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A STUDY ON DIRECT ANTITUMOR ACTIVITY OF BROPIRIMINE (ORAL INTERFERON INDUCER) FOR RENAL CELL CARCINOMA

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Aryl pyrimidinones, including bropirimine, exert anti-tumor activity through the induction of interferon (IFN)-alpha. Herein, the direct anti-tumor effect of bropirimine on 17 renal cell carcinoma (RCC) surgically obtained was examined using an organ culture system closely resembling the in vivo state, and also using the heterotransplanted nude mouse system. The findings obtained using the organ culture system showed that bropirimine inhibited ^{3}H-thymidine uptake significantly in 15 of the 17 RCC (88.2%) compared with the control. Furthermore, the ^{3}H-thymidine uptake was dose-dependently inhibited in 3 of the 17 tumors (17.6%). The oral administration of bropirimine against RCC heterotransplanted in nude mice (JRC 901: an erythropoietin-producing strain) tended to inhibit tumor growth dose-dependently, but not significantly (mean tumor weight ratio: T/C ratio: over 43%, degeneration degree of tumor: incomplete). However, the production of erythropoietin from the JRC 901 was significantly inhibited. These findings suggest that bropirimine has a direct anti-tumor activity, without the mediation of IFN-alpha induction, against human renal cell carcinoma.

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Key words: Bropirimine, Direct action, Renal cell carcinoma

INTRODUCTION

Bropirimine (U-54, 461S) is a derivative of aryl pyrimidinones developed by the Upjohn Company of U.S.A. It produces cytokines including interferon-alpha (IFN-alpha) after administration in animals or humans[1] [2]. In Japan, bropirimine is currently being tested as a new drug which provides anti-tumor activity through the induction of IFN-alpha in phase II study for patients with renal cell carcinoma (RCC) and with bladder cancer. In this study, we examined the direct anti-tumor activity of bropirimine using renal cell carcinoma obtained from surgery.

MATERIALS AND METHODS

1) Materials

We examined 17 RCC tumors derived from operations at Jikei University Hospital between January and September 1992. All patients received nephrectomy except for one who received resection of metasta-

sized tumor in the retroperitoneal cavity. Over 70% of the patients had low stage and low grade tumors.

2) Methods

a) Organ culture system: Cultures were conducted according to the method of Hoffman et al.[3]. After placing the sliced collagen gel matrix (2×1 cm square: spongostan) in a 6-well plate, we added 4 ml of Dulbecco's modified eagle medium (MEM) containing 20% faetal calf serum (FCS) and allowed it to stand for 6 hours. The tumor was cut into 2×2 mm pieces, and placed on the spongostan. Each experimental group consisted of 14 tumors. After 7 days of incubation with CO_2 at 37°C, the anti-tumor efficacy was evaluated by comparison of the ^{3}H-thymidine uptake between treated tumors and the control.

Bropirimine was used at two doses, i.e., 24.58 and 49.16 μg/ml based upon the peak blood level of bropirimine determined in the phase I trial (Cmax.: 51.94 ± 17.5 μg/

ml)[2]. In the control group, physiological saline was added to the medium.

b) Nude mouse system: To establish the RCC tumor heterotransplanted in nude mice, we inoculated the 17 tumors which had been tested in the in-vivo-like culture system to nude mice. One of the 17 tumors proved serially heterotransplantable in nude mice (JRC 901) (Fig. 1a). Furthermore, the JRC 901 tumor not only showed positive staining with the anti-Epo antibody (Fig. 1b), but also produced erythropoietin (Epo) at a high level after the inoculation of the tumor.
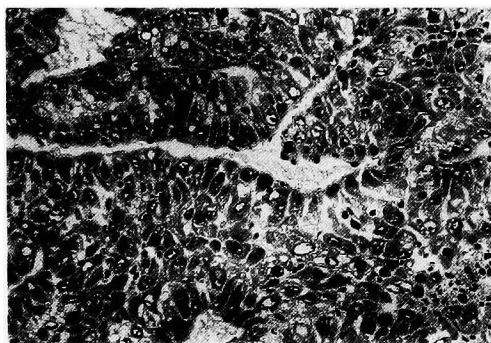

Fig. 1a. Histological feature of JRC 901 (H & E staining, ×100) JRC 901 tumor consisted of granular cell type, alveolar pattern and grade II renal cell carcinoma.

