Enhancing Effect of Thoracotomy and/or Laparotomy on the Development of the Lung Metastases in Rats After Intravenous Inoculation of Tumor Cells

Takao HATTORI, Yuichiro HAMAI, Tatsuji HARADA,
Hiroshi, IKEDA and Toshiyuki IKEDA

ABSTRACT: To evaluate the effect of operative stress on the tumor growth, thoracotomy and/or laparotomy were performed 48 hours after the inoculation of Sato lung cancer cells in Donryu rats. The survival time, the number of metastatic foci on the surface of the lung and the per cent-area of metastatic foci in the frontal section through the pulmonary hilus were observed. Thoracotomy and laparothoracotomy reduced significantly the survival time as compared with the control but no significant difference was found between the two test groups subjected to operative stress. The number and percent-area of metastatic foci found were inversely related to the length of the survival time.

KEY WORDS: Operative stress, thoracotomy, laparothoracotomy, Sato lung cancer, Donryu rats, lung metastases, enhancing effect.

INTRODUCTION

In a previous report,[7] the enhancing effect of operative stress on the tumor growth such as thoracotomy and/or laparotomy was examined in Donryu rats bearing ascitic form of Sato lung cancer (SLC). Thoracotomy and laparothotacotomy reduced significantly the survival time of the rats as compared with the control but no significant difference was observed between the two groups subjected to different operative stress. This report constitutes the continuation of it, presenting the influence of the operative intervention on the development of the lung metastases in Donryu rats inoculated intravenously with various numbers of SLC cells. As reported in previous paper,[7] the system of Donryu rat and SLC cells presently used is an excellent syngeneic tumor-host system and the result obtained shows only a few deviation in a small range.

MATERIALS AND METHODS

Inbred Donryu rats, two months old males weighing about 170–200 g, were purchased from the breeding center of Nippon Rat Co., Saitama. The tumor cells utilized in this study were Sato lung cancer (SLC), which was kindly provided by Dr. H. Niitani of National Cancer Center Hospital, Tokyo. The technics used for the preparation of tumor cell suspensions have been previously described in detail.[7] Numbers of SLC cells inoculated intravenously in Donryu rats were 1.0×10^5 and 1.0×10^4, respectively. Rats were

From the Department of Surgery, Research Institute for Nuclear Medicine and Biology, Hiroshima University, Hiroshima, Japan.
Supported in part by Grants-in Aid from the Ministry of Education, Science and Culture and the Ministry of Health and Welfare.

lightly anesthetized and subjected to thoracotomy and/or laparotomy on the day two after the tumor inoculation using the same technics reported in the previous paper.[7] The survival time of the rats was expressed as T/C, the ratio of mean survival time of the test group to that of the control. Rats surviving over 50 days were designated as 50 day survivor for calculation of mean survival time. The number of the metastatic nodules on the surface of the lung was calculated macroscopically on the day 50 by sacrificing the rats. The number was expressed for each lobe and totalized. Then, the lung was sectioned in the frontal segment through the pulmonary hilus and stained with Hematoxylin-Eosin. The segmental area was measured on the section using planimeter. The percentage of the metastatic foci to the total lung was calculated in the segmental area and the percent-area was expressed for each lobe and the mean value of the five lobes was calculated.

Results

Survival Time

The survival time in each group was shown in Table 1 (1.0×10^5 cells inoculation) and Table 2 (1.0×10^4 cells inoculation). In both experiments, the survival time was

Table 1. Survival time of Donryu rats inoculated with SLC (1.0×10^5) intravenously in relation to operative stress

Experimental group	No. of rats	Individual days of death[a]	Average survival days (mean± SD)[b]	T/C[c] (%)	Survivors at day 50
Laparotomy	10	17, 18 (3), 19 (2), 20, 21 (2)	22.1± 9.9	84.4	1/10
Thoracotomy	10	15, 16 (2), 17, 18 (2), 19 (2), 20, 30	18.8± 4.2	71.8	0/10
Laparo-thoracotomy	10	15, 16, 17 (2), 18 (4), 19 20	17.6± 1.4	67.2*[d]	0/10
Control	10	18, 19 (3), 20, 21, 22, 24	26.2±12.7	/	2/10

[a] Figures in parenthesis indicate the number of rats.
[b] Rats surviving over 50 days were calculated as 50 day survivors in the determination of mean survival time.
[c] T/C=Percentage of the ratio of the mean survival time of the test groups to that of the control.
[d] Significant difference from the control: *$p<0.05$

