The Transplantation of Human Epidermoid Carcinoma (H.Ep. #3) into Conditioned Adult Dogs*

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SUMMARY

Transplantation studies revealed that a human epidermoid carcinoma, H.Ep. #3, can grow in conditioned adult dogs; but toxic, or near-lethal, doses of x-radiations and cortisone are required for transplants to grow. Because of these limitations, cortisone-treated and x-radiated adult dogs bearing human tumor transplants are not amenable for long-term experimental chemotherapy studies.

Human neoplastic tissue has been successfully grown in the eye and brain of nonconditioned animals (21), fetuses and newborn rats (8), and in the fertile chick egg (3, 6). In the main, however, heterologous transplantation experiments, with human tumors, have required host conditioning. Successful conditioning for the growth of human tumors in animals has been achieved with x-radiation and cortisone (20) or hydrocortisone (10); other procedures used in experiments on heterologous transplantation of human tumors have involved thorotrast or trypan blue (4, 17), zymosan (15), corticoids other than cortisone and hydrocortisone (16), and germ-free guinea pigs (14). Sufficient uniformity of tumor growth and reproducibility of results have permitted certain human tumors growing in conditioned mice (12, 13), rats (18, 19), and hamsters (5) to be used in experimental cancer chemotherapy studies.

In his review, Woglom (22) cited the unsuccessful attempts of Pyrille in 1775, Langenbeck in 1840, Libert in 1851, and Bilbroth in 1867 to transplant human neoplastic tissue or extracts into nonconditioned dogs. Allam and associates (1) recently used conditioned, mixed-breed puppies to establish a transplant line of canine thyroid carcinoma. In view of the potential usefulness of a human tumor-dog system for chemotherapy and biochemical studies, experiments were designed to study the transplantability of a human epidermoid carcinoma (H.Ep. #3) in the conditioned dog. This tumor was first reported by Toolan (20) to grow well in the conditioned mouse, rat, and hamster. It is routinely maintained at the Sloan-Kettering Institute and has been used for chemotherapy (12, 13, 16, 18, 19), biochemical (2), and biological (7, 9) studies.

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MATERIALS AND METHODS

Seventeen mongrel dogs of either sex and from 1 to 5 years old were selected for this study. One to 3 days prior to transplantation, animals were given 150 r total-body x-radiation. The irradiation was performed at 250 kvP and 30 ma. at a T.S.D. of 100 cm.; added filtration of 0.33 mm. copper provided a half-value layer of 1 mm. copper.

H.Ep. #3 tissue was obtained from 10 to 14-day-old tumors growing intramuscularly or subcutaneously in x-radiated and cortisone-treated rats (20). Two tumors, individually minced and diluted to 50–70 per cent suspensions, were used for each transplantation experiment. The diluting fluid consisted of physiological saline fortified with potassium penicillin G (1,000 units/ml) and streptomycin sulfate (2 mg/ml). Inoculations of tumor suspensions (1.0–2.0 ml.) were made in two to four subcutaneous sites. Immediately after transplantation, and on alternate days thereafter (Saturdays and Sundays excluded), dogs were treated with cortisone acetate. External diameter measurements were made on the 7th, 14th, and 21st days after transplantation, or at death.

RESULTS

Nonconditioned dog.—H.Ep. #3 failed to grow when transplanted into a nonirradiated and noncortisone-treated dog (Table 1). Although a meas-

