KAWASAKI MED J. VOL. 11 : NO. 3, 135-141 (1985)

The Effects of Intravenous Feeding on Tumor Growth —An Autoradiographic Analysis—

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Accepted for Publication on June 13, 1985

ABSTRACT. Effects of parenteral nutrition on tumor growth were examined by using autoradiographic procedures. Growth rate of the Sato's lung cancer inplanted subcutaneously in the back of the rats of malnourished group was significantly lower (2.68 ± 0.82, $p < 0.001$) than that of intravenously alimented group and orally fed group (5.19 ± 1.50, 5.72 ± 1.69, respectively). Both mitotic index and labeling index of the tumors from malnourished rats were significantly lower compared with orally fed rats, and this tendency was remarkable in the central region of tumor. Labeling index of parenterally fed group was significantly higher than that of orally fed group. Even in the central region of tumor in this group, labeling intensity was not decreased compared with subcapsular region. Percentage of labeled mitoses of the tumors from the intravenously fed animals was lower than orally fed one's.

Based on these findings, it is anticipated that parenteral nutrition may increase or maintain the tumor cells being mobilized in the tumor proliferation phase. This point seems to be useful for the anti-tumor chemotherapy.

Key words : Parenteral nutrition — Tumor growth — Cell kinetics — Autoradiography

There are many classical reports concerned with nutrition in relation to cancer. However, oral feeding was used in these studies, and, thus, precise control of the quality and quantity of the diet was difficult because of the influence of the digestive tract.

Intravenous feeding of rats, which was established by Steiger and his colleagues in 1972, has allowed for the precise control of nutrient intake and the accurate assessment of the effects of nutrition on tumor growth. Despite increasing interest, the role of nutritional support in the tumorhost relationship remains poorly defined. The present experiment examines the effects of intravenous feeding on the growth characteristics of a transplantable rat cancer, using autoradiographic analysis.

MATERIALS AND METHODS

Thirty-one young Donryu rats weighing about 120 g were inoculated

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subcutaneously in the back with about 2 mm^3 of tissue taken from the cortex of one parent tumor of Sato's Lung Cancer. The transplants were uniformly palpable 7 days after the inoculation.

After one additional week of observation for evaluation of tumor growth, the animals were divided into three groups : A) the intravenously hyperalimented group of 10 rats which received a solution of 20% dextrose and 4.0% amino acids, B) the orally fed group of 13 rats which were supplied with regular rats chow ad libitum and C) the control group of 8 rats which received an iv solution of 5% dextrose without nitrogen.

All animals of Groups A and C were anesthetized with Ketamine by intraperitoneal administration, and then underwent surgical placement of a silastic catheter into the superior vena cava via the right jugular vein. A special harness and fluid infusion swivel apparatus allowed for unrestricted activity of the rats in their individual metabolic cages.

All intravenous solutions contained supplemental electrolytes (Table 1) to avoid hyperglycemia, and were administered by means of a Holter model 903 Infusion Pump (Extracorporeal Medical Specialities, King of Prussia, PA). Daily urinary glucose determinations were carried out with testing paper, and if an animal continued to have glycosuria for prolonged periods, it was eliminated from the study.

TABLE 1. Composition of intravenous solutions

	IVH	C
Glucose	200.0 g	50.0 g
Amino acids	40.0 g	—
Na	66.7	66.7
K	25.0	25.0
Cl	66.7	66.7
Mg	6.7	6.7
Ca	13.3 mEq/L	13.3 mEq/L

The tumor was measured three dimensionally by calipers at the beginning of infusion and the end of the experiment (1 week after cannulation), and the growth rate was calculated. A skin-thickness correction was applied to the original measurement.

Before they were killed, each animal was injected intraperitoneally with one μci/g body weight of tritiated methylthymidine (^{3}H-TdR, Japan R.I. Society, Tokyo). Two hours after the injection, the rats were sacrificed by ether asphyxiation. The tumors were carefully excised, dissected from surrounding tissues and fixed with 10% formaldehyde solution. Four micron histologic mid-cross sections of the tumors were made, and the mitotic index, labeling index and percentage of labeled mitoses were determined.

The mitotic index (M.I.) was measured in more than 2000 tumor cells from 10 random areas of the subcapsular (SC) and middle (M) regions of the tumors. The SC versus M ratio for each specimen was also calculated. A modified Matsuzawa's autoradiographic technique was used to determine the percentage of tumor cells in the synthetic phase. After 12 weeks of exposure

at 4°C in a light-tight box, the slides were developed in Konidol X (Sakura Co., Japan), fixed and then stained with hematoxylin. Since the background grain count was less than 0.1 grain per cell, a nucleus was judged positive if it had five or more grains about it. The labeling index (L.I.) of the subcapsular and middle regions of tumors, and the SC vs M ratio, were determined similarly. The percentage of labeled mitoses 2 hours after injection of ^{3}H-thymidine was determined by examination of 100 or more mitoses, dividing the tumors into two regions as described above. The same grain count criterion was used as above.

