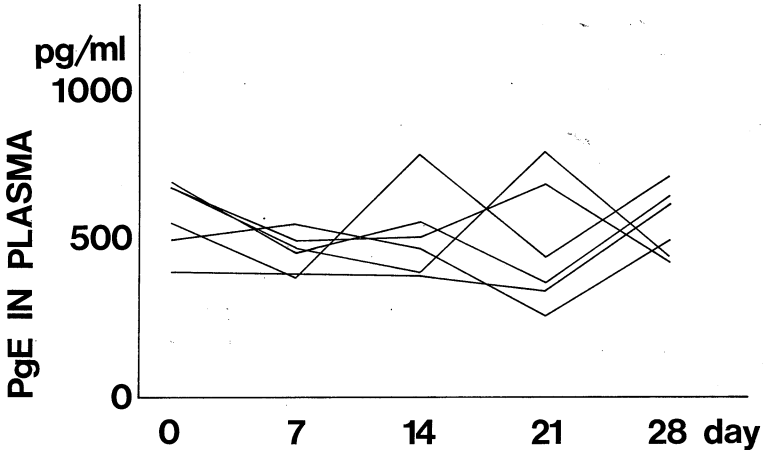

Fig. 1. PgE plasma level vertus time in normal rabbits.

TABLE 1. PgE level in plasma of rabbits following intrahepatic or intravenous transplantation of VX-2 carcinoma

Concentration and volume of VX-2	Method of implantation	Sites of tumor	PgE level in plasma (pg/ml)		
			Before implantation	After implantation	
1.0 % 0.1 ml	intrahepatic	Liver	487±304	3W*	928±414
10 % 0.1 ml	(n=6)		514±199	2W*	1180±570
1.0 % 0.1 ml	intravenous	Lung	543±118	2W	865±372
	(n=8)			3W	1140±565
10 % 0.1 ml			661±197	1W	1113±129
				2W	1110±322

* Represents time when abnormal scan was first observed (weeks).

TABLE 2. PgE level and bone scan findings in rabbits receiving indomethacin and/or MMC administration after intramedullary transplantation of VX-2 cell suspension (5%, 0.1 ml)

Indomethacin and/or MMC (dose)	PgE level in plasma (pg/ml)			Day when positive bone scan was obtained
	Before implantation	17 days later	21 days later	
control (n=4)	716±165	2332±1490	2685±1123	16.3±1.5
MMC 0.2 mg/kg every other day (n=5)	514± 99	885± 422	1238± 242	19.4±2.2
indomethacin 50 mg/day (n=5)	371±179	444± 372	470± 212	24.4±1.3
indomethacin with MMC (n=3)	468±117	712± 328	909± 479	26.7±2.3

Changes of PgE level in intrahepatic VX-2-bearing rabbits (Table 1): Although Tc-99m phytate liver scans revealed SOLs, the elevation of PgE was slight, compared with that of intramedullary implantation.

Changes of PgE level in intravenous VX-2 injected rabbits (Table 1): Intravenous injection of VX-2 caused multiple lung tumors. However, PgE level was not higher than in cases of intramedullary implantation.

Effects of treatments on both PgE and scintigraphy (Table 2, Fig. 2,3): In the MMC-treated group, the therapeutic effect was not evident. In the indomethacin-treated group, PgE did not increase, and a delay of bone scan positivity was noted. In a combination-treatment group, the therapeutic effects were remarkable.

