jnm/CONCISE COMMUNICATION

PROSTAGLANDINS IN MICE WITH NEUROBLASTOMA

Savita Puri, Robert E. Belliveau, Richard P. Spencer, and Nicholas R. Bachur

Yale-New Haven Hospital, New Haven, Connecticut
Baltimore Cancer Research Center, National Cancer Institute, Baltimore, Maryland

Male albino mice, with and without transplanted neuroblastoma C1300, had tissues analyzed for prostaglandins by radioimmunoassay. Highest prostaglandin concentrations occurred in tumor tissue, lungs, spleen, and kidneys. Tritiated prostaglandins A_1 or E_1 and tritiated arachidonic acid were injected intravenously. The concentration (organ/tumor) was higher at 1 hr than at 6 or 24 hr. Prostaglandin E_1 and arachidonic acid localized principally in the kidney (likely the excretory organ) whereas prostaglandin A_1 showed highest concentration in the liver.

Recent reports, which describe a radioimmunoassay specific for different prostaglandins (PG), have noted significant elevations in plasma levels of prostaglandin E (PGE) in patients with neuroblastoma tumors (*1*) as well as increased production by neuroblastoma cells grown in tissue culture (*2*). The purpose of the present investigation was to measure chemically converted PGE and PGA in terms of PGB equivalents (*3*) in the tissues of mice carrying a neuroblastoma. In addition, comparative distribution and possible use of administered ^{3}H-PGE$_1$, ^{3}H-PGA$_1$, and their precursor, ^{3}H-arachidonic acid (*4*), for tumor localization was assessed.

MATERIALS AND METHODS

A group of 2-month-old male albino mice (A/J strain, Jackson Laboratory, Bar Harbor, Me.) and a similar group of the same strain with previously transplanted neuroblastoma tumor (C1300) were studied. The tumor had been transplanted subcutaneously and was used after 4–5 weeks growth (1–2 cm in size). At the time of the experiments, the tumor weighed between 1–3 gm. Mice from each group were sacrificed and samples of tumor, heart, lungs, liver, spleen, and kidney were obtained. Weighed tissue samples of approximately 100–200 mg were removed and quickly placed in chilled saline for homogenization (Omni-Mixer, Sorval, Norwalk, Conn.). The homogenate was transferred to centrifuge tubes to which a tracer of ^{3}H-PGB of 4,000 cpm (for estimation of recovery), and 1 cc of 1 *M* KOH was added. After incubation and conversion of prostaglandins to PGB (*3*), extraction was accomplished as described previously for plasma samples (*5*). Aliquots of the PGB fraction obtained from column separation were quantified by utilizing a commercially available radioimmunoassay (Clinical Assays, Cambridge, Mass.) as previously adapted (*5*). Results were expressed as PGB equivalents per gram of wet tissue weight. In another series of experiments, tumor-bearing mice were each injected through a tail vein with 2.5 μCi of ^{3}H-labeled prostaglandins A_1 (110 Ci/mmole) or E_1 (87 Ci/mmole), or ^{3}H-arachidonic acid (80 Ci/mmole) (New England Nuclear, Cambridge, Mass.). For this purpose, the materials were dissolved in 0.1 cc etha-

Received July 1, 1974; revision accepted Aug. 22, 1974.
For reprints contact: Richard P. Spencer, Dept. of Nuclear Medicine, University of Connecticut School of Medicine, Farmington, Conn. 06032.

TABLE 1. TISSUE PGB EQUIVALENTS IN NORMAL AND C1300 TUMOR-BEARING MICE (ng/gm TISSUE) (5 MICE PER VALUE)

Tissue	Nontumor	Tumor	P values: Tumor/nontumor*	P values: Tumor/other tissue in same animals†
Tumor		503.0 ± 75.8		
Heart	33.3 ± 3.5	67.0 ± 11.3	<0.05	<0.001
Lungs	215.5 ± 13.0	332.6 ± 36.1	<0.02	N.S.
Liver	85.4 ± 8.0	64.0 ± 22.6	N.S.‡	<0.001
Spleen	241.4 ± 56.6	168.4 ± 11.7	N.S.	<0.010
Kidney	82.4 ± 14.2	101.2 ± 5.8	N.S.	<0.001

