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Effect of E7010 on liver metastasis and life span of syngeneic C57BL/6 mice bearing orthotopically transplanted murine Colon 38 tumor

Abstract Purpose: E7010 is an orally active sulfonamide antitumor agent showing good activity against various subcutaneously inoculated rodent tumors and human tumor xenografts. The purpose of this study was to evaluate the effect of E7010 on liver metastasis and life span of mice bearing orthotopically transplanted murine Colon 38 tumor. Methods: Orthotopic transplantation of murine Colon 38 tumor as intact tissue yielded hepatic metastasis with a high incidence in about 1 month in C57BL/6 mice, and the mice died in about 2 months with cachexia. In this model, the maximum tolerated dose of E7010 (100 mg/kg per day) was administered orally on various schedules, including for 14 days or daily until death, starting at 14 days after transplantation, or for 8 days from 21 days after transplantation. Results: E7010 showed tumor growth inhibition (T/C = 40%) at the orthotopic site similar to that at the subcutaneous site (T/C = 32%) when administered from 14 days after transplantation. When E7010 was started from 21 days after transplantation, it significantly decreased the number of hepatic metastases (control 17.1 ± 20.8, E7010 2.6 ± 5.3), although inhibition of tumor growth at the orthotopic site was only moderate (T/C = 60%). The administration of E7010 until death produced a significant increase in life span (control 49.8 ± 8.9 days, E7010 62.5 ± 6.1 days). Although the tumor weight of the E7010-treated group on the day of death was similar to that of the untreated group (control 1.166 ± 0.507 g, E7010 1.211 ± 0.632 g), there were significantly fewer liver metastases in the E7010-treated group (control 41.3 ± 31.1, E7010 2.0 ± 2.0). Conclusion: E7010 suppressed tumor growth at both primary and metastatic sites and increased life span in an orthotopic transplantation model of murine Colon 38 tumor in syngeneic C57BL/6 mice. Hepatic metastasis was inhibited more effectively than the growth of the primary tumor.

Key words E7010 · Antimitotic · Orthotopic transplantation · Survival · Liver metastasis

Introduction

E7010 is an orally active, novel sulfonamide anticancer agent [19], which exhibits a broad spectrum of antitumor activity against human tumor xenografts [8]. It increases the percentage of mitotic cells and inhibits tubulin polymerization in a dose-dependent manner, and these activities correlate well with its cell growth-inhibitory activity [18]. Binding of E7010 to purified tubulin is inhibited by colchicine, but not by vincristine, although the binding properties are different from those of colchicine. Furthermore, E7010 is effective against tumor cells which are multidrug-resistant due to overexpression of P-glycoprotein. E7010-resistant P388 cell lines show no cross-resistance to vincristine or paclitaxel [18].

In a phase I study of single or 5-day repeated administration of E7010 [17], reduction of spinal cord metastasis in patients with uterine sarcoma, a minor response in patients with pulmonary adenocarcinoma and decreases in carcinoembryonic antigen and squamous cell carcinoma antigen in patients with stomach cancer and recurrent uterine cervical carcinoma, respectively, were observed. The dose-limiting toxicity in the single-dose study was peripheral neuropathy and that in the 5-day repeated-dose study was peripheral neuropathy together with intestinal paralysis. Pharmacokinetic analysis showed that E7010 has a favorable absorption and elimination profile and does not accumulate. The maximum allowable doses were 320 mg/mm² for the single-dose study and 200 mg/mm² for the 5-day repeated-dose study. A divided dose study is needed to see whether the blood level of E7010 can be better controlled.

Recent clinical studies of anticancer agents have tended to focus on parameters such as clinical benefit,
time to progression, overall survival and quality of life [14, 15]. Compared to the subcutaneous (s.c.) xenograft model which has generally been used for preclinical evaluation of anticancer agents, an orthotopic transplantation model may be more appropriate for such evaluations because it should better reflect the behavior of clinical tumors [3]. An orthotopically transplanted colon cancer model has been found to be useful for evaluating effect on life span [12, 16]. Moreover, inhibition of metastasis, which is difficult to evaluate in a clinical study, but is important to improve the outcome of cancer therapy, can also be evaluated [4] in an orthotopic transplantation model.

We have previously reported that orthotopically transplanted murine Colon 38 tumor rapidly metastasizes to the liver in syngeneic C57BL/6 mice [6], as occurs in colorectal cancer patients, and the mice die in about 2 months with small variation. Therefore, we considered that this was an appropriate model to evaluate the effects of drugs on metastasis of colon cancer and survival. In this study, we used the model to investigate the effects of E7010 on liver metastasis and survival, in addition to primary tumor growth.