Table 2. Survival time of Donryu rats inoculated with SLC (1.0×10^4) intravenously in relation to operative stress

Experimental group	No. of rats	Individual days of death[a]	Average survival days (mean± SD)[b]	T/C[c] (%)	Survivors at day 50
Laparotomy	10	19, 23 (3), 24, 27, 30, 38 39	29.6± 9.8	84.3	1/10
Thoracotomy	10	18, 19 (2), 20 (3), 23, 24, 27	24.0± 9.6	68.4*[d]	1/10
Laparo-thoracotomy	10	18, 19 (3), 20 (3), 24, 26, 29	21.4± 3.7	61.0**	0/10
Control	10	20, 24, 25 (2), 27, 30	35.1±13.1	/	4/10

[a], [b] and [c] See footnote for Table 1.
[d] Significant difference from the control: *$p<0.05$, **$p<0.01$

significantly reduced by thoracotomy and laparothoracotomy as compared with the control. The difference became most apparent in small number inoculation of 1.0×10^4 cells. The laparotomy also reduced the survival time by about 16 per cent as compared with the control in both experiments but the difference was not significant.

Number of Metastatic Nodules

The results were given in Tables 3 (1.0×10^5 cells inoculation) and 4 (1.0×10^4 cells inoculation). In 1.0×10^5 cells inoculation laparotomy did not increase the number of metastatic foci in the lungs but did thoracotomy and laparothoracotomy, giving the significant increase as compared with the control. In 1.0×10^4 cells inoculation, however, the increase of metastatic foci in number was demonstrated in all three test groups, with significant difference in thoracotomy and laparothoracotomy groups as compared with the control but no differences between themselves.

Table 3. Number of metastatic nodules on the lung of Donryu rats inoculated with SLC (1.0×10^5) intravenously in relation to operative stress

Experimental group	No. of rats	No. of metastatic nodules (mean±SD)						T/C[b] (%)
		Right lobe[a]				Left lobe	Total	
		1	2	3	4			
Laparotomy	10	17.5± 9.8	18.7±10.6	32.0±20.7	17.0±11.4	45.1±25.2	130.3± 71.7	87.3
Thoracotomy	10	29.0±12.9	26.9±13.7	48.8±21.4	27.6±12.6	72.6±36.3	204.9± 84.8	137.3
Laparo-thoracotomy	10	42.5±20.1	42.0±19.4	71.5±25.7	41.1±18.3	94.7±57.0	291.8±132.9	195.6*[c]
Control	10	15.7±16.8	28.9±28.7	51.1±60.2	20.6±20.9	32.9±32.5	149.2±141.9	/

[a] 1: upper lobe, 2: middle lobe, 3: lower lobe, 4: deep median lobe.
[b] T/C=Percentage of the ratio of the mean number of total metastatic nodules of the test groups to that of the control.
[c] Significant difference from the control: *$p<0.05$

Table 4. Number of metastatic nodules on the lung of Donryu rats inoculated with SLC (1.0×10^4) intravenously in relation to operative stress

Experimental group	No. of rats	No. of metastatic nodules (mean±SD)						T/C[b] (%)
		Right lobe[a]				Left lobe	Total	
		1	2	3	4			
Laparotomy	10	2.0±1.7	5.5±5.2	8.9±7.2	6.5±5.7	9.9± 6.3	32.8±21.2	145.8
Thoracotomy	10	7.4±5.9	10.3±8.4	16.3±14.2	6.1±7.0	17.7±13.1	57.8±45.6	256.9*[c]
Laparo-thoracotomy	10	9.9±7.5	8.6±4.4	14.1±8.6	8.5±3.7	18.8±12.3	59.9±30.9	266.2**
Control	10	2.5±3.2	4.1±5.1	5.8±8.4	4.7±5.9	5.3± 6.9	22.5±25.8	/

[a] and [b] See footnote for Table 3.
[c] Significant difference from the control: *$p<0.05$, **$p<0.01$

Percent-Area of Metastatic Nodules

As shown in Tables 5 (1.0×10^5 cells inoculation) and 6 (1.0×10^4 cells inoculation), the results obtained were quite similar to those of the number of metastatic nodules. Thoracotomy and laparothoracotomy increased significantly the percent-area of metastatic foci as compared with the control and the difference became remarkable in small number inoculation of 1.0×10^4 cells.

