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# Inhibitory effect of a spicamycin derivative, KRN5500, on the growth of hepatic metastasis of human colon cancer-producing tissue polypeptide antigen

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**Abstract** The inhibitory effect of KRN5500, a spicamycin derivative, on the growth of hepatic metastasis of the tissue polypeptide antigen (TPA)-producing human colon cancer COL-1 was examined in severe combined immunodeficient (SCID) mice. Prior to this chemotherapeutic study, we confirmed the high correlation coefficient ($r = 0.86$, $P < 0.01$) between plasma TPA levels in athymic nude mice bearing COL-1 and tumor volume. In the chemotherapy of experimental hepatic metastasis induced by intrasplenic injection of COL-1 cells, KRN5500 at 12 mg/kg per day was administered i.v. three times at 4-day intervals. From the start of chemotherapy (day 1), plasma TPA levels in the mice were significantly decreased from 8332 U/l to a minimum of 494 U/l on day 16 and were within the range for intact SCID mice (409–634 U/l). The mean tumor weight was 4.87 *g* in the liver of untreated mice on day 19 and 0.74 *g*, in the liver of KRN5500-treated mice, a significant difference, representing a tumor growth inhibition rate of 85%. These results suggest the usefulness of TPA as a tumor marker in an experimental xenograft model. The chemotherapeutic efficacy of KRN5500 against experimental hepatic metastasis indicates that it may be a useful drug for the treatment of patients with hepatic metastases of colon cancer.

**Key words** Spicamycin derivative · KRN5500
Human colon cancer · Hepatic metastasis
Tissue polypeptide antigen

## Introduction

Hepatic metastasis is the most common cause of death in patients with colon cancer [1, 4]. About 70% of patients with advanced colon cancer develop hepatic metastasis [12, 16]. Russell et al. [8] reported that 80% of first relapse sites of colon cancer involved the liver. Although the efficacy of many kinds of antitumor drugs in patients with hepatic metastasis of colon cancer has been investigated [9], most drugs show little effectiveness [9, 15]. Thus, drugs impeding the growth of hepatic metastasis of colon cancer are important to improve chemotherapy of colon cancer.

Recently, we described the marked efficacy of a spicamycin derivative, KRN5500 (6-[4-deoxy-4-(tetradeca-2(*E*), 4(*E*)-dienoylglycyl)amino-L-*glycero*-β-L-*manno* heptopyranosyl]amino-9H-purine, Fig. 1), against human tumor xenografts. In particular KRN5500 shows an inhibitory effect on growth of human colon cancer. Indeed, KRN5500 has been found to cause a tumor mass reduction in 46% of tested colon cancers [6]. This finding suggests the potential application of KRN5500 in the treatment of hepatic metastases of colon cancers.

In this study, we investigated the effects of i.v. KRN5500 on experimental hepatic metastasis of human colon cancer COL-1 by successive determinations of a plasma tumor marker, tissue polypeptide antigen (TPA), which is produced by COL-1. Hepatic metastasis was induced in severe combined immunodeficient (SCID) mice by intrasplenic (i.s.) injection of COL-1 cells [7, 10].

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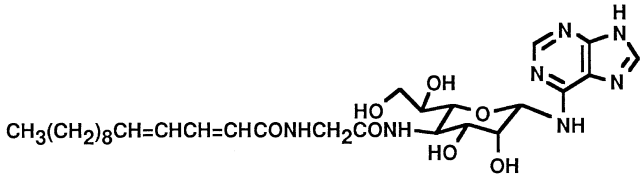


**Fig. 1** Structure of KRN5500

## Materials and methods

### Animals

Female SCID mice (Fox Chase C.B17/icr-Scid Jcl) at 6–8 weeks old were purchased from Nihon CLEA Co., Tokyo, Japan. Female athymic nude mice (BALB/c nu/nu Slc) of the same age were purchased from Japan SLC, Shizuoka, Japan. Mice were housed in laminar flow cabinets under specific pathogen-free conditions and fed irradiated basal CE-2 diet (Nihon CLEA Co., Tokyo, Japan) and water *ad libitum*.

### Reagents and drugs

KRN5500 was semisynthesized from spicamycin in our laboratory. KRN5500 was dissolved in dimethylsulfoxide and Cremophor EL (Sigma Chemical Co., St. Louis, Mo.), and 0.9% saline was added to give a final concentration of 1% of both dimethylsulfoxide and Cremophor EL.

### Measurement of plasma TPA level

To obtain plasma, 100 μl blood was taken into heparinized capillary tubes after cutting the orbital plexus vein of the mice. The plasma was then separated by centrifugation at 12 000 *g* for 15 min. Plasma TPA levels were analyzed by radioimmunoassay [3] using a Prolifigen TPA Kit Daiichi II (Daiichi Radioisotope Laboratory, Tokyo, Japan) by the Biomedical Laboratory Co., Tokyo, Japan.

