

Experimental Transplantation Models of Mouse Sarcoma 180 in ICR Mice for Evaluation of Anti-Tumor Drugs

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ABSTRACT. Two new experimental models of transplantable mouse sarcoma 180 were developed in ICR mice in order to examine the optimum transplantation sites and methods. The cervicodorsal hypoderm was evaluated as the best transplantation site for mouse sarcoma 180 among seemingly usable transplantation sites such as groin, armpit, cervicodorsal, abdominal and lumbodorsal hypoderms by hypodermic transplantation. In addition, the lung transplantation model was established by monitoring the survival period as a reliable parameter for evaluation of anti-tumor effects.—**KEY WORDS:** cervicodorsal hypoderm, intravenous transplantation, lung tumorigenesis method, mouse sarcoma 180, solid tumor.

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Transplantable mouse sarcoma 180 (S-180) has worldwide been used in many research institutes since Dr. Walgom of Crocker Lab., U.S.A. found it in the right armpit of a male white mouse in 1914 [9].

S-180, which had initially been used in the solid type, was transformed into the ascites type by Goldie *et al.* in 1952 [3] and since then it has been employed for anti-tumor screening of many types of drugs [6]. Relatively poor popularity of S-180, which is reflected by very few papers dealing with this tumor, seems to stem from its many unclear characteristics [12], which results in the U.S. classification of S-180 in the group of undifferentiated tumors [2].

In the present studies, we examined the optimum transplantation sites and methods for S-180 in ICR mice. We found that the cervicodorsal hypoderm was the best transplantation site for the cells. Further we developed the lung transplantation system. This is the first report on evaluation of anti-tumor effects of drugs on lung tumorigenesis of S-180 which was transplanted in the tail vein of ICR mice.

MATERIALS AND METHODS

Tumor cells: S-180 was kindly provided by National Institute of Cancer. The tumor cells were transplanted intraperitoneally in ICR mice every seven days and the cells employed in this paper were collected after the 343rd–347th transfer.

Animals: Male SPF ICR mice (aged 4–5 weeks and weighing 21–26 grams) were purchased from Japan SLC, Inc. and Charles River Japan Inc., and health-monitored for 4 days prior to tests. Each test group or control group consisted of 5–10 mice and were observed for 42 days at maximum length. Mice were maintained at a room temperature of $23 \pm 1^\circ\text{C}$ and a relative humidity of $55 \pm 5\%$, and were fed *ad libitum* with solid feed MF (Oriental Yeast Co., LTD) and tap water.

Drugs: Aclacinomycin (ACM, Mercian K. K.), THP-adriamycin (THP, Mercian K. K.), Adriamycin (ADM, Kyowa Hakko K. K.) and Daunomycin (DM, Meiji Seika K. K.) were dissolved in physiological saline (Fuso Kogyo K. K.). Saline was used as vehicle control.

Preparation of the cell suspension: The ascites type of S-180 was recovered from ICR mice with a sterile syringe 7 days after intraperitoneal transplantation. A drop of the ascites was diluted with physiological saline and an erythrosin B solution in a leucocyte melangeur, and was visually enumerated on a blood cell counter under a light microscope.

Based on the cell count, the ascites was appropriately diluted with physiological saline to give an S-180 suspension as follows. In hypodermic tumorigenesis experiments, for determination of the optimum inoculum size and for experiments, for determination of the optimum inoculum size and for experimental therapy, 2.5×10^6 , 5×10^6 , 2.5×10^7 and 5×10^7 cells/ml S-80 suspensions were prepared. In lung tumorigenesis experiments, for determination

of the optimum inoculum size, 4×10^5 , 2×10^6 , 4×10^6 , 8×10^6 , 1.6×10^7 , 2.4×10^7 , 3.2×10^7 and 4×10^7 cells/ml S-180 suspensions were used. For enumeration of nodes and experimental therapy, 4×10^4 , 2×10^5 , 4×10^5 , 2×10^6 , 4×10^6 and 2×10^7 cells/ml S-180 suspensions were prepared.

