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Effect of Growth Hormone on Growth and Cytokinetics of Ehrlich Ascites Tumor in Hypophysectomized Mice*

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Summary

Growth of Ehrlich ascites tumor cells was inhibited in hypophysectomized in ICR-JCL male mice. The total cell number in the hypophysectomized animals was about a half of that in control on the 5th day after tumor cell inoculation. This inhibitory effect in hypophysectomy was more evident as the tumor age increased, and the doubling time of the tumor cells was prolonged as the time after the inoculation elapsed. Administration of mouse growth hormone showed a recovery of the inhibited tumor growth in hypophysectomized animals, whereas that of prolactin did not show any affect on the growth of the tumor. The tumor cells transplanted into the thyroidectomized, adrenalectomized, and castrated mice grew in a manner comparable to that in intact animals.

Regarding the retardation of growth of the tumor cells in hypophysectomized mice, cytokinetic experiments revealed that the inhibition of the growth in hypophysectomy was caused by an prolongation of the DNA synthetic phase in the cell cycle time.

Introduction

A number of studies have been made regarding the involvement of endocrine factors in carcinogenesis and growth of tumors including transplantable tumors[1~3]. As to the effect of growth hormone (GH) on the growth of trasplantable tumors in intact mice, only a few reports are available, and the finding and conclusions in these investigations were conflicting[4~6].

On the other hand, several studies have been hitherto reported on the inhibition of the tumor growth in hypophysectomized animals; however, the machanism which underlies the inhibition by hypophysectomy has not yet been elucidated.

We examined, therefore, the effects of the purified GH on Ehrlich ascites tumor growth, and also analysed the cytokinetics of the tumor cells in the hypophysectomized mice.

Materials and methods

Animals: ICR-JCL male mice were supplied from the Nihon Clea, Tokyo. Hypophysectomized, thyroidectomized, and castrated mice, 30 g in body weight, operated

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at an age of 5–6 weeks were obtained from the Nihon Hypox Laboratories, Tokyo. Adrenalectomy was conducted from the back of mice in our laboratory. These animals were used on the 4th or 5th day after the operation. The completeness of the operation was checked by macroscopic observations when the animals were killed. These animals, with free access to a pellet diet (No. NMF, Oriental Yeast Ind., Tokyo) and water, were housed in a Coitotron EA at 24–26°C with a light : dark cycle of 12 : 12 throughout the experiments. Animals were killed daily until the 5th day after tumor cell inoculation.

Tumor cells: Hyperdiploid Ehrlich ascites tumor cells have been maintained in our laboratory by weekly intraperitoneal transplantation into ICR–JCL mice[7]. A seven day-old tumor was inoculated in doses of 10^7 cells intraperitoneally into mice. For estimation of the total number of the tumor cells in ascites, the ascites was drained with a syringe and the peritoneal cavity was washed with 20–50 mℓ of phosphate buffered saline (PBS). The ascites and washings were pooled. The tumor cells were counted using a haemocytometer.

Chemicals: Tritium labeled thymidine with a specific activity of 12.0 mCi/mmol was purchased from Daiichi Pure Chemical Co. Ltd., Tokyo.

Sheep prolactin (LTH) was purchased from Sigma Chemical Co. Ltd. St. Louis, U.S.A. Mouse GH was purified from ICR–JCL mouse pituitaries according to the method of Raben[8]. The recovery of GH was about 3% of the pituitary acetone powder. The purity of the preparation was examined by the aid of polyacrylamide gel electrophoresis as described by Davis *et al.*[9] and the biological activity was tested in terms of the increase in body weight of hypophysectomized mice (Fig. 1 a and 1 b).

GH and LTH administrations: Daily injections of 6 μg of the purified GH and 5.0 I.U. of LTH per mouse were made subcutaneously from the 4th day after hypophysectomy, respectively. Tumor cells were inoculated on the second day after injection of GH or LTH. Cell counting was done on the 5th day after inoculation.

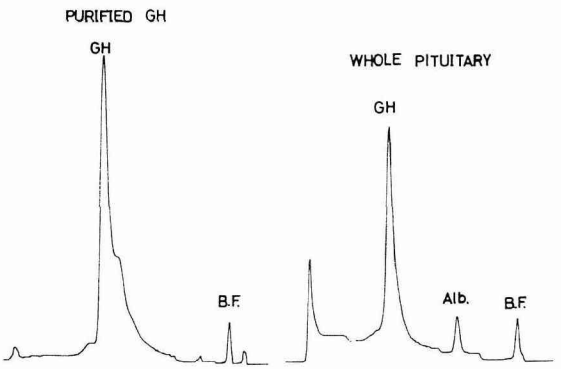

Fig. 1 a Densitometrical diagrams of a preparation of mouse GH on polyacrylamide gel electrophoresis (7.5%, pH 9.5). The purified GH shows a single peak and a shoulder of faster migrating substance. This faster migrating substance was reported to possess full biological activity.