### Tumors

COL-1 human colon cancer was supplied by the Central Institute for Experimental Animals, Kanagawa, Japan. The tumor was maintained by serial s.c. transplantation of tumor fragments into athymic nude mice as described by Inaba et al. [5].

### Liver metastasis

Liver metastasis of COL-1 was induced by i.s. injection according to a previously reported method [11]. A tumor specimen of COL-1 was minced in culture medium for 15 min at 37° C. The cell suspension was then filtered through a two-layer sterile gauze and $1 \times 10^5$ viable single cells in 0.1 ml medium were injected into the spleen of SCID mice. The spleen was immediately excised and the abdominal cavity was closed [13].

### KRN5500 chemotherapy

After about 60 days from the i.s. injection of COL-1 cells into SCID mice, the plasma TPA level in each mouse was measured. Mice showing more than 5000 U/l plasma TPA were selected for the chemotherapy study. Mice in the treatment group were given 12 mg/kg per day KRN5500 i.v. three times at 4-day intervals. The control group was given vehicle i.v. according to the same schedule as the group treated with KRN5500. From the start of chemotherapy (day 1), plasma TPA levels were measured once or twice a week. On day 19, all mice were sacrificed and the tumor nodules in the liver of each mouse were excised and weighed. The tumor growth inhibition rate was calculated as previously reported [6].

### Statistical analysis

The significance of differences between the treated group and the control group was calculated using Student's *t*-test.

## Results

### Plasma TPA levels and tumor volume in athymic nude mice bearing COL-1

Prior to this study, we measured the levels of tumor markers in the plasma of athymic nude mice bearing COL-1. Among nine tumor markers (α-fetoprotein, carcinoembryonic antigen, CA19-9, CA72-4, TPA, $\beta_2$-macroglobulin, immunosuppressive acidic protein, pheritin, and cyclic acid), only plasma TPA showed high levels (data not shown). As shown in Fig. 2, a high correlation coefficient ($r = 0.86$, $P < 0.01$) between the plasma TPA levels in these mice and tumor size was observed.

In addition, the plasma TPA levels and tumor size of COL-1-bearing athymic nude mice were measured and expressed as a function of time after implantation (Fig 3). The plasma TPA levels began to increase 10 days after implantation and increased with growth of tumor volume. These results indicate that it is possible to monitor tumor growth in the liver by measuring the plasma TPA level.

### Effect of KRN5500 on experimental hepatic metastasis of TPA-producing COL-1

About 60 days after i.s. injection of COL-1 cells into SCID mice, chemotherapy of the hepatic metastasis by KRN5500 was commenced. At this time, the mean plasma TPA level of the KRN5500-treated group and the control group were 8332 and 13 202 U/l, respectively. From the start of chemotherapy by KRN5500, the plasma TPA levels decreased rapidly and reached a minimum level (494 U/l) which was within the range of intact SCID mice (409–634 U/l) on day 16. However, the plasma TPA levels in control mice increased and reached about $8 \times 10^5$ U/l on day 19. The maximum difference between the plasma TPA levels in the control and treatment groups was about 1040-fold on day 16 (Fig. 4).

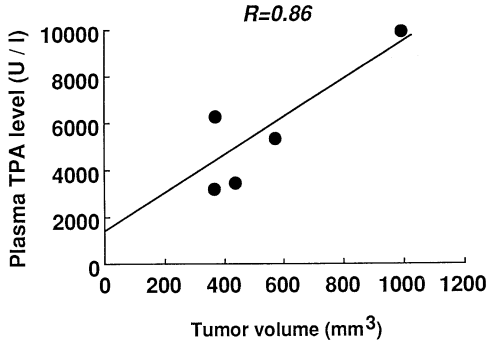


**Fig. 2** Correlation between plasma TPA levels and tumor volume. COL-1 was implanted s.c. into five athymic nude mice. When the tumor volume reached about 400–1000 $mm^3$, the plasma TPA levels of these mice were measured. The line represents the regression line obtained by the least squares method

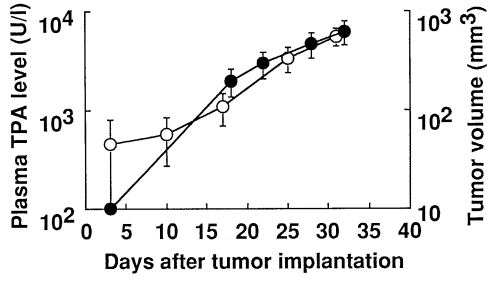


**Fig. 3** Changes in plasma TPA levels and tumor volume. COL-1 was implanted s.c. into athymic nude mice on day 1. After implantation of the tumor, the plasma TPA levels of athymic nude mice bearing COL-1 (*open circles*) and the tumor volume (*closed circles*) were measured once or twice a week. Data points are the means ± SE from five mice