Table 5. Percent-area of metastatic nodules on the lung of Donryu rats inoculated with SLC (1.0×10^5) intravenously in relation to operative stress

Experimental group	No. of rats	Percent-area of metastatic nodules[b] (mean±SD)						T/C[c] (%)
		Right lobe[a]				Left lobe	Average	
		1	2	3	4			
Laparotomy	10	24.3±14.4	22.9±12.0	23.0±12.8	22.2±14.4	23.1±14.8	23.1±11.9	113.2
Thoracotomy	10	46.5±19.8	41.0±21.2	43.4±13.9	37.4±13.8	41.1±18.5	41.9±14.5	205.2*[d]
Laparo-thoracotomy	10	52.8±19.6	55.1±19.4	57.6±17.3	51.6±17.7	57.5±23.5	54.9± 9.7	269.1*
Control	10	21.8±20.1	22.6±18.7	18.4±20.2	20.4±18.0	20.8±17.0	20.4±17.0	/

[a] Percentage of metastatic nodules to the total lung on the frontal segmental area through the pulmonary hilus.
[b] 1: upper lobe, 2: middle lobe, 3: lower lobe, 4: deep median lobe.
[c] T/C=Percentage of the ratio of the average of the five lobes of the test groups to that of the control.
[d] Significant difference from the control: *$p<0.01$

Table 6. Percent-area of metastatic nodules on the lung of Donryu rats inoculated with SLC (1.0×10^4) intravenously in relation to operative stress

Experimental group	No. of rats	Per cent-area of metastatic nodules[b] (mean±SD)						T/C[c] (%)
		Right lobe[a]				Left lobe	Average	
		1	2	3	4			
Laparotomy	10	8.9±10.7	23.0±18.4	21.3±12.0	18.4±16.0	14.8± 9.8	17.3± 7.7	161.7
Thoracotomy	10	35.5±27.2	31.1±25.7	34.0±20.4	29.9±24.6	36.4±12.8	32.0±17.6	299.0*[d]
Laparo-thoracotomy	10	48.0±23.5	38.8±22.0	41.6±17.5	26.5±12.7	31.0±12.8	37.2±10.6	347.7*
Control	10	10.0±13.5	10.8±15.4	11.0±12.5	7.5±11.5	14.1±13.6	10.7±11.8	

[a], [b] and [c] See footnote for Table 5.
[d] Significant difference from the control: *$p<0.01$

Discussion

In an experimental program for the study of lung metastases, an accurate and reproducible method of measuring the extent of the tumor spread is necessary. The best way is the microscopical identification using serial sections of the lung. As they are expensive and time-consuming, we used in this study both the gross calculation of the metastatic foci and microscopical identification on one frontal section through the pulmonary hilus. Because of the similarity in color to normal parenchyma, light pigmentation method with India ink was recommended by Wexler[16] to facilitate the gross identification of the lung metastases. This method, however, interferes with the later staining of the section with Hematoxylin-Eosin. In practice the gross identification of the metastatic foci can be made satisfactorily in our SLC and Donryu rats without using Wexler's method. The results demonstrated a highly positive correlation between the number of the metastatic foci and their percent-area in the frontal section.

With improvement in cytologic technics an increased number of investigators have found a remarkably higher incidence of cancer cells in the venous blood draining tumors

and in the peripheral blood of patients with various malignant neoplasms. Even after curative operation, tumor cells were found in the blood. On the other hand, that the passage of tumor cells into the blood stream does not necessarily cause metastases has been frequently suggested since Goldmann[4] in 1897 first enunciated this opinion. While the speculation along this line has been advanced, no conclusive evidence is presented as to why circulating tumor cells might form secondary foci in some instances and not in others. Many factors have been known to increase the number of metastases in vivo, i.e., massage,[8,10,14] local roentgen irradiation,[9,11] total body irradiation,[11] trypan blue inoculation of host,[3] pregnancy,[5] elevated environmental temperature[13] and surgical stress. Although there has always been speculation on the possibility that surgical trauma resulted in a more rapid growth of tumor, little factual evidence is available. Schatten[15] or Lewis and Cole[12] reported the increased incidence of pulmonary metastases following total removal of primary leg tumor or amputation of limb with tumor. Experiments conducted by Buinauskas[1] et al. indicated the increased "take" of Walker 256 cells by the stress of celiotomy which was performed just before the subcutaneous inoculation of cells in rats. Fisher and Fisher[2] investigated the influence of operative stress in relation to the incidence of hepatic metastases after cell inoculation into the portal vein. The increased incidence was observed when inoculation was immediately followed by partial hepatectomy. Partial hepatectomy 24 hours after the inoculation had less effect and 48 hours after resulted in no greater incidence of metastases than was found in the control. They emphasized a transient period after the operation as important during which the stress of hepatectomy influences the rapid growth of tumor cells.