Alb.: albumin and LTH, B.F.: buffer front

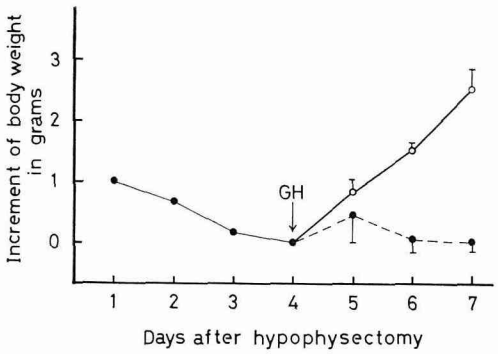

Fig. 1 b Effect of purified GH (6 μg/mouse/day) on increase in body weight of hypophysectomized mice. Mean values and standard deviations are plotted.
(○——○) GH injected (●- - -●) PBS injected

Dilution rate of pre-labeled radioactivity: Four i.p. injections of ^{3}H-thymidine (0.2 μCi/g body weight) separated by 6 hr intervals were given to mice bearing a 4 day-old tumor. This procedure was designed to label all the cells in the cycle including mitosis. Actually, the labeled cells were about 92% as determined by autoradiography. Labeled tumor cells were transplanted in doses of 10^{7} cells into mice. Five days after the transplantation of the labeled tumor cells, were harvested and washed with ice-cold trichloroacetic acid. The radioactivity of the acid-insoluble materials was determined in a liquid scintillation spectrometer using a toluene-triton scintillator.

Cytokinetic experiments: Cytokinetic time parameters were calculated from percent labeled mitoses curve (PLM)[11]. The PLM experiments were carried out on the 4th day after transplantation. After puls-labeling with ^{3}H-thymidine (20 μCi/mouse), animals were killed at 1 hr intervals up to a period of 33 hr. For autoradiography, smears were fixed in absolute ethanol and dipped in 2×diluted Sakura NR-M2 emulsion (Konishiroku Photo Ind., Tokyo) pre-warmed to 45°C. After one week exposure at room temperature these were developed and stained with Giemsa. The fraction of labeled mitoses was determined from 100 mitotic cells per slide, the fraction of labeled cells was determined in 1,000 cells per slide, and mitotic index obtained by counting 1,000 tumor cells.

Examinations were repeated twice or three times for each experiment, and 3 to 6 animals were used in a group in each examination. The statistical probability, P, of a significant was derived from Fisher's table of t values.

Results

Tumor growth: Growth curves for this tumor are shown in Fig. 2. The growth rate was exponential for the first few days and its decline from the exponential mode was notable from the 4th day. On the first day after tumor cell inoculation, the difference in the cell growth between hypophysectomized and intact mice was not significant, but on the 2nd day and thereafaer this difference became remarkable. On the 5th day,

the effectiveness of hypophysectomy was evident and the difference in the cell growth between the two groups was statistically significant. To assess whether the inhibition of the tumor growth in the hypophysectomized is caused by disappearance of the hormones from the target endocrine organs of hypophysis, the total tumor cell number in the thyroidectomized, adrenalectomized, and castrated mice was examined on the 5th day after transplantation. The results are summarized in Table 1. The influences of the extirpation of the target endocrine organs were not significant. Since specific target endocrine glands of GH and LTH have not been defined, these hormones were administered to the hypophysectomized animals with results as shown in Table 2. The total cell number in the GH-injected group was about by 85% greater than in the hypophysectomized animals, whereas the injected sheep LTH had no effect on recovery of the tumor growth inhibited by hypophysectomy.

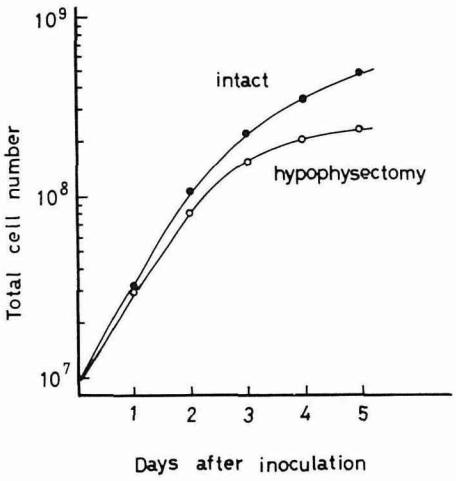

Fig. 2 Tumor growth in hypophysectomized and intact mice. Mean values are plotted.