Fig. 1b. Immunohistochemical staining feature of anti-Epo antibody (×100). Positive reaction was observed within the cytoplasms of all JRC 901 tumors.

The JRC 901 tumor was inoculated subcutaneously into both lateral abdomens of the mice, and after the tumor had grown to the logarithmic phase (5 weeks after inoculation), bropirimine was administered orally. Two doses were tested (500 mg/kg and 1,000 mg/kg) based upon the basic data on blood drug levels at which IFN-alpha could be induced[4]. Bropirimine was dissolved in 0.5% sodium carboxylmethyl cellulose (CMC), the solution was adjusted to yield a prescribed drug concentration (500mg/kg or 1,000 mg/kg), and the volume of the single dose was 0.15 ml/body (25 g). Bropirimine was administered every 4 days orally for a total of 8 times. In the control mice, 0.15 ml physiological saline was orally administered similarly.

The mice used in this study were male BALB/C nu (+)/nu (+), mice, 6–8 weeks of age (Clea Japan). Five mice were used in the treated group (control group consisted of 8 mice), and maintained in a specific-pathogen-free (SPF) environment in the Laboratory Animal Center of our university.

c) Evaluation of efficacy: Anti-tumor activity was assessed according to the NCI's protocol (Battle Columbus Laboratory protocol[5]). After the inoculation of the tumor, its short (S) and long (L) diameters were measured and from the volume (V mg) = $L \times S^2/2$ according to the spheroidal theory, the relative mean tumor weight ratio (T/C ratio) was determined as follows.

The equation for the calculation of T/C

$$\text{ratio} = \frac{Tn/T0}{Cn/C0} \times 100 \ (\%)$$

Tn: tumor weight in the therapeutic group on day n,

T0: tumor weight in the therapeutic group on day 0,

Cn: tumor weight in the control group on day n,

C0: tumor weight in the control group on day 0,

When the calculated T/C ratio for any group was 42% or lower, the response was defined as positive; when it was 43% or higher, the treatment was judged to be ineffective.

Simultaneously, a histological evaluation was made according to the classification of Shimosato et al. (grade I: no degeneration

grade IIa: partial degeneration; under 50 %, grade IIb: partial degeneration; over 50%, but viable cells still remaining, grade III: no viable cells are detected, grade IV: no viable cells are detected with the replacement of fibroblasts or other reactive histological changes after all tumor cells were killed[6]). Degeneration equal to or higher than grade III, wherein no viable tumor cells are observed, was considered an indication of histological effectiveness. Furthermore, since the JRC 901 tumor produced Epo, especially 5 weeks after inoculation, the inhibition of production of Epo was also examined to evaluate the efficacy of bropirimine.

d) Analysis of Epo level: Serum specimens were collected from the tumor-bearing mice every week after the tumor were inculated in the two treated groups (all mice) and the control group (5 of 8 mice), and stored at $-80°C$. At analysis, the serum specimens were thawed and 50 mcl sera were used in duplicate for the radio-immunoassay conducted by SRL Company Ltd., Japan. Robson's[7] staging system of

RCC and Bannayan's four-step[8] grading system were used. Student's t-test was used to determine the significance of differences among the tested groups.

RESULTS

1) Organ culture system

^{3}H-thymidine uptake was significantly inhibited in 15 of the 17 RCC tumors (88.2 %) after the treatment with bropirimine at either high or low dose or both compared with the control $(0.01 < P < 0.05)$. In 3 of these 17 tumors (17.6%), the inhibition of ^{3}H-thymidine was dose-dependent, but no dose-dependency was observed in 9 tumors (52.9%). One tumor responded to the high dose (5.9%), two (11.8%) to the low dose and another two (11.8%) to neither of the doses.