In the previous paper,[7] we demonstrated that thoracotomy and laparothoracotomy 48 hours after the intraperitoneal inoculation of SLC cells in Donryu rats significantly reduced the survival time as compared with the control. In the present paper, the reduction of survival time and the increased number and percent-area of metastatic foci of the lung seemed to be related to the stress of thoracotomy and laparothoracotomy performed after intravenous inoculation of tumor cells. At first glance, these data might appear to be a point against thoracotomy in cancer treatment. However, if these results are sustained by the human subjects, we might improve our operative results by eliminating the deterious effect of the thoracotomy on host resistance. One attempt on this line is our new by-pass procedure for the esophagocardial cancer, which consists of the transabdominal removal of esophagocardial lesion and the re-establishment of alimentary continuity at cervical esophagus, thus obviating thoracotomy.[6]

(Received for publication on April 8, 1977)

References

1. Buinauskas, P., McDonald, G.O. and Cole, W.H.: Role of operative stress on the resistance of the experimental animal to inoculated cancer cells, *Ann. Surg.* 148: 642–648, 1958.
2. Fisher, E.R. and Fisher, B.: Experimental studies on factors influencing hepatic metastases, *Cancer.* 12: 926–932, 1959.
3. Foulds, L.: The effect of vital staining on the distribution of the Brown-Pearce rabbit tumor. Tenth Scientific Report on Investigations of the Imperial Cancer Research Fund. Taylor and Francis (London), pp 21–31, 1932.
4. Goldmann, E.E.: Anatomische Untersuchungen ueber die Verbreitungswege boesartiger Geschwuelste, *Beitr. z. klin. Chir.* 18: 595, 1897.
5. Greene, H.S.N.: The heterologous transplantation of human melanomas, *Yale J. Biol. and Med.* 89: 269–278, 1949.
6. Hattori, T., Hamai, Y. and Ishii, T.: A new procedure of transabdominal resection of

esophagocardial cancer and cervical anastomosis obviating thoracotomy, *Jap. J. Surg.* 5: 211–221, 1975.
7. Hattori, T., Hamai, Y., Harada, T., Ikeda, H. and Ikeda, T.: Enhancing effect of thoracotomy and /or laparotomy on the growth of ascitic tumor in rats, *Jap. J. Surg.* in press.
8. Jonescu, P.: Ueber das Vorkommen von Geschwuelstzellen in stroemenden Blut von Tieren mit Impftumoren, *Ztschr. f. Krebsforsch.* 75: 129–142, 1922.
9. Kaplan, H.S. and Murphy, E.D.: The effect of local roentgen irradiation on the biological behavior of a transplantable mouse carcinoma, *J. Natl. Cancer Inst.* 9: 407–413, 1949.
10. Knox, L.C.: The relationship of massage to metastases in malignant tumors, *Ann. Surg.* 75: 129–142, 1922.
11. Krebs, C.: The effect of roentgen irradiation on the interrelation between malignant tumors and their hosts, *Acta Radiol., Suppl.* 8: 1–133, 1929.
12. Lewis, M.R. and Cole, W.H.: Experimental increase of lung metastases after operative trauma (Amputation of limb with tumor), *Arch. Surg.* 77: 621–629, 1958.
13. Lucke, B. and Schlumberger, H.: Induction of metastases of frog carcinoma by increase of environmental temperature, *J. Exper. Med.* 89: 269–278, 1949.
14. Marsh, M.C.: Tumor massage and metastases in mice, *J. Cancer Res.* 11: 101–107, 1927.
15. Schatten, W.E.: An experimental study of post-operative tumor metastases, *Cancer* 11: 455–468, 1958.
16. Wexler, H.: Accurate identification of experimental pulmonary metastases, *J. Natl. Cancer Inst.* 36: 641–645, 1966.