TABLE 1

OBSERVATIONS ON H. EP. #3 TRANSPLANTS IN MONGREL DOGS CONDITIONED* WITH X-RADIATION AND CORTISONE ACETATE

Conditioning			Tumor sizes—days after transplantation						Biopsy grades‡			Comments
			7th day		13th–14th day		21st day		Days after transplantation			
X-Ray	Cortisone (mg/kg×3/wk)	Dog no. and sex	Tumors†	Av. diam. (cm.) (range)	Tumors†	Av. diam. (cm.) (range)	Tumors†	Av. diam. (cm.) (range)	7th	13–14th	21st	
None	None	1 ♀	2/2		1/2	2.2	1/2		− to +		−	Regression started on 18th day.
	60	2 ♀	2/2	1.7 (1.5–1.8)	2/2	2.7# (2.3–3.0)	2/2	2.5 (2.2–2.8)		− to +#	− to >+	Dog edematous, toxic 21st day.
		3 ♀	4/4	1.2 (0.7–1.8)	4/4	1.9 (1.7–2.2)			+to++	+to++		Sacrificed 14th day.
	30	4 ♂	3/4	1.4 (1.3–1.6)	0/4							Regressions started 9th day.
		5 ♂	3/4	2.3 (1.5–3.5)	0/4				+			Sacrificed 14th day.
150 r	60	6 ♂	1/1	2.3			1/1	2.2	+		+	Pneumonia 28th day; Biopsy grade 28th day++.
		7 ♂	4/4	2.3 (2.2–2.7)	4/4	2.9 (2.3–3.3)			+	++		Died 14th day—pneumonia.
		8 ♀ §	4/4	1.6 (1.5–1.7)	4/4	2.1				− to +**		Died 10th day—edematous.
		9 ♀ §	4/4	1.7 (1.3–2.0)	4/4	2.0 (1.3–2.3)						Sacrificed 14th day—edematous.
	30	10 ♀	4/4	2.0 (1.8–2.3)	4/4	2.6 (2.5–3.1)	0/4					Tumors suppurative—14th day.
		11 ♀	2/4	1.8 (1.7–1.8)	4/4	2.0 (1.5–2.8)	4/4	2.5 (1.9–3.3)			−	Tumors suppurative—23d day.
		12 ♂	4/4	1.5 (1.2–1.6)	4/4	2.1 (1.9–2.3)	0/4					Tumors suppurative—21st day.
		13 ♀	3/4	1.6 (1.2–2.0)	4/4	1.9 (1.6–2.3)				+#		Died 16th day, four tumors weighed 25 gms.
		14 ♂	4/4	2.2 (2.0–2.3)					++			Died 8th day—pneumonia.
		15 ♂	4/4	2.1 (1.5–2.9)	2/4	1.8 (1.6–1.9)				++		Dog sacrificed: two tumors weighed 8 gm. on 14th day.
		16 ♂	2/2	1.5 (1.1–1.9)	2/2	2.1 (1.7–2.5)						Sacrificed on 14th day.
	15	17 ♂	4/4	1.6 (1.2–1.8)	0/4							Regression—14th day.

* = Cortisone acetate given 3 ×/week until final observation.
† = No. tumors measured/no. tumors transplanted.
‡ = None or poor quality tissue, −; Fair quality, +; Good quality, #.
§ = Conditioned with four doses of cortisone, given over 7 days.
# 16th day observations.
** 10th day observations.

urable mass (2.2 cm.) was present 14 days after transplantation, a biopsy made on the 7th day revealed degenerated tumor cells.

Nonirradiated dogs, conditioned with 60 mg/kg cortisone acetate.—On the 7th day after transplantation, tumors averaged 1.2–1.7 cm. in diameter. A biopsy revealed tumor tissue in active mitosis (three to four figures/high-power field [h.p.f.]) with negligible host inflammatory reaction. By the 14th to 16th days, tumors were 1.9–2.7 cm., and biopsy material showed extremely vascular, newly formed connective tissue at the periphery of tumor nodules. Biopsy specimens, taken from a 21-day-old transplant which measured 2.5 cm., revealed 50–75 per cent necrobiotic tumor tissue. In the areas having viable tumor, mitotic activity was two to three figures/h.p.f.

