ANTI-TUMOR ACTIVITY OF 1-ACETYL-3-*o*-TOLUYL-5-FLUOROURACIL AGAINST MURINE HEPATOMA MH134 AND ITS EFFECTS ON TISSUE WEIGHTS FOLLOWING SUBCUTANEOUS AND ORAL ADMINISTRATION

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Anti-tumor activity of 1-acetyl-3-*o*-toluyl-5-fluorouracil (A-OT) against MH134 solid tumors in mice was studied following subcutaneous and oral administration and compared with that of 5-fluorouracil (5-FU) administered subcutaneously at doses of 0.2, 0.4 and 0.6 mmol/kg/d. Oral administration of A-OT demonstrated a remarkable effect on MH134 solid tumors, the effect being more marked than that of subcutaneous administration of A-OT.

Anti-tumor activity of oral administration of A-OT at a dose of 0.2 mmol/kg/d was comparable to that of subcutaneous administration of 5-FU at the same dose.

The level of decrease in thymus weight and the magnitude of increase of spleen weight following oral administration of A-OT at any dose were smaller than those by subcutaneous administration of 5-FU (0.2 mmol/kg/d).

Keywords — 1-acetyl-3-*o*-toluyl-5-fluorouracil; 5-fluorouracil derivative; anti-tumor activity; murine hepatoma MH134; oral administration; subcutaneous administration

INTRODUCTION

5-Fluorouracil (5-FU) which was synthesized in 1957 by Heidelberger *et al.*¹⁾ has been used as an effective compound for the clinical treatment of human solid tumors including lung cancer, breast cancer, and cancers of the digestive organs. A large number of 5-FU derivatives have been synthesized to date, but there is none which is superior to 5-FU.²⁾

The authors have also synthesized various 5-FU derivatives^{3,4)} and 1-acetyl-3-*o*-toluyl-5-fluorouracil (A-OT) has been confirmed to possess effective anti-tumor activity against Ehrlich ascites cells, thyroid cancer cells, Hela cells and leiomyosarcoma cells *in vitro*⁵⁾ and also has been confirmed to increase the life span of mice bearing Ehrlich cancer cells,^{5,6)} L-1210 leukemia,⁶⁾ P-388 Leukemia⁶⁾ and MH134 hepatoma.⁶⁾

The present study has shown that the oral administration of A-OT has a greater anti-tumor

activity than by its subcutaneous administration and it has an effect comparable to the subcutaneous administration of 5-FU.

MATERIALS AND METHODS

Animals — Eight-week-old C₃H strain female mice (body weight ranging from 18.5 to 23.8 g) were employed.

Tumor Cells — Murine hepatoma MH134 provided initially by the Department of Microbiology, Kitasato University School of Medicine were used. The hepatoma has been maintained by serial inoculation in C₃H mice.

Anti-tumor Agents — 5-FU and A-OT provided by Taiho Pharmaceutical Co. Ltd. were employed. Chemical structure of A-OT is shown in Chart 1.

Experimental Procedures — Preparation of Tumors: MH134 tumor cells suspended (5×10^7 cells/ml) in physiological saline solution were

inoculated subcutaneously on the left dorsal scapular region of 100 C₃H strain female mice at a rate of 5×10^6 cells per animal. Mice inoculated with tumor cells were randomly divided into 10 groups each with 10 mice. Moreover, 10 mice bearing no tumor were used as non-tumor bearing control.

Of the 10 groups of tumor bearing mice, subcutaneous administration of 5-FU or A-OT was made at a dose of 0.2, 0.4 and 0.6 mmol/kg/d on six groups in the buttock region. In three groups, oil solution of A-OT was administered orally with a stomach tube at a dose of 0.2, 0.4 and 0.6 mmol/kg/d. The remaining one group was used as tumor bearing control.