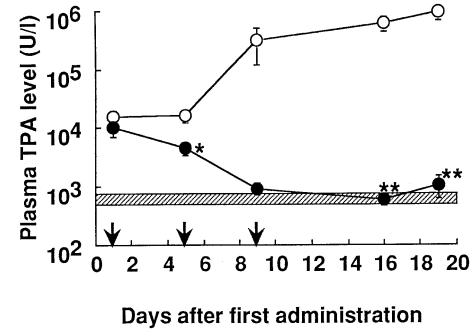


**Fig. 4** Changes in plasma TPA levels of SCID mice which had COL-1 liver metastasis. COL-1 cells ($1 \times 10^5$) were implanted into SCID mice by i.s. injection. About 60 days after tumor implantation (day 1), mice were treated either with vehicle (*open circles*) or with KRN5500 (12 mg/kg per day, *closed circles*). Vehicle or KRN5500 was given every 4 days for a total of 3 i.v. injections (*arrows*). Plasma TPA levels were measured once or twice a week. Data points are means ± SE of from three or four mice. The *hatched area* in indicates the range of the plasma TPA levels of ten normal SCID mice. $*P < 0.05$, $**P < 0.01$ versus the control group

**Table 1** Tumor weight in the livers of SCID mice treated i.s. with COL-1. COL-1 cells ($1 \times 10^5$) were implanted into SCID mice by i.s. injection and about 60 days after tumor implantation, chemotherapy was started. Vehicle or 12 mg/kg per day KRN5500 was injected i.v. three times at 4-day intervals. After 18 days from the start of chemotherapy, mice were sacrificed and their hepatic tumor nodules were weighed. Values are means ± SE of three or four mice per group

| | Number of mice | Tumor weight (g) |
|---|---|---|
| Control | 3 | 4.87 ± 1.27 |
| KRN5500 | 4 | 0.74 ± 0.41** |

** $P < 0.01$ compared with control

The tumor weights in the livers of both the control and the treated mice on day 19 are shown in Table 1. The mean tumor weight of untreated mice reached 4.87 *g*, but the mean tumor weight in the liver of KRN5500-treated mice was 0.74 *g* and the tumor growth inhibition rate in KRN5500-treated mice compared with untreated mice was 85%.

## Discussion

Tumor markers are useful as a means of detecting tumor metastases in viscera and in the assessment of the antitumor efficacy of drugs against tumor metastasis. In experimental metastasis models, several tumor markers are used to monitor the growth of human tumors at the site of metastasis [10, 14].

Prior to the study on the antitumor activity of KRN5500 against experimental hepatic metastasis, we measured several tumor markers in the plasma of athymic nude mice bearing COL-1. Only the TPA level was significantly higher in the tumor-bearing mice than in normal mice. TPA is a tumor-associated antigen which is present in the membranes of human cancer cells and is released from a wide spectrum of cancerous tissues [2]. A high correlation between the plasma TPA level and tumor volume demonstrated in this study (Figs. 2, 3), indicated that TPA may be a good marker by which we can detect the growth of COL-1 in mice. In further studies, high plasma TPA levels were detected in athymic nude mice bearing other human tumors including stomach and colon cancers (data not shown). TPA may be a useful tumor marker in experimental xenograft models.

By monitoring the plasma TPA levels, we were able to demonstrate the inhibitory effect of KRN5500 on the growth of hepatic metastasis of COL-1 in SCID mice (Table 1, Fig. 4). Treatment with KRN5500 resulted in significant suppression of plasma TPA levels and reduced the levels to within the normal range by 15 days after treatment started. It is suggested that tumor cells showed minimum or no viability. A significant difference in tumor weight on day 19 also indicates the inhibitory effect of KRN5500 on tumor growth. These findings suggest that KRN5500 is effective not only

against s.c.-implanted human tumor xenografts but also against tumors in viscera.

In our previous study, KRN5500 showed antitumor activity by inhibiting protein synthesis in tumor cells [6]. The reduction in plasma TPA observed in this study may be a result of the inhibitory effect of KRN5500 on protein synthesis in COL-1. Although it is not clear whether TPA is essential for the survival of COL-1, KRN5500 might inhibit the growth of COL-1 by inhibiting the production of TPA.

We conclude that TPA may be a useful tumor marker for assessment of the chemotherapeutic efficacy of antitumor drugs in experimental xenograft studies especially in metastasis and in in situ transplantation models. The chemotherapeutic efficacy of KRN5500 on the growth of hepatic metastasis of COL-1 in SCID mice, as assessed by the plasma TPA level, indicates that KRN5500 is an interesting candidate new drug for the treatment of patients with hepatic metastases of colon cancer.

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