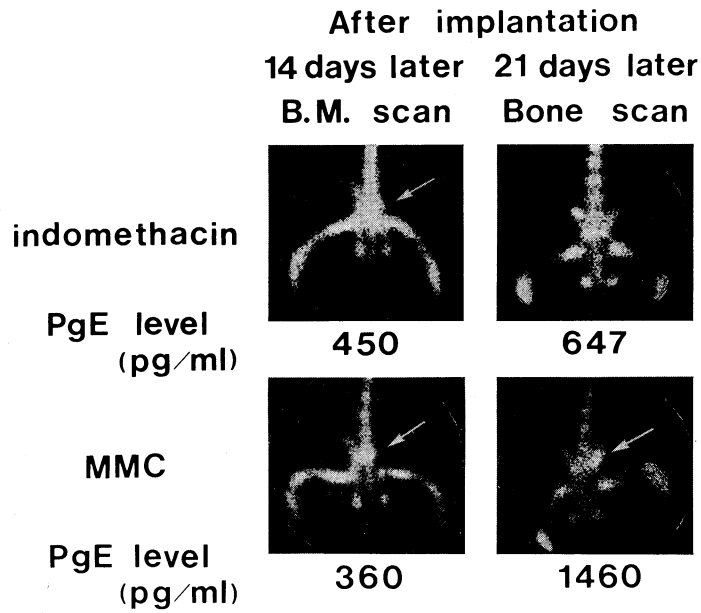

Fig. 2. Effects of indomethacin on MMC on the PgE level and scintigraphy.

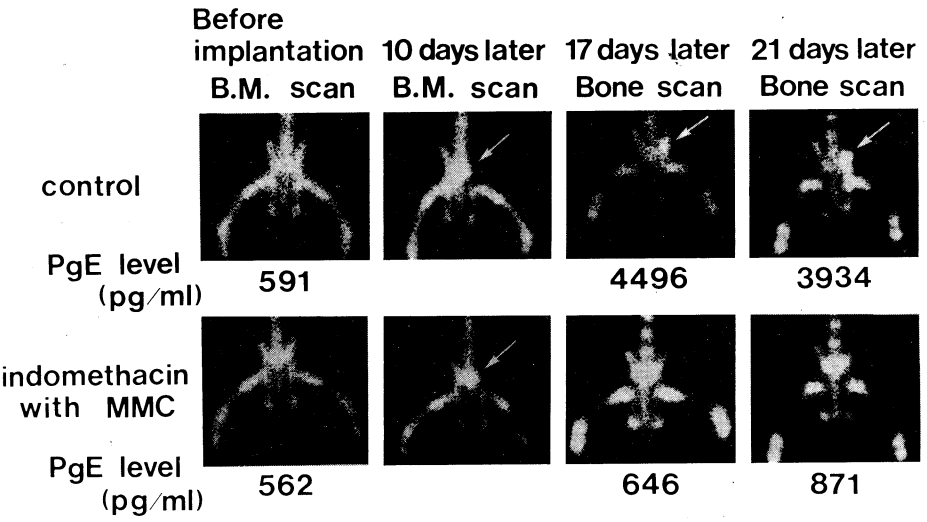

Fig. 3. Effects of chemotherapy (indomethacin with MMC) on the PgE level and scintigraphy.

DISCUSSION

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.[3,4] However, the mechanism of development of bone metastases remains unclear. Recently, prostaglandin has been recognized as one of the factors concerned in the bone metastases from breast carcinoma.[5–8] Briefly, malignant tumors product larger quantities of Pg, and it may play a potential role in osteolysis due to bone metastasis. On the other hand, noting that bone metastasis in most cases occurs through the arterial blood flow, Ito et al.[9] stressed the importance of bone marrow scintigraphy in early diagnosis, as compared with scintigraphy, radiography, and pathological findings. Therefore, a systematic study of the relationships of the bone and marrow scintigraphy, combined with therapeutic techniques and PgE plasma levels, would be useful in elucidating the mechanism of development of bone metastases.

Our previous study reported[1] correlative changes of PgE levels with bone and bone marrow scintigraphy following formation of process of bone metastasis on VX-2 rabbits in order to clarify the role of PgE in the development of bone metastasis. In these experiments we concluded that PgE level increased when intramedullary implanted VX-2 developed invasion of bone cortex.

In our present experiment we observed the infiltration of the tumor to the liver and lung after the intrahepatic and intravenous transplantation by scintigraphy. Compared with PgE level in intramedullary transplantation, intrahepatic and intravenous implantation of VX-2 did not cause marked elevation of PgE.

For the purpose of establishing a therapeutic plan in experimental bone tumors, a study was made about PgE level and bone scintigraphy in rabbits treated with indomethacin and/or MMC. 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. On the other hand, in the indomethacin administrated rabbits the PgE level was low, and effective in delaying of bone metastasis. Also, the combination of indomethacin and MMC was much effective in preventing bone metastasis than indomethacin alone. From these experiments, it is concluded that PgE might a more important role in a development of bone metastasis than in extraosseous metastasis.

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