Tumor transplantation: Hypodermic tumorigenesis experiments: One test group or control group consisted of 5 mice, and were hypodermically inoculated with 0.2 ml/mouse of an S-180 cell suspension with a sterile syringe. Cell densities of the S-180 suspensions tested were as follows: 5×10^6 cells/mouse for comparison of the armpit, groin, cervicodorsal, abdominal and lumbodorsal hypoderms as transplantation site. 5×10^5 , 1×10^6 , 5×10^6 and 1×10^7 cells/mouse for determination of the optimum inoculum size and other transplantation conditions in the cervicodorsal hypoderm, and 5×10^6 cells/mouse for experimental therapy.

Lung tumorigenesis experiments: S-180 was transplanted by injection of 0.25 ml of a cell suspension into the tail vein under the following conditions. For determination of the optimum inoculum size, 1×10^5 , 5×10^5 , 1×10^6 , 2×10^6 , 4×10^6 , 6×10^6 , 8×10^6 or 1×10^7 cells/mouse were transplanted in 5–10 mice/group. For enumeration of nodes, 1×10^4 , 5×10^4 , 1×10^5 , 5×10^5 , 1×10^6 or 5×10^6 cells/ml were transplanted in 5 mice/group. For experimental therapy, 5×10^6 cells/mouse were transplanted in 5 mice/group.

Drug administration: Both transplantation systems were therapeutically tested by administration of ACM, THP, ADM or DM into the tail vein once a day for 7 consecutive days starting from 24 hr after transplantation. Tested doses of an anthracycline antibiotic were 30, 60 and 90 $\mu\text{g}/0.25 \text{ ml/mouse/day}$.

Excision, weighing and node enumeration of tumor blocks: Hypodermic tumorigenesis experiments: Mice were killed with carbon dioxide gas 16 days after transplantation in order to compare the weights of the excised tumors in the armpit, groin, cervicodorsal, abdominal and lumbodorsal hypoderms, 5, 10, 15 and 20 days after transplantation in order to compare the mean daily increase in weight, volume and maximum sectional area of tumor in those sites, 5, 10, 15 and 20 days after transplantation in order to determine the optimum inoculum size on the basis the weight of excised tumor blocks, and 15 days after transplantation to perform experimental therapy. Using scissors, excised tumor blocks were removed from the healthy

tissues of the transplantation site. The weight of a fresh tumor block excised was measured by a Mettler electronic balance PL1200, and its volume was measured by a hand-made volumetric analyser containing mercury. The major and minor axes of a tumor block were measured with a caliper to calculate the maximum sectional area of that tumor block by multiplication [1, 5, 10].

Lung tumorigenesis experiments: Mice were killed with carbon dioxide gas 42 days after inoculation for determination of the optimum inoculum size and experimental therapy based on the survival period. Numbers of mice which had died during the observation period of 41 days were recorded every day. The survival period of 1 day covers the time span of 24 hr from zero to 24 hr and more after transplantation. In comparative experiments using the weight of the excised lung, mice were killed with carbon dioxide gas 2, 5, 8, 11 and 14 days after transplantation, and the wet weight and volumes of the excised lungs were measured as described above. For enumeration of nodes on the excised lung, mice were killed 10 days after transplantation. The excised lungs were fixed with a Bouin's solution [7] so that the number of nodes formed on the lung surface might be counted by naked eyes.

Anti-tumor effect: The anti-tumor effect in experimental therapy was evaluated as follows: In hypodermic tumorigenesis experiments, the anti-tumor effects in the control and test groups were calculated based on the mean wet weight of the excised tumor blocks and were expressed as the percent tumor growth inhibition by using the following equation [8]: Percent tumor growth inhibition = $(A-B) \times 100/A$

Wherein A and B are the mean wet weights of the excised tumor blocks in the control and the test drug groups, respectively. In lung tumorigenesis experiments, the anti-tumor effects in the control and test groups were calculated based on the mean survival periods of the control and test animals and were expressed as the percent survival elongation by using the following equation [11]: Percent survival elongation = $(B-A) \times 100/A$

Wherein A and B are the mean survival periods (expressed in days) of the control and the test drug groups, respectively.