**Materials and methods**

**Drugs**

E7010 was synthesized at Eisai Company (Tsukuba Research Laboratories, Ibaraki, Japan). E7010 was suspended in 0.5% methylcellulose and administered orally. 5-Fluorouracil (5-FU) was purchased from Kyowa Hakko Kogyo Company, Tokyo, Japan. It was diluted with 0.9% NaCl and administered orally. The control group was given 0.5% methylcellulose orally. The oral administration was accomplished by using a stainless steel gavage tube.

**Animals**

Female C57BL/6 mice were obtained from Charles River, Atsugi, Japan. They were given food (MF; Oriental Yeast Company) and UV-irradiated water ad libitum and maintained under specific pathogen-free conditions. They were used for experiments when they were 6 to 8 weeks old.

**Tumor cells**

Murine Colon 38 tumor was supplied by the Cancer Chemotherapy Center, Japan Foundation for Cancer Research, Tokyo, and maintained by serial s.c. inoculation in female C57BL/6 mice.

**Orthotopic transplantation of Colon 38 intact tissue**

Orthotopic transplantation of colon cancer intact tissue was conducted as described previously [6]. Briefly, Colon 38 tumor growing subcutaneously in C57BL/6 mice was resected and the tumor tissues were cut into pieces weighing 25 mg in Hank’s balance salt solution after aseptic removal of necrotic portions. Mice were anesthetized with a 2.5% solution of a 1:1 mixture of 2,2,2-tribromoethanol (Aldrich, Milwaukee, Wis.) and t-amyl alcohol (Wako, Osaka, Japan). An incision was made in the left lower abdomen. Then the cecum was gently exposed and one of the tumor pieces was fixed onto the surface of the cecum with a 6-0 Dexon II suture (Davis-Geck, Manati, Puerto Rico). The cecum was returned to the abdominal cavity and the incision was closed with a Dexon II suture.

**Experimental chemotherapy of subcutaneously inoculated Colon 38**

About 25 mg of Colon 38 tumor was inoculated s.c. with a trocar into the right flank of each mouse on day 0. The animals were divided into vehicle- and drug-treated groups consisting of eight animals each on day 14 after transplantation. Each tumor was measured with a sliding caliper. The volume of the cuboid mass was calculated from the major dimension (L) and minor dimension (S) using the following equation: tumor volume = L × S²/2. Antitumor activity in terms of tumor growth was determined by expressing the mean tumor weight of the test group (T) as a percentage of that of the control group (C) (T/C × 100).

**Experimental chemotherapy of orthotopically transplanted Colon 38**

Mice were transplanted orthotopically with about 25 mg of Colon 38 on day 0 and divided into vehicle- and drug-treated groups on either day 14 or day 21 after transplantation. E7010 was administered orally daily according to the indicated regimen for each experiment, at a dose of 100 mg/kg which was the maximum tolerated dose (MTD) with the 8-day daily schedule. 5-FU was administered orally daily at a dose of 30 mg/kg, which was the MTD in our experiment with the 8-day daily schedule. Mice were killed on the indicated days and the locally growing tumor and liver were resected. The excised tumors were weighed and the metastatic nodules in the liver were counted in a blind manner under a dissecting microscope after staining the liver with Bouin’s solution.

For determination of life span, orthotopically transplanted mice were observed until 75 days after transplantation and were autopsied on the day of death or at the end of the observation period. If mice died with only local small tumor and death was accompanied by a loss of body weight, we judged the death as toxic. Antitumor activity in terms of tumor growth was determined by expressing the mean tumor weight of the test group (T) as a percentage of that of the control group (C) (T/C × 100). Efficacy in terms of life span was determined as the percentage increase in life span (ILS(%)) calculated in terms of mean survival times (MST): ILS(%) = [(MST of the test group / MST of the control group) − 1] × 100.

**Statistical analysis**

Differences in tumor weight and relative tumor volume were analyzed for significance using Student’s t-test. The Mann-Whitney U-test and Kruskal-Wallis h-test followed by the Steel test were used to compare the number of metastatic nodules in the livers. Differences in survival time were analyzed for significance using the log-rank test.

**Results**

**Antitumor activity of E7010 against subcutaneously inoculated murine Colon 38 tumor**

We first examined the antitumor activity of E7010 against subcutaneously inoculated murine Colon 38 tumor in syngeneic C57BL/6 mice. About 25 mg of tumor was inoculated s.c. on day 0. E7010 was administered orally at a dose of 100 mg/kg daily for 8 days from 14 days after inoculation, and then on day 23 the efficacy