Administration of Drug: 5-FU was administered only subcutaneously. A-OT was administered both subcutaneously and orally. i) Subcutaneous Administration: An amount corresponding to a dose of 0.2, 0.4 and 0.6 mmol/kg of 5-FU and A-OT, respectively, was dissolved in 0.05 ml dimethylsulfoxide (DMSO, Wako Chemicals) immediately prior to administration. A volume of 0.05 ml of the respective solutions was subcutaneously administered in the buttock region. ii) Oral Administration: A mixture of 17 ml of triglyceride of medium chain fatty acid (ODO®, Nissei Seiyu Company) and 3 ml of triacetin (Tokyo Chemical Ind. Co. Ltd., purity more than 99%) was heated 110–120 °C and thereafter A-OT was dissolved herein so that the amount of A-OT per 0.05 ml of the mixture would be 0.2, 0.4 and 0.6 mmol/kg. The solution was apportioned into ampules at 1 ml and kept at room temperature until the experiment. The stability of A-OT in this oil solution has been confirmed.⁷⁾

CHART 1. Chemical Structure of A-OT

The administration of the agents both subcutaneously and orally was commenced on the fourth day after tumor transplantation and continued for 15 d at one administration per day.

Evaluation of Anti-tumor Activity and Effect on Other Tissues: During the period of the experiment body weight was measured every 3 d and the long and short diameters of the tumor were measured daily. The superficial general condition was observed. Forty eight hours after the final drug administration, all the cases were subjected to autopsy and the weight of the tumor, liver, kidney, adrenal gland, spleen, thymus and lung were measured. Furthermore, specimens were prepared of various tissues according to routine procedure and following

FIG. 1. The Chronological Changes of Tumor Size after Administration of the Agents at a Dose of 0.2 mmol/kg/d

- , tumor bearing control,
- △, 5-FU, subcutaneous administration,
- , A-OT, subcutaneous administration,
- , A-OT, oral administration.

Tumor size was tentatively shown as a value obtained by multiplying the long diameter with the short diameter.

hematoxylin–eosin staining and microscopically examined.

RESULTS AND DISCUSSION

As the number of deaths due to the toxicity of 5-FU in the groups administered 0.4 and 0.6 mmol/kg/d was high, only the data of the group administered 5-FU at a dose of 0.2 mmol/kg/d are shown in the Table I and Fig. 1. In the group administered A-OT both subcutaneously and orally at the dose used in the experiment, no case of death was observed. The chronological changes of tumor size after administration of the agents at a dose of 0.2 mmol/kg/d as an example are shown in Fig. 1. To estimate the tumor size *in vivo* the following formula has been proposed:⁸⁾

$$\text{tumor size} = \frac{\text{long diameter} \times (\text{short diameter})^2}{2}$$

However, in the present study, the tumor size was tentatively shown as a value obtained by multiplying the long diameter with the short diameter because of the flat shape of the tumors. Suppression of the increase in the tumor size was observed in the groups administered 5-FU subcutaneously and A-OT orally. These findings are supported by the results shown in the Table I.

Shown in the Table I are the weights of the various tissues measured 48 h after the final drug administration. Through the administration of 5-FU at a dose of 0.2 mmol/kg/d, the tumor weight showed a 48.5% decrease in comparison with that of the tumor bearing control group ($p < 0.01$). In the groups subcutaneously administered A-OT at doses of 0.4 and 0.6 mmol/kg/d, a decrease in tumor weight of 48.1% ($p < 0.01$) and 71.9% ($p < 0.01$) was observed, respectively, while in the group administered A-OT at a dose

TABLE I. *Weight of Tissues 48 h after the Final Administration of Drugs and Initial and Final Body Weight*