The means and standard errors (S.E.) were calculated for all experimental data and parameters in each group and then subjected to the *t*-test among the groups concerned.

RESULTS

Hypodermic Tumorigenesis Experiments

Comparison of the transplantation sites in the tumor growth: Among the tested sites of the groin, armpit, abdominal, cervicodorsal and lumbodorsal hypoderms, the cervicodorsal hypoderm group produced the mean wet weight of the tumor block of 5 g, which is about 5 times heavier than that of the groin hypoderm group, and about 4 times heavier than that of the armpit hypoderm group. The cervicodorsal hypoderm group showed statistically significant difference from the other four groups at $p < 0.01$. Variance of the wet weight data was also the best in the cervicodorsal hypoderm group. (Table 1).

Comparative time course studies of the tumor growth in the mean wet weight, mean volume and mean maximum sectional area in the groin, armpit and cervicodorsal hypoderms: The mean wet weight, mean volume and mean maximum sectional

area values of the tumor solids in the cervicodorsal hypoderm group on day 15 were 1.5–2.7, 2.0–2.7 and 1.3–1.7 times, respectively, higher than those in the other two groups (Fig. 1).

Optimum inoculum size: Among the four inoculum sizes of 2.5×10^6 , 5×10^6 , 2.5×10^7 and 5×10^7 cells/ml, no significant difference was observed in the daily wet weight increase of the tumor block in the cervicodorsal hypoderm, but 5×10^6 cells/0.2 ml/mouse was considered the optimum depending on the highest gross increase in the wet weight (data not shown). These findings indicated that the cervicodorsal hypoderm transplantation system was highly efficient in tumorigenesis and probably useful for evaluation of the anti-tumor effects of drugs.

Anti-tumor evaluation: Table 2 shows that the maximum tumor growth inhibition percentages of ACM, THP, ADM and DM are 33–63%, 78–84%, 78–85% and 44–71%, respectively, at the same doses.

Lung Tumorigenesis Experiments

Table 1. The wet weights of S-180 solids in different transplantation sites

Transplanted site ^{a)}	Solid weight (mg) ^{b)} Mean \pm S.E.	Comparative ratio	S.E./Mean $\times 100$ (%)
Groin	945 \pm 113 ^{c)}	1.0	11.9
Armpit	1272 \pm 240 ^{c)}	1.3	18.8
Abdomen	581 \pm 101 ^{c)}	0.6	17.3
Cervicodorsum	5003 \pm 507	5.3	10.1
Lumbodorsum	781 \pm 79 ^{c)}	0.8	10.1

a) 5×10^6 cells/mouse were transplanted at different subcutaneous sites in 5 ICR mice.

b) Solids were weighed at 16 days after transplantation.

c) Significantly different from the cervicodorsal group ($p < 0.01$).

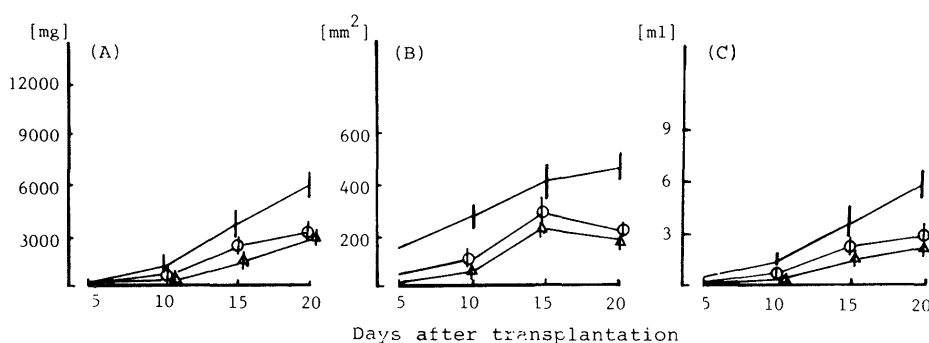


Fig. 1. The weights, volumes, and cut areas of S-180 solids at the different subcutaneous sites in ICR mice.