2) Effects using JRC 901 strain

Of the 3 tumors in which ^{3}H-thymidine uptake was inhibited by bropirimine in the organ culture system dose-dependently (Fig. 2), 1 tumor was serially heterotransplantable in nude mice (JRC 901) (Fig. 1a). We examined the anti-tumor activity
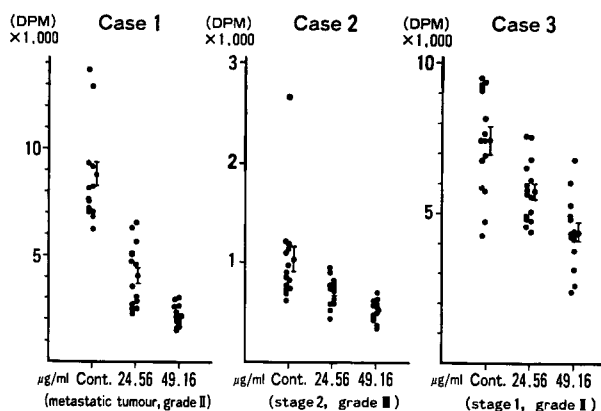

Fig. 2. Three tumors where ^{3}H-thymidine uptake was significantly suppressed dose-dependently by treatment with Bropirimine. Case 1 represents a tumor which was heterotransplantable in nude mice (JRC 901). (Case 1: 24.56 μg/dl vs control, 49.16 μg/dl vs control, 24.56 μg/dl vs 49.16 μg/dl: 0<P<0.02, Case 2: 24.15 μg/dl vs control; 0.02<P 0.05, 49.16 μg/dl vs control; o<0.002, 24.56μg/dl vs 49.16 μg/dl; o<P 0.02, Case 3: 24. 58 μg/dl vs control; 0.002<P<0.02, 49.16 μg/dl vs control; 0<P<0.002, 24.58 μg/dl vs 49.16 μg/dl; 0.002<P<0.02). (DPM: Disintegration per minutes)
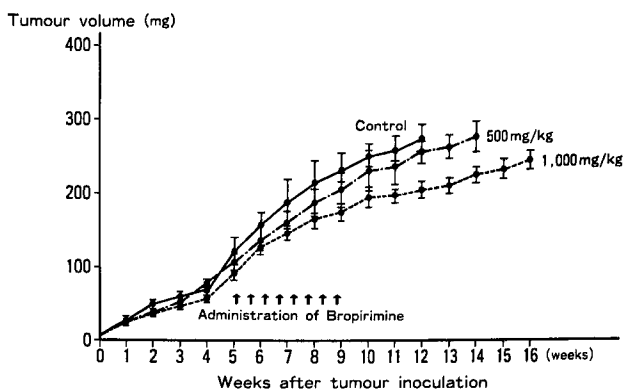
Tumour volume (mg)

Fig. 3. Anti-tumor efficacy of Bropirimine against JRC
901 strain heterotransplanted to nude mice.
Dose-dependent suppression of tumor growth
was noted following oral administration of bropi-
rimine. However, the rate of T/C was not signif-
icant because of its rate was over 42% (500
ng/kg: 91.8%, 1,000 mg/kg: 67.7%). (each growth
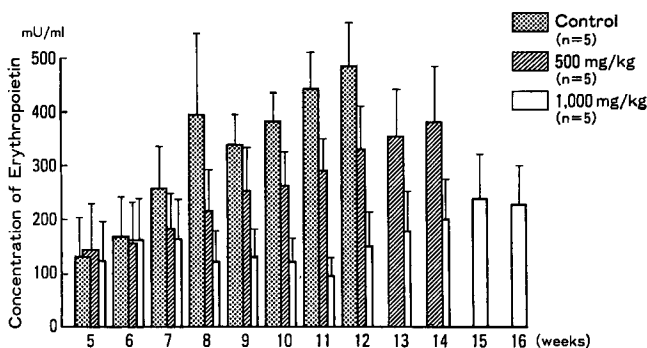point was mean value with standard deviations.)

Fig. 4. Time-course changes in serum level of erythropoietin after
oral administration of bropirimine. A significant suppres-
sion of erythropoietin production was noted 3 weeks after
the start of treatment with bropirimine (1,000 mg/kg) (3
weeks after the start of treatment to 4 weeks: $P < 0.05$, after
5 weeks: $0.002 < P < 0.005$).

of bropirimine using the JRC 901 strain.
Fig. 3 shows the growth curves for the
treatment groups and the control.

Bropirimine at the dose of 500 mg/kg
inhibited tumor proliferation, but not
significantly compared with the control.
The T/C ratio was 91.8%, indicating no
positive response. The degree of histol-
ogical degeneration was grade IIa with
viable tumor cells remaining in over 50%
of the total tumor area. Therefore, this
dose of bropirimine was also ineffective

from the histological viewpoint.