Nonirradiated dogs, conditioned with 30 mg/kg cortisone acetate.—These dogs had measurable masses (1.4–2.3 cm.) 7 days after transplantation, but biopsy material revealed host reaction in the form of a heavy inflammatory infiltrate. Small foci of the tissue were completely necrotic, but large areas showed degenerative changes of varying degree (clumping of chromatin, heteroploid cells). The moderately viable parts of the tumor contained little intercellular substance and showed only approximately 1 mitotic figure/h.p.f. By the 14th day after transplantation, tumors were barely palpable.

Irradiated dogs, conditioned with 60 mg/kg cortisone acetate.—Four dogs were treated with this combination of conditioning. Three died within 10–14 days. Tumors in all four dogs were, on the average, 1.7–2.3 cm. by the 7th day after transplantation; biopsy material revealed moderate (three to four figures/h.p.f.) to high (7 figures/h.p.f.) mitotic activity. Approximately 25–50 per cent of the tumor tissues were necrotic. Biopsy specimens of 10- and 14-day-old tumor tissue, taken from either dead or toxic dogs, revealed viable areas of moderate to high mitotic activity. Histological examination of a 21-day-old transplant revealed moderate mitotic activity with slight to moderate inflammatory infiltration. A piece of this tumor grew exceedingly well when transplanted back into the conditioned rat.

Irradiated dogs, conditioned with 30 mg/kg cortisone acetate.—On the average, 7-day-old transplants were 1.5–2.2 cm. in diameter. Examination of biopsy material revealed extremely viable tumors containing nine to ten mitotic figures/h.p.f. In one dog bearing four tumor transplants, two tumors had regressed by the 14th day after transplantation, but two others remained and, when excised, weighed a total of 8 gm. A biopsy specimen of a 21-day-old tumor showed completely necrobiotic tumor tissue, but no inflammatory infiltrate or fibroblastic activity. Of seven dogs treated with this combination of conditioning, only 1 died prior to the 14th day; the remaining animals were healthy and free of edema.

Irradiated dogs, conditioned with 15 mg/kg cortisone acetate.—Tumorous masses averaging 1.6 cm. in diameter were present on the 7th day after transplantation, but by the 14th day barely palpable nodules were present at the inoculation sites.

DISCUSSION

In an effort to combine the uniqueness of transplantable human neoplastic tissue with a more useful preclinical animal than the mouse, rat, or hamster, experiments were designed to study the fate of human tumor transplants in conditioned adult dogs.

In total, seventeen adult dogs of mixed breed and either sex were used. Data indicate that the conditioning regimen of cortisone acetate at 30 mg/kg, in combination with 150 r total-body x-radiation, is of moderate usefulness. Of seven dogs treated with this combination, one died by the 8th and one on the 16th day after transplantation. Tumors tended to regress after the 14th day, even though cortisone was administered during the entire period of observation; by the 21st day inoculation sites were usually suppurative, and tumor tissue was necrotic. Other conditioning regimens were either exceptionally toxic or lethal (60 mg/kg cortisone acetate, with or without x-radiation), or ineffective in allowing tumor to grow (30 mg/kg cortisone acetate without x-radiation; 15 mg/kg cortisone acetate with x-radiation).

The sequence of histologic changes observed for 7–21-day-old H.Ep. #3 transplants growing in conditioned adult dogs are similar to those observed during the growth and regression of transplantable animal tumors (22). At times, toxic or near-toxic combinations of cortisone and x-radiation did not effectively block host reaction to the transplants. These observations are in agreement with those of Takayama and Woolley (17), who described the appearance of inflammatory reaction tissue in and about H.Ep. #3 transplants growing in cortisone-conditioned mice. However, the presence of host inflammatory infiltrate (plasma cells and lymphocytes) does not imply that tumor tissue is not viable and capable of growth; a backtransplant of H.Ep. #3 grew exceedingly well in the conditioned rat; no attempt was made to pass the tumor through succeeding generations of rats.

In view of these considerations, and since tumors regress and inoculation sites may become

suppurative after the 14th day, it would be difficult to interpret chemotherapy and other experimental data obtained from a dog-human tumor system.

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