Table 1 *Influences of the extirpation of the endocrine organs on the tumor growth*

Operation	Total tumor cell number ($\times 10^8$) on the 5th day
Intact	4.81 ± 0.08*
Hypophysectomy	2.33 ± 0.26 ($P<0.01$)
Thyroidectomy	4.65 ± 0.20 (n.s.)
Adrenalectomy	4.07 ± 0.28 (n.s.)
Castration	4.46 ± 0.27 (n.s.)

* Standard error of the mean.
n.s.: not significant

Table 2 *Effect of administration of GH or LTH on the tumor growth in the hypophysectomized mice.*

Treatment	Total tumor cell number ($\times 10^8$) on the 5th day
Hypophysectomy +PBS	2.33 ± 0.26*
+LTH (5 I.U./day)	2.30 ± 0.24 (n.s.)
+GH (6 μg/day)	4.32 ± 0.28 ($P<0.01$)

* Standard error of the mean.
n.s.: not significant

Table 3 *Effect of diet on the tumor growth in intact mice*

Dietary manipulations	Tumor growth on the 5th day
Free diet	100
Food restriction	99.4
High-calory diet*	97.9

In the Table are relative vlues normalized with free diet as 100%.
* Clea-B.F pellet diet.

Table 4 *Dilution of radioactivity of the ^3H-thymidine pre-labeled tumor cells*

Inoculum	On the 5th day	
	Hypophys-ectomy	intact
Exp. 1. 34942	1682 (21.1)	742 (47.2)
Exp. 2. 62155	2680 (23.2)	1310 (47.4)

The values are radioactivies (c.p.m./10^7 cells) of the tumor cells with ^3H-thymidine, and the values in parentheses are the dilution rates of radioactivities on the 5th day compared with the values at inoculation.

Since hypophysectomized animals were fed a lesser amount than the intact animals, and the body weight did not increase, the effect of the restriction of the food intake on the growth of the tumor was examined. As shown in Table 3, the food restriction resulting in a mere body weight maintainance did not affect the tumor cell growth.

Decrease of pre-labeled radioactivity: The results in Table 4 showed that the dilution rates of radioactivity were almost equal to that of multiplication of the tumor cells. The rates were about 22 in hypophysectomized animals and 47 in the intact animals on the 5th day.

Cell kinetics: Median cell kinetic parameters on the 4th day tumor computed from the PLM curves (Fig. 3) were given in Table 5, and the growth fraction was determined from the relationship by Mendelsohn

$$f = (Ns/N)/(Ns/Ng)$$

where Ns=number of DNA synthetic phase, N=total number in or out of a mitotic cycle and Ng=number in a mitotic cycle. In intact mice, the duration of cell cycle time (G) was 22 hr, of which 18 hr were spent in DNA synthetic phase (S) and 4 hr in G-2+mitosis. These periods of the parameters were consitent with those calculated by Lala and Patt[13] on 7 day-old Ehrlich ascites tumor cells inoculated in doses of 10^6 cells. In hypophysectomized mice, G was 27, S was 23, G-2+mitosis was estimated as

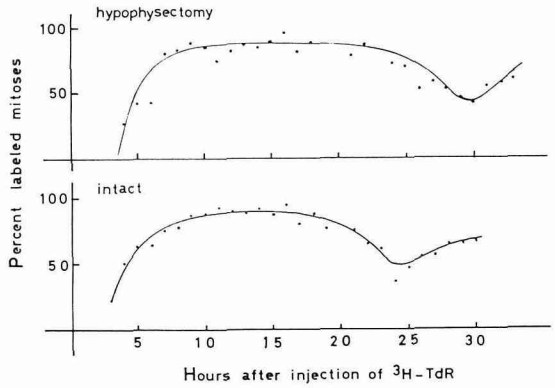

Fig. 3 Frequency of labeled mitoses at different times after ^{3}H-thymidine (TdR) injection into mice with the 4th day-old tumor.

Table 5 *Growth parameters of ascites tumors on the 4th day*

Parameters		Hypophysectomy	Intact
Median duration (hr) of:	Doubling time	145	57
	Cell cycle time	27	22
	DNA synthesis	23	18
	G-1	—	—
	G-2+mitosis	4	4
0.5 hr ^{3}H-thymidine labeling index (%)		57	61
Computed proliferating fraction (%)		67	74

4 hr. It was noted that prolongation of cell cycle time in the hypophysectomized depend on the expended *S* phase. The *G*–1 phase of cell cycle was either very small or absent.