	Control		5-FU, s.c.		A-OT, s.c. (dose: mmol/kg/d)		A-OT, p.o.		
	Non-tumor bearing	Tumor bearing	0.2	0.2	0.4	0.6	0.2	0.4	0.6
Initial body wt. (g)	21.22	21.92	22.58	21.71	21.92	21.48	21.49	21.82	21.15
S.D.	1.50	1.20	1.64	0.81	1.17	0.96	1.27	1.03	1.13
Terminal body wt. (g)	23.31	24.51	24.14	24.09	24.01	22.40	23.84	24.12	23.50
S.D.	1.62	1.48	1.96	1.37	1.25	1.59	1.39	0.99	0.96
Tumor (mg)	—	811	418	925	421	228	422	289	251
S.D.		236	161	405	137	77	161	109	103
Liver (g)	1.300	1.503	1.523	1.568	1.577	1.539	1.465	1.589	1.532
S.D.	.141	.167	.174	.139	.143	.162	.080	.113	.094
Kidney (mg)	178	185	193	182	177	165	170	172	158
S.D.	17	17	17	17	15	16	13	11	17
Adrenal Gland (mg)	63	61	49	62	55	48	68	75	64
S.D.	12	20	17	18	18	15	15	20	15
Spleen (mg)	84	135	208	231	239	202	149	158	167
S.D.	12	11	21	45	24	68	27	22	23
Thymus (mg)	305	360	126	239	174	66	311	288	262
S.D.	68	64	58	42	51	28	27	62	66
Lung (mg)	171	182	180	183	180	176	173	171	165
S.D.	13	14	27	15	16	19	16	7	15

of 0.2 mmol/kg/d, no decrease in tumor weight could be observed. These findings indicate that anti-tumor activity of A-OT administered subcutaneously against MH134 murine hepatoma is about one half that of 5-FU. However, inasmuch as there were no deaths in the group given subcutaneous administration of A-OT at any dose, it is suggested that the side effects are considerably smaller in comparison with 5-FU. In comparison with the non-tumor bearing control group, the weights of spleens or thymus in the group administered the agents showed an increase or a decrease of varying levels, respectively, while tumor bearing control groups showed a slight in-

crease in spleen weight and did not show significant changes in thymus weight. These findings suggest that the increase in spleen weight and the decrease of thymus weight were proportional to the amounts of the agents given. The increase in spleen weight and the decrease of thymus weight may serve as an index of the side effects, but we consider that further studies should be done in relation to changes in peripheral and bone marrow white cells. Unlike the case of subcutaneous administration, oral administration of A-OT showed an activity equivalent to 5-FU at a dose of 0.2 mmol/kg/d. At that dose, spleen weight did not show any significant difference

FIG. 2 (a, b). Thymus from the Group of Tumor Bearing Control Mice

FIG. 3 (a, b). Thymus from the Group Administered 5-FU Subcutaneously at a Dose of 0.2 mmol/kg/d

from that of the tumor bearing control group. The decrease in thymus weight was 13.8%. This rate of decrease is smaller than that of the group given subcutaneous administration of 5-FU at the same dose.

The decrease rate of tumor weight in the group given oral administration of A-OT was greater than that of the group given subcutaneous administration at a dose of 0.2 and 0.4 mmol/kg/d and at all dose levels oral administration proved to be effective. On the other hand, the decrease rate of thymus weight in the group given oral administration of A-OT was remarkably smaller than that of the groups given subcutaneous administration. This is also identical for

the increase rate of spleen weight. These findings suggest that oral administration of A-OT in comparison with subcutaneous administration has a strong anti-tumor activity against MH134 murine hepatoma and smaller effects on other tissue weights at the same dose level.

Forty eight hours after the completion of the experiment, all the cases were subjected to autopsy and after evaluating macroscopically the presence of superficial changes, tissue specimens were prepared for microscopic examination. Except for the thymus, hardly any histological difference could be observed in the tissues between the groups administered the agents and the non-tumor bearing control or tumor bearing control groups. Corresponding to the level of weight decrease of thymus, changes in the histological pictures were observed. Some examples of the histological pictures of the thymus of the groups administered the drugs and the tumor bearing control group are shown in Fig. 2–4. In the group given subcutaneous administration of 5-FU at a dose of 0.2 mmol/kg/d, a remarkable decrease in thymus weight was observed. The thymus was markedly atrophic with serious atrophy of the cortex and the structure of the tissue was also poorly defined with a marked decrease in small lymphocytes (Fig. 3). On the other hand, in the group administered A-OT orally, decrease in thymus weight was small and histologically, at all dose levels, there was no large difference from the tumor bearing control group (Fig. 2–4).

(a)

50 ×

(b)

200 ×

FIG. 4 (a, b). Thymus from the Group Administered A-OT Orally at a Dose of 0.2 mmol/kg/d

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