* Tissue from tumor and nontumor-bearing mice was compared by Student's t-test.
† PGB levels were compared with those in other tissues of tumor-bearing mice.
‡ N.S. is non-significant or >0.05.

nol. At 1, 6, and 24 hr following injection, mice were sacrificed; weighed tissue and tumor samples (100–200 mg) were obtained and homogenized in chilled saline. Aliquots were then removed and incubated at 56°C with ProtoSol (New England Nuclear) for 24 hr. Subsequently, scintillation counting fluid was added (Aquasol, New England Nuclear) and the samples were counted with quench correction. After determination of the cpm/gm tissue, the results were expressed as ratios of tissue-to-tumor activity.

RESULTS

The levels of PGB equivalents were significantly higher in the tumor than other tissues (Table 1). Following injection of tritiated prostaglandins or arachidonic acid, the highest ratios of activity (organ/tumor) were observed at 1 hr (Table 2). The distribution of ^{3}H-labeled PGs and arachidonic acid showed greater activity in the kidney, liver, and lung as compared with tumor tissue. The highest levels were obtained in the kidney with ^{3}H-PGE$_1$. Kidney levels were greater than liver levels with ^{3}H-PGE$_1$ and ^{3}H-arachidonic acid. The reverse was seen with ^{3}H-PGA. The percent of the injected dose in major tissues is given in Table 3.

TABLE 2. RATIOS OF ACTIVITIES OF ^{3}H-PGE$_1$, ^{3}H-PGA$_1$ AND ^{3}H-ARACHIDONIC ACID (5 MICE PER GROUP)

	Time: 1 hr	6 hr	24 hr
	^{3}H-PGE$_1$		
Kidney:tumor	6.3 (5.5–7.2)*	1.8 (1.3–2.2)	1.6 (0.8–2.1)
Liver:tumor	3.2 (2.7–3.8)	1.5 (1.1–2.0)	1.1 (0.8–1.3)
Spleen:tumor	1.1 (0.9–1.2)	1.1 (0.7–1.8)	0.9 (0.7–1.0)
Heart:tumor	1.3 (0.9–1.7)	1.1 (0.8–1.3)	1.2 (0.9–1.6)
Lung:tumor	2.4 (1.9–2.8)	1.2 (0.8–1.8)	1.0 (0.8–1.3)
	^{3}H-PGA$_1$		
Kidney:tumor	3.2 (1.5–4.9)	1.7 (1.4–1.9)	1.4 (1.1–1.5)
Liver:tumor	4.7 (2.4–7.5)	1.9 (1.1–2.7)	1.4 (1.3–1.4)
Spleen:tumor	1.4 (0.5–2.4)	1.1 (0.9–1.3)	1.2 (1.1–1.3)
Heart:tumor	1.5 (1.0–2.1)	1.3 (0.9–1.9)	1.3 (1.2–1.4)
Lung:tumor	2.6 (1.3–4.5)	1.3 (1.2–1.4)	1.3 (0.9–1.5)
	^{3}H-arachidonic acid		
Kidney:tumor	3.7 (2.8–5.5)	1.8 (1.6–2.3)	1.6 (1.4–1.8)
Liver:tumor	2.5 (2.0–3.5)	1.4 (1.4–1.8)	1.4 (1.0–1.9)
Spleen:tumor	1.3 (0.8–1.5)	1.1 (0.7–1.4)	1.5 (1.0–2.4)
Heart:tumor	1.4 (0.6–3.1)	0.8 (0.6–1.0)	1.1 (0.7–1.7)
Lung:tumor	1.5 (1.0–2.1)	0.9 (0.8–1.3)	1.3 (1.0–1.8)

* Average value with range in parenthesis.