Discussion

A number of studies have been made on the effects of pituitary hormones on the growth of tumors and the course of tumorigenesis[1~3,14]; however, only a few reports are available which are concerned with the tumor growth in hypophysectomized animals. A report by Talaley *et al.*[15] showed that hypophysectomy inhibited the growth of Walker tumor in rats to about a half of the tumor weight in control. Huggins and Oka[16] reported that hypophysectomy resulted in a profound regression of stem-cell erythroblastic leukemia in rats. Our results on Ehrlich ascites tumor in mice are consistent with these reports in that hypophysectomy inhibited the growth of the tumor. On the 5th day after the inoculation, the tumor cell number reached only about one-half in hypophysectomized mice as compared with that in intact animals. Thyroidectomy, adrenalectomy and castration of mice, in contrast to hypophysectomy, did not produce inhibitory effects on the tumor, in a similar manners to the results by Kodama[17]. It is thus appropriately suggested that the inhibitory effect of hypophysectomy is not ascribed to the ablation of target organs of hypophysis. Since it was further shown that administration of GH, but LTH, to hypophysectomized mice enhanced the tumor growth, GH is emphasized as a crucial endocrine factor that affects the growth of Ehrlich ascites tumor.

Since hypophysectomized animals were found to ingest food in less than a half of the normal amount, it was surmized that the effects of dietary manipulations may play a role in the tumor growth. In this respect, Watanabe and Oka[18] reported recently that the growth of Ehrlich solid tumor was not affected either by the feeding schedule or by the type of diet. Our results on Ehrlich ascites tumor were in good agreement with this report by Watanabe and Oka, in that food restriction did not suppress the tumor growth.

There are only a few reports on the influence of administration of GH on the growth of transplantable tumors in intact mice. These reports seem to be in contrast with each other; namely a promotion of tumor growth by Smith *et al.*[5], a nullification by Schulman *et al.*[4], and an inhibition by Smith *et al.*[6]. In these experiments, they used transplantable mammary adenocarcinoma and the injected GH was in strikingly large dose (100–400 times) as compared with our experiments. A complete separation of LTH activity from GH preparations was reported to by virtually impossible[19,20]. It is therefore suggested that the LTH effect on the tumor growth is superimposed on the GH effect, when a large dose of the GH preparation is used. Six micrograms, in our experiments, are considered as the minimal effective dose. Furthermore, hypophysectomy is a nessesary postulate for examining the effects of GH. In this connection the discrepancies among the assumptions mentioned above may also be based on the fact that non-hypophysectomized mice were used in these investigations.

Considerable information has been accumulated regarding the influences of GH on

various enzymes, proteins, RNA, immunocapacity, and lymphocyte transformation[21~26]. However, the mechanism of GH action on tumor growth has not been clarified, and there is no information available regarding the inhibitory mechanism on tumor growth in hypophysectomy. The retardation in tumor growth in hypophysectomized animals may have resulted from 1) the appearance or elongation of the lag-phase, 2) an increase in the rate of cell loss, 3) a decline in the proliferating fraction and 4) a prolongation of the mitotic cell cycle. In our studies, the inhibited growth of Ehrlich ascites tumor in hypophysectomized mice is considered to be mainly caused by the prolongation of cell cycle time, directly by lengthening of the DNA synthetic phase, but is probaly affected by the decrease in the growth fraction to a slight extent.

A previous cytokinetic analysis of Ehrlich tumor made in non-hypophysectomized mice by Lala[27] showed that a lengthening of cell cycle time due to a prolongation of the *S* phase was found to occur in addition to a decrease in the growth fraction in the later stages of the tumor development. The prolongation of the *S* phase was also emphasized by Dombernowsky *et al.*[28] as relevant to retardation of JB-1 ascites tumor in mice with the increase of the tumor age. The results in the present experiments on hypophysectomized mice are considered to be consistent with these previous reports.

Hypophysectomy may provide a microenvironment in ascites which is disadvantageous for the tumor cell growth such as in ascites in the later stages of tumor development in the non-operated animals.

Another possible interpretation on the action mechanism of the tumor inhibiting effect of hypophysectomy may be that GH stimulates the DNA synthesis or mitosis, through specific RNA and protein, as was also suggested by Baserga[29]. We have in program further experiments to investigate whether GH potentiates the cell cycle stimulating effects on Ehrlich tumor cells, in order to contribute to the answer of the above-mentioned question.

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