The S-180 solids were removed and measured weight (A), maximum cut area (B) and volume (C) on day 5, 10, 15, and 20 after 5×10^6 cells/mouse of S-180 had been transplanted at the cervicodorsal (—), armpit (○) and groin (△) hypoderm sites in ICR mice. Animals of 5 mice per group were used each site. These values (Mean \pm SE) of solids of the armpit and groin hypoderm in each of three experiments (A, B, C) were shown.

Table 2. Anti-tumor effect of anthracycline drugs on solid of S-180 at the cervicodorsal hypoderm in ICR mice

Drug ^{a)}	Dose (mg/kg/day × 7)	Solid weight (g) ^{b)} Mean±S.E.	Inhibition (%)
Control	—	5.48±0.78	0
ACM	1.2	3.67±0.57	33.0
	2.4	2.59±0.41 ^{c)}	52.7
	3.6	2.04±0.53 ^{d)}	62.8
THP	1.2	1.21±0.32 ^{e)}	77.9
	2.4	0.86±0.18 ^{e)}	84.3
	3.6	toxic death	—
ADM	1.2	1.23±0.10 ^{e)}	77.6
	2.4	0.83±0.11 ^{e)}	84.9
	3.6	0.84±0.25 ^{e)}	84.7
DM	1.2	3.07±0.57 ^{c)}	44.0
	2.4	1.70±0.40 ^{d)}	69.0
	3.6	1.60±0.25 ^{e)}	70.8

a) ACM: Aclacinomycin. THP: THP-adriamycin.

ADM: Adriamycin. DM: Daunomycin.

b) Animals were sacrificed on day 15 after inoculation (5×10^6 cells/mouse of S-180).c) Significantly different from the tumor control group ($p < 0.05$).d) Significantly different from the tumor control group ($p < 0.01$).e) Significantly different from the tumor control group ($p < 0.001$).

Optimum inoculum size: In preliminary tests using the survival period as evaluation index, an inoculum size of 1×10^6 cells/0.25 ml/mouse was found to be too small, as some mice survived after the observation period of 42 days, whereas an inoculum size of 1×10^7 cells/0.25 ml/mouse was considered to be too large, as some mice died on transplantation. When S-180 was injected at an inoculum size of 5×10^6 cells/0.25 ml/mouse, all the mice died during the predefined observation period of 42 days, showing a mean survival period of 18 days (data not shown). Based on these results, the inoculum sizes ranging from 1×10^5 to 1×10^7 cells/0.25 ml/mouse were examined again in more details. Table 3 shows that the optimum inoculum size is in the range of 4×10^6 to 6×10^6 cells/0.25 ml/mouse. In practice, the inoculum size of 5×10^6 cells/0.25 ml/mouse was considered to be best for the lung tumorigenesis and so employed in the subsequent experiments.

Comparison of the control lung and the S-180 tumor-bearing lung: The time course experiments revealed that the mean wet weights of the tumor-bearing lungs 8 and 14 days after transplantation were about twice and three times heavier than that 2 days after transplantation, respectively. The tumor-bearing lung showed significant daily increases in weight up to 8 days after transplantation, and the increase rate became reduced after that. Compared with the mean wet weight 2 days after transplanta-

tion, the control lung showed only 14–17% increases up to 14 days. On 14 days after transplantation, the tumor-bearing lung was about 4 times heavier than the control lung at $p < 0.01$ (Table 4).

These results suggest a close cause-effect relationship between tumorigenesis and death. In addition, the weight increase of the tumor-bearing lung and the survival period of the mice were considered to be reliable parameters for lung tumorigenesis tests.