Bropirimine at the dose of 1,000 mg/kg
inhibited tumor proliferation significantly,
and compared with the control, it reduced
the mean tumor weight from the 3rd week
after the treatment was started (Fig. 3).
However, the T/C ratio was 67.7%, indi-
cating that the treatment was ineffective.
As to the degree of histological degenera-
tion, viable tumor cells remained in 39%
of the area (grade IIb). Therefore, bro-
pirimine was ineffective from the histol-

ogical viewpoint.

After three weeks of treatment with bropirimine at a dose of 500 mg/kg, the serum concentration of Epo tended to be lower than that in the control group. Furthermore, the production of Epo after three weeks of treatment with 1,000 mg/kg was significantly suppressed (Fig. 4). Thus, by inhibiting the production of Epo, the administration of bropirimine was effective against the JRC 901 tumor.

DISCUSSION

Chemotherapy and radiotherapy have been applied in the treatment of patients with advanced RCC, but these treatment modalities have been far from encouraging so far[9][10]. With new clinical application of cytokines including IFNs due mainly to the development of techniques made possible by genetic recombination in the 1980s, various kinds of cytokine therapy have been applied on many patients with advanced cancer. Although satisfactory results have not been obtained[11]. IFN clearly produces a higher response rate in the treatment of RCC than the conventional treatment modalities mentioned above. Therefore, cytokine therapy including IFN is promising in the treatment of patients with advanced RCC. Bropirimine is a drug developed based on a totally new concept. It produces various effects including the induction of endogenous IFN-alpha, activation of natural killer cells (NK cells), activation of macrophages, induction of interleukin-1 (IL-1) and enhancement of antigen-antibody interaction in animal experiments[1]. In Japan, phase II trials targeting RCC and bladder tumors are underway on the basis of the results of the phase I trials. In addition to inducing endogenous cytokines, bropirimine is excreted in intact form in the urine at high concentrations[2].

Encouraged by these pharmacological properties, we examined whether bropirimine had a direct anti-tumor activity against RCC. In the experiment utilizing the organ culture system where bropirimine was brought in direct contact with tumor tissues, ^{3}H-thymidine uptake was significantly reduced, which indicated the suppression of DNA synthesis of the tumor cells, in 88.2% of all treated tumors. Therefore, these findings suggested that bropirimine had direct anti-tumor activity against RCC. Furthermore, orally administered bropirimine induced not only an anti-proliferative action against the JRC 901 strain, but also suppressed its erythropoietin production heterotransplanted in nude mice. These findings also indicated the presence of direct anti-tumor activity against RCC, because even if endogenous mouse IFN-alpha was produced by the administration of bropirimine, mouse IFN had no anti-tumor activity against human tumors.

The findings of experiment using tumor-bearing nude mice suggested that bropirimine could not totally eliminate the xenografted tumors. Therefore, the development of more effective treatment modalities with enhanced anti-tumor activity of bropirimine, such as the combination of bropirimine with chemotherapy, IL-2 or radiation is awaited[12]. Furthermore, in addition to the direct anti-tumor activity against RCC which shown here, the excretion of intact bropirimine at high concentrations in urine, as reported in the phase I study[2], appears to support the high efficacy of bropirimine against superficial bladder cancer in the clinical setting[13], which will be an interesting subject for future study.

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和文抄録

Bropirimine（経口インターフェロン産生薬剤）の腎細胞癌に対する直接的抗腫瘍効果に関する検討

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腎細胞癌症例17例の腎摘時採取した腫瘍を用いて器官培養法，ならびにヌードマウス移植継代可能株となった1株を用いて，bropirimine の直接的抗腫瘍効果に関して検討した．その結果，器官培養法では，17例中15例（88.2%）が対照群に比較して ^{3}H-thymidine uptake が有意に抑制され，そのうち3例（17.6%）は bropirimine の濃度依存性に ^{3}H-thymidine の uptake が抑制された．さらに，ヌードマウス可移植性（erythropoietin: Epo 産生株）となった1株（JRC 901）に対する bropirimine の経口投与では，bropirimine の濃度依存性に腫瘍増殖が抑制されたが，対照群間に有意差はなく，かつ組織学的にも via-ble な腫瘍細胞の完全消失はえられなかった．しかし，JRC 901 株の Epo 産生量は，対照群に比較して有意に抑制された．以上の結果，bropirimine は腎細胞癌に対し直接的抗腫瘍活性を有することが明らかとなった．