DISCUSSION

The neuroblastoma tissue levels of PGs [as expressed by PGB equivalents (*3*)] corroborated the results of other investigators who found elevations of PGE in the plasma of patients with neuroblastoma (*1*) and in tissue culture fluid of neuroblastoma cells grown in vitro (*2*). The next highest levels had been observed in spleen and lung. These observations did not correlate with our findings on the kinetics of distribution of labeled PGs and arachidonic acid. This suggests that breakdown and synthesis of the compounds proceed by different pathways; that is, there

TABLE 3. PERCENT OF TOTAL INJECTED ^{3}H-PGE$_1$, ^{3}H-PGA$_1$, AND ^{3}H-ARACHIDONIC ACID IN MAJOR ORGANS, 1 HR FOLLOWING INJECTION

Tissue	^{3}H-PGE$_1$ (%)	^{3}H-PGA$_1$ (%)	^{3}H-arachidonic acid (%)
Tumor	7	5	8
Kidney	2	<1	2
Liver	12	11	13
Spleen	<1	<1	<1
Heart	<1	<1	<1
Lung	<1	<1	<1

are likely different sites or enzymes for production and degradation.

The distribution of ^{3}H-PGE at 1 hr revealed the highest activity in the kidney followed by the liver and other organs, similar to the findings of previous workers (*6–8*). These high renal levels were attributed to concentration in the major organ of excretion (*6–8*). The major cellular localization of PGE and its metabolites was found in the soluble fraction of homogenized tissue. Nakano (*6*) speculated that PGE was most likely bound to plasma membrane components and not intracellular organelles. Our studies also showed that arachidonic acid, a precursor of prostaglandin synthesis, showed relatively less activity in the kidney but demonstrated a distribution similar to PGE_1. Tritiated PGA_1 showed more activity in the liver than in the kidney at 1 hr and thus a pattern of distribution dissimilar from the others. These results suggest that the PGs and perhaps precursors have specific affinities for tissues which are based on minute molecular alterations. Apparently tissue levels of activity fall rapidly with time (6 and 24 hr) as 60% excretion of the administered dose has been found primarily in the urine with minor amounts in the feces at 40 hr after injection (*7*).

The pattern of uptake following injection of tagged compounds failed to demonstrate tumor activity significantly in excess of that of the kidney, liver, and other tissues. However, the differences of distribution among molecular forms of PG suggest that there should be a further search for species of PG, perhaps a smaller-sized precursor, which might concentrate in this tumor sufficiently to allow detection.

ACKNOWLEDGMENT

This work was supported by USPHS CA 14969 and GM 2094, and by DT 34D from the American Cancer Society.

REFERENCES

1. JAFFE BM, BEHRMAN HR, PARKER CW: Radioimmunoassay measurement of prostaglandins E, A, and F in human plasma. *J Clin Invest* 52: 398–405, 1973

2. JAFFE BM, PARKER CW, PHILPOTT GW: Prostaglandin release by human cells in vitro. In *Prostaglandins in Cellular Biology*, Ramwell PW, Pharriss BB, eds, New York, Plenum Press, 1972, pp 207–226

3. ZUSMAN RM: Quantitative conversion of PGA or PGE to PGB. *Prostaglandins* 1: 167–168, 1972

4. SAMUELSSON B: Biosynthesis of prostaglandins. *Prog Biochem Pharmacol* 5: 109–128, 1969

5. BELLIVEAU RE, BACHUR NR: Prostaglandin B equivalents in human plasma. *Prostaglandins* 5: 241–248, 1974

6. NAKANO J: Tissue distribution and subcellular localization of ^{3}H-prostaglandin E_1 in rats and dogs. *Arch Int Pharmacodyn* 187: 106–119, 1970

7. SAMUELSSON B: Synthesis of tritium-labeled prostaglandin E_1 and studies on its distribution and excretion in the rat. *J Biol Chem* 239: 4091–4096, 1964

8. HANSSON E, SAMUELSSON B: Autoradiographic distribution studies of ^{3}H-labeled prostaglandin E_1 in mice. Prostaglandins and related factors 31. *Biochem Biophys Acta* 106: 379–385, 1965