Enumeration of nodes on the tumor-bearing lung: Figure 2 shows that the inoculum sizes of 1×10^4 , 5×10^4 , 1×10^5 , 5×10^5 and 1×10^6 cells/0.25 ml/mouse produced 7–64 nodes on the lung surface, whereas the inoculum size of 5×10^6 cells/0.25 ml/mouse gave 322–484 nodes. This indicated that the number of nodes observed on the lung surface is considered to be a reliable parameter for lung tumorigenesis experiments. If this parameter is valued, the optimum inoculum size should be in the range from 1×10^6 to 5×10^6 cells/0.25 ml/mouse.

Anti-tumor evaluation: The maximum survival elongation percentages of ACM, THP, ADM and DM at the same dose were 15.4%, 6.7%, –11.5% and 15.4%, respectively (Table 5). These results indicated that none of the anthracycline antibiotics has a significant survival-elongating effect on the lung tumor.

Table 3. Mortality and its survival times of the tumor animals transplanted intravenously with different inoculum sizes of S-180

Inoculum size (cells/mouse)	Survival time ^{a)}		S/T ^{b)}
	days	Mean±S.E.	
1×10^5	19,21,26,35,42	$28.6 \pm 4.34 <$	1/5
5×10^5	18,20,21,32,42	$26.6 \pm 4.56 <$	1/5
1×10^6	16,24,24,25,42	$26.2 \pm 4.27 <$	1/5
2×10^6	17,19,19,22,23	20.0 ± 1.10	0/5
4×10^6	14,16,17,17,21	17.0 ± 1.14	0/5
6×10^6	15,15,16,18,19	16.6 ± 0.81	0/5
8×10^6	0,10,14,16,17	11.4 ± 3.09	0/5
1×10^7	0,0,11,12,16	7.8 ± 1.74	0/5

a) Animals were observed for 42 days.

b) Survived animal per treated animals.

Table 4. The daily lung weight after intravenous transplantation of S-180 (5×10^6 cells/mouse) in ICR mice

Removed day	Normal lung (A)		Tumor lung (B)		Comparative increase	
	Weight (mg) (Mean±S.E.)	Increase (%)	Weight (mg) (Mean±S.E.)	Increase (%)	B-A (mg)	B-A/A ×100(%)
Day 2	176 ± 14.8	0	$246 \pm 15.0^a)$	0	70	39.8
Day 5	181 ± 8.6	2.8	$299 \pm 14.8^c)$	21.5	118	65.2
Day 8	195 ± 11.1	10.8	$606 \pm 107.4^a)$	146.3	411	210.8
Day 11	206 ± 12.7	17.0	$660 \pm 129.8^a)$	168.3	454	220.4
Day 14	200 ± 6.2	13.6	$780 \pm 93.3^b)$	217.1	580	290.0

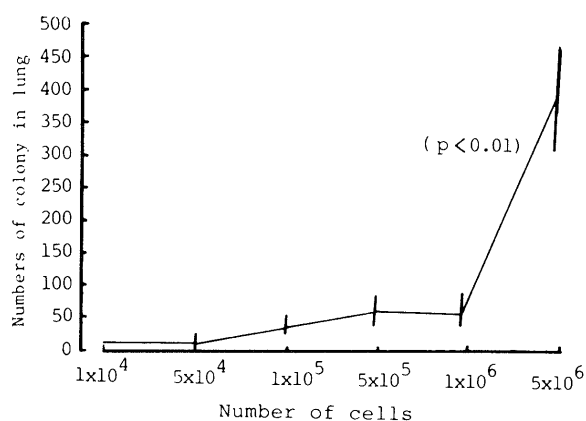
a) Significantly different from the tumor control ($p < 0.05$).b) Significantly different from the tumor control ($p < 0.01$).c) Significantly different from the tumor control ($p < 0.001$).

Fig. 2. The sarcoma tissue colonies on lung surfaces by different inoculum sizes

Tumor lungs were removed and the numbers of S-180 tissue colonies were counted on day 10 after different inoculum sizes of S-180 had been transplanted intravenously in ICR mice. Each group consisted of 5 mice. Data represent the mean±SE on colony numbers of each group.