Throughout the study, Student's t-test was used to test statistical differences between means.

RESULTS

The mean body weights of the three groups of rats at the beginning of the experiment were nearly identical: 173.2 g (Group A), 171.4 g (Group B) and 179.0 g (Group C). Tumors were not significantly different in size at the beginning of experiment. The rats given the regular diet gained 26.8±8.4 g over 7 days, while the rats which received an iv solution of 5% dextrose lost 59.4 ± 10.9 g in the same period, and hyperalimented rats lost a little weight (−17.2 ± 11.2) (Table 2).

TABLE 2. Effects of nutritional manipulations on tumor growth and weight change

	IVH Group	PO Group	C Group
Weight Change	−9.9±14.6	+29.9±10.4	−49.6±14.0 g/week
Caloric Intake	236.1±37.3	265.7±31.5	40.9±10.0 Cal/kg·day
Cell Count	61.8± 3.2	70.0± 7.9	75.0± 4.0
Growth Rate	5.19±1.50	5.72±1.69	2.68±0.89
Tumor/Carcass	2.02±1.05	2.21±1.00	2.36±0.89 %

Urinary glucose determinations were slightly positive on the first day in hyperalimented rats, but negative from the 2nd to 7th day, which suggests that rats adapted to the infusion of large doses of glucose.

Table 3 shows the growth rate of tumors in the three groups. There was

TABLE 3. Effects of nutritional manipulation on tumor growth and weight change in one-week experiments

	Group A	Group B	Group C
Growth rate	5.19±1.50	5.72±1.69	2.68±0.82*
Weight change	−17.2±11.2 g	+26.8±8.4 g	−59.4±10.9 g
Caloric intake	276.6±27.2		57.2±13.2

*P <0.001

significantly lower tumor growth in the malnourished group which received 5% dextrose (2.68 ± 0.82, $p<0.001$) compared to the other two groups, but not significant difference in the growth rate between the rats of Groups A and

B (5.19 ± 1.50 vs 5.72 ± 1.69).

In the subcapsular region, the mitotic index of tumors from the parenterally fed rats, was significantly higher than that from the rats infused with 5% dextrose (1.66 ± 0.22 vs 0.77 ± 0.21), but nearly indentical to that from orally fed rats (1.63 ± 0.32). In the middle region of tumors, the mitotic index remained the same in Groups A and B (1.60 ± 0.21 and 1.64± 0.41, respectively), but was significantly lower in the control group (0.64 ± 0.18, $p<0.001$) (Table 4).

TABLE 4. Effect of nutritional manipulation on the mitotic index

	Group A	Group B	Group C
SC	1.66 ± 0.22	1.63 ± 0.32	0.77 ± 0.21*
M	1.60 ± 0.21	1.62 ± 0.41	0.64 ± 0.18*
SC/M	1.06 ± 0.21	1.03 ± 0.17	1.30 ± 0.44**

*P <0.001 relative to the other two groups
**P <0.1 relative to Group A

Analysis of the SC versus M ratio revealed that there was a significantly high ratio in the tumors from the rats which received 5% dextrose, which means that there is less mitotic activity in the middle region of the tumors than the subcapsular region in malnourished hosts.

The labeling index of the subcapsular region of the tumors from parenterally alimented rats was slightly, but significantly higher than that from orally fed rats (26.1±2.37 vs 23.8±2.86, $p<0.1$). Tumors from control rats infused with 5% dextrose, showed a remarkably suppressed mean labeling index (16.8 ± 3.59, $p<0.001$). In the determination of the labeling index of the middle region of the tumors, the same differences were observed in the three groups (Table 5). The SC vs M ratio of the labeling index showed the same tendency

TABLE 5. Effect of nutritional manipulation on labeling index

	Group A	Group B	Group C
SC	26.1 ± 2.37*	23.8 ± 2.86**	16.8 ± 3.57
M	22.5 ± 3.13*	19.6 ± 3.67**	12.8 ± 2.83
SC/M	1.18 ± 0.12***	1.23 ± 0.17	1.32 ± 0.15

*P <0.01 relative to Group B
**P <0.001 relative to Group C
***P <0.05 relative to Group C

as shown in the analysis of the mitotic index. There was no significant difference in the appearance of grains between the tumors from orally and parenterally fed rats, both in the subcapsular and middle regions of tumors.

The percentage of labeled mitoses of the tumors from rats of the hyperalimented group was significantly lower than that of the orally fed group, both in the subcapsular and middle regions of tumors. In the control group, there was no labeled mitosis two hours after the injection of radioisotopes.