DISCUSSION

It is apparent in the present studies that the cervicodorsal hypoderm is more suitable than the other sites conventionally used sites for tumorigenesis. The clear superiority to the armpit and groin hypodermis was proved by significant difference in the wet weight increase of tumor blocks, as the mean wet weight of tumor solids in the cervicodorsal hypoderm group was 3.9 and 5.3 times heavier than those in the armpit and groin hypoderm groups, respectively. Variance of the mean wet weight of tumor blocks also supports superiority of the cervicodorsal hypoderm to the other transplantation sites.

In total, the cervicodorsal hypoderm is concluded to be the best among the tested sites for S-180 transplantation, based on better and more stable tumorigenesis.

It is of interest to indicate that the intravenous transplantation of S-180 induces the tumor cells to stay and grow at a high frequency in the lung, which

Table 5. Anti-tumor effect of anthracycline drugs on the lung sarcoma in ICR mice

Drug ^{a)}	Dose (mg/kg/day×7)	Survival time ^{b)}		Inhibition (%)
		Days	Mean±S.E.	
Control	—	17,20,20,23,24	20.8±1.24	0
ACM	1.2	14,15,28,28,28	22.6±3.31	8.7
	2.4	16,17,18,25,28	20.8±2.40	0.0
	3.6	9,20,24,31,36	24.0±4.66	15.4
THP	1.2	20,20,20,25,26	22.2±1.36	6.7
	2.4	10,10,12,16,28	15.2±3.38	-26.9
	3.6	7, 7, 7, 7, 8	7.2±0.20 ^{c)}	-65.4
ADM	1.2	14,16,18,20,24	18.4±1.72	-11.5
	2.4	9,18,18,21,21	17.4±2.20	-16.3
	3.6	8, 9,11,20,21	13.8±2.78	-33.7
DM	1.2	15,17,17,18,31	19.6±2.89	-5.8
	2.4	14,16,16,17,18	16.2±0.66 ^{d)}	-22.1
	3.6	17,20,20,21,42	24.0±4.55	15.4

a) ACM: Aclacinomycin THP: THP-adriamycin ADM: Adriamycin DM: Daunomycin

b) Animals were treated intravenously drugs from day 1 to 7 after having transplanted intravenously with 5×10^6 cells/mouse of S-180.

c) Significantly different from the tumor control group ($p < 0.001$).

d) Significantly different from the tumor control group ($p < 0.05$).

leads to the node formation. Although not yet experimentally proved, this phenomenon seems to be due to some specific factors. Macroscopic examination 6 weeks after intravenous injection revealed that the lung was the sole organ which contained the tumor. This finding and other general symptoms suggest that the main cause of death in ICR mice injected with S-180 in ICR mice may be useful as an experimental model for human lung cancer.

The anti-tumor effects of ACM, THP, ADM and DM which were observed by the cervicodorsal hypoderm tumorigenesis system are dose-dependent. Ranking of these anthracycline antibiotics by a groin hypoderm tumorigenesis system [4] is comparable to the above-described results, which demonstrates that the cervicodorsal hypoderm tumorigenesis system is as dependable as conventional systems.

Contrary to the expectation, the results of evaluation of the anthracycline antibiotics by the lung tumorigenesis system using intravenous transplantation showed that the mean survival periods of the drug treatment groups were rather shorter than that of the drug-free control group, even though the antibiotics were given at the same doses as observed to be effective by the cervicodorsal hypoderm tumorigenesis system. No dose-effect correlationship was recognized, but the anthracycline antibiotics except for ACM and DM seemed to

exhibit toxic patterns. ACM and DM are assumed to become effective at higher doses. THP and ADM, if given at lower doses or for a shorter period, may be effective in this lung tumorigenesis system. The lung tumorigenesis system is worth further study and refinement, although its practical utility has not yet been proved in evaluation of anti-tumor drugs.

In conclusion, the two new tumorigenesis models which have been developed in ICR mice by transplantation of S-180 in the cervicodorsal hypoderm for solid tumor formation and by intravenous injection of S-180 for lung tumor formation are reliable and useful. They are also expected to be useful in evaluation of anti-tumor effects of drugs.

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