DISCUSSION

It is well established that spontaneous tumor growth occurs more readily in well-nourished than in malnourished animals.[1-4] Recently, interest has focussed on nutritional support as an adjunct treatment for malignancy. Pareira and his colleagues have stressed the possibility of temporary significant reversal of cancer cachexia in terminal patients by tube feeding. Schwartz and his coworker, and Copeland and his associates have already combined parenteral iv feeding and chemotherapy for selected groups of cancer patients.

In spite of their good results, other investigators suggest that intravenous nutritional support might stimulate the growth of malignancy. For example, Steiger and his colleagues, using a mammary carcinoma, reported an increased growth rate, as measured by changes in tumor volume in animals that were parenterally nourished with nitrogen-containing fluids in contrast to those given 5% dextrose.

The present study offers no conclusive evidence from analysis of the tumor growth rate that intravenous feeding stimulates more rapid tumor growth in the host, since tumor growth in rats which received intravenous hyperalimentation might have been influenced by the calorie and/or nitrogen intake. Goodgame *et al.*, who employed an iv infusion of 25% dextrose and 4.25% amino acids on methylcholanthrene induced rat sarcoma, reported a significantly higher growth rate than in rats fed orally. Hasegawa *et al.* investigated the effect of a one-week iv infusion of 21% dextrose and 4% amino acids on the same tumor as in the present study, and reported a slightly higher growth rate (5.72) than in the present study. Cameron and Pavlat demonstrated a significant increase in tumor weight in intravenously hyperalimented rats during the fourth and fifth experimental weeks, despite the fact that the caloric intake of the orally fed rats decreased 127.0 and 109.0 calories as compared to 149.8 and 184.8 calories in hyperalimented rats during the fourth and fifth weeks, respectively. The estimated tumor weight in the 2nd week of total parenteral nutrition increased 81% compared to 116% in orally fed controls. Previous experience summarized in Table 2, shows the relationship between growth rate and total caloric (nitrogen) intake in a one-week infusion of 20% dextrose and 4.0% amino acids.

From these investigations, it might be considered that when caloric and/or nitrogen intake are increased by intravenous hyperalimentation, tumor growth proceeds more quickly.

In the present study, there was no significant difference in the mitotic index of tumors between intravenously and orally fed rats, although there was a singnificant decrease in the mitotic index of tumors from malnourished rats which received 5% dextrose. Cameron has reported that the mitotic index of tumors from rats parenterally fed for 2 weeks was significantly higher than that of tumors from orally fed rats. However, the difference was not true, because his measurements were carried out on the tumors from rats whose caloric intake had already decreased, as previously mentioned. Mitotic activity of tumros perhaps is stimulated or maintained when nutritional support by intravenous feeding is at high or normal levels.

Estimation of the growth characteristics of tumors by determining the incorporation of ^{3}H-TdR is not an adequate procedure, because there may be

an undefined endogenously synthetized precursor pool in the host which could compete with exogenously administered ^{3}H-TdR for incorporation into DNA. Autoradiographic analysis may be the most reliable method to determine the changes in cell proliferation of tumors due to nutritional changes in the host.

It is well known that cells rest in the G_1 period of the cell cycle in most instances. But Basegra and Wiebel, using an autoradiographic anaylsis for Ehrlich ascites tumor cells, have reported a markedly prolonged G_2 period after 48 hours of fasting, and even 12 hours fasting produced a delay in G_2. In the present experiment, the percentage of labeled mitoses of tumors in hyperalimented hosts, which correlates to the G_2 period was lower than that in orally fed hosts. A low percentage of labeled mitoses and weight loss of intravenously hyperalimented rats might be due to the intravenous hyperalimentation being relatively under-caloric compared to normal feeding.

In spite of these findings, tumors from intravenously alimented rats showed higher labeling intensity both in the subcapsular and middle regions of tumors compared with those of orally fed rats, which suggests that intravenous hyperalimentation might have stimulated tumor growth. If this presumption is true, cell cycle specific chemotherapeutic agents might be more effective when combined with intravenous hyperalimentation.

SUMMARY

The effects of intravenous hyperalimentation on tumor growth were examined using autoradiography. There was no significant difference in the growth rate and mitotic index between the tumors from the rats which received 20% dextrose and 4.0% amino acids iv and those which were fed orally. In spite of a significantly lower percentage of labeled mitoses, there was a significantly higher labeling index of tumors from intravenously fed rats both in the subcapsular and middle regions of tumors, compared with orally fed rats. Intravenous feeding probably stimulates tumor growth by increasing the growth fraction of the tumor. Therefore, it may be advantageous to combine parenteral nutrition with other treatments of malignancy.

Acknowledgment

I should like to thank the Assistant, Mrs. Y. Nagahata for kind support to this experiment. I also appreciate Dr. A. Ueki and Professor K. Sano for their continuous help and advice.

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