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Antineoplastic Effect of Erbstatin on Human Mammary and Esophageal Tumors in Athymic Nude Mice

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The growth of MCF-7, a human mammary carcinoma, in athymic nude mice was inhibited by intraperitoneal administration of erbstatin for 14 days in combination with an iron chelator, foroxymithine, which inhibits the decomposition of erbstatin. Another human mammary carcinoma, Br-10, was not affected. Foroxymithine alone had no anti-tumor activity. In four esophageal tumors, erbstatin retarded tumor growth. There were no side-effects in any erbstatin-treated group. Levels of epidermal growth factor receptors were not changed throughout treatment with erbstatin at any dose. Erbstatin, a tyrosine kinase inhibitor, may have an antineoplastic effect against human mammary and esophageal tumors.
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INTRODUCTION

THE regulation of cell division by growth factors involves activation of tyrosine protein kinases [1, 2] and most growth factor receptors possess intrinsic tyrosine kinase activity capable of autophosphorylation [3, 4]. In human mammary and esophageal tumors, the expression of epidermal growth factor receptors (EGFRs) is a poor prognostic indicator [5, 6]. Binding of EGF to the external domain of its receptor initiates tyrosine specific kinase activity [7], which has an important role in the growth of tumors possessing EGFRs.

Erbstatin, isolated from culture filtrates of actinomyces MH435-hF by Umezawa *et al.* [8], is a specific inhibitor of EGFR tyrosine kinase [8]. The tyrosine kinase activity of EGFR in cultured A431 cells and the autophosphorylation of the *src* gene product $pp60^{src}$ in Rous sarcoma virus-infected normal rat kidney cells was specifically inhibited by erbstatin [9]. In addition, erbstatin suppressed the growth of L-1210 leukemia cells in mice [10]. We have investigated the antineoplastic activity of erbstatin against human mammary and esophageal tumors and its effects on EGF binding to receptor in athymic nude mice.

MATERIALS AND METHODS

Human mammary tumors MCF-7 and Br-10 and human esophageal tumors TEN-11, EH-1, EH-4 and EH-10 were used. MCF-7 cell line was given by Dr Y. Nomura (Kyushu Cancer Center Hospital, Fukuoka) and maintained in the estrogenized female athymic nude mice. Five-week-old female athymic nude mice (BALBc/nu nu) were purchased from Nihon Kurea (Tokyo) and bred in an aseptic room. Br-10 was established by Dr Hirohashi *et al.* at the National Cancer Center, Tokyo in 1974 from pleural effusion and given by Dr T. Kubota (Keio Gijuku University, Tokyo). Both tumors were passed about every 5 weeks in estrogenized female athymic mice. EH-1, EH-4 and EH-10 were established in our institute and TEN-11 was a gift from T. Nishihira (Tohoku University, Sendai). The EGFR content of these tumors is shown in Table 1.

MCF-7 cells were cultured in RPMI medium containing 5% charcoal-stripped fetal calf serum (FCS) and 17β-estradiol 1 nmol/l at 37°C in humidified incubators in 5% CO_2. For cell growth experiments, 2×10^5 cells per well were seeded and 24 h later the medium was removed and replaced with fresh medium and additives. Several concentrations of erbstatin were incubated with cells for 24 h. At different times, cells were counted in triplicate after harvesting with gentle trypsinization.

Erbstatin (molecular weight 179) was a gift from Dr T. Takeuchi (Institute of Microbial Chemistry, Tokyo) and Dr K. Umezawa (Keio Gijuku University, Tokyo). Erbstatin decomposes in serum by a pathway requiring oxygen and ferric ion; therefore, we simultaneously administered foroxymithine, a potent chelator for ferric ions [11]. Foroxymithine had no

Table 1. Antineoplastic activity of erbstatin against human mammary and esophageal tumors

Tumor	Antineoplastic effect (%)*	EGFR content (fmol/mg protein)	K_d (nmol)
Mammary tumor			
MCF-7	41.0	35.5	0.56
Br-10	98.0	—	—
Esophageal tumor			
EH-1	65.9	59.8	0.27
EH-4	54.4	80.5	0.54
EH-10	67.8	192.0	0.72
TEN-11	61.3	147.0	0.49

*Tumor volume of treated (erbstatin 4 mg per mouse) vs. non-treated.

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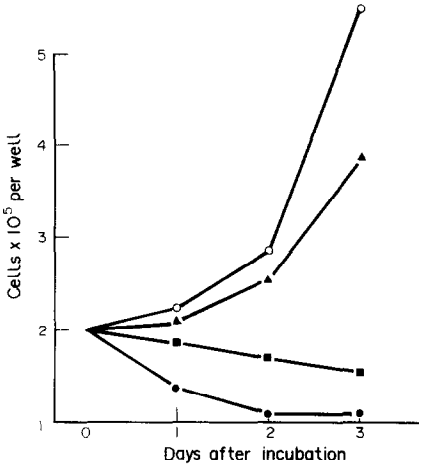

Fig. 1. Effect of erbstatin on proliferation of MCF-7 cells. ○ = control, ▲ = 10^{-8}, ■ = 10^{-6} and ● = 10^{-4} mol/l.

antineoplastic effect on cultured MCF-7 cells (data not shown). Erbstatin was administered at three grading doses of 1, 2 and 4 mg per mouse intraperitoneally for 14 days in combination with 1 mg per mouse of foroxymithine [10].

Tumor diameters were measured at least twice weekly and tumor volume was calculated by tumor volume (TV) = $(\text{width})^2 \times (\text{length}) \div 2$.

EGFR assay was done with [^{125}I]EGF [12]. EGFR contents were calculated by Scatchard plot.

Tumors were dissected free from subcutaneous tissues and fixed in 10% formalin. Paraffin-embedded tumors were processed for histological examination and stained with hematoxylin–eosin.

RESULTS

In culture with 5% FCS with estradiol, the growth of MCF-7 cells was inhibited by erbstatin 1 μmol/l (Fig. 1). The IC_{50} of erbstatin for MCF-7 cells was 5 μmol/l by the [^{3}H]TdR uptake inhibition test (data not shown).

The maximum inhibition of the growth of MCF-7 tumor was obtained on day 18 after the initial treatment (Fig. 2a). No difference in the growth of MCF-7 tumors was found between erbstatin treatment at 2 and 4 mg, but a significant difference was found between that at 1 mg and 2 mg. Foroxymithine did not show any antineoplastic activity.

The effect of erbstatin against EH-4 tumor was dose-dependent (Fig. 2b). The maximum effect was seen on day 10. The anti-tumor effects of erbstatin (4 mg per mouse) against three other esophageal tumors combined with 1 mg per mouse foroxymithine are shown in Table 1. Erbstatin was not active against Br-10 tumor.

Change of body weight was examined in erbstatin-treated mice. Significant body weight loss was not seen at any dose. There were no other adverse effects.

No significant histological changes were found after erbstatin treatment, including examination of the liver, lung, heart, adrenal gland and ovary.

[^{125}I]EGF binding to its receptor was measured in the tumors dissected after final administration of erbstatin. The change of EGFR was examined in EH-4 esophageal tumors from animals treated with 1, 2 or 4 mg erbstatin (Table 2). EGFR content and K_d values were consistent with known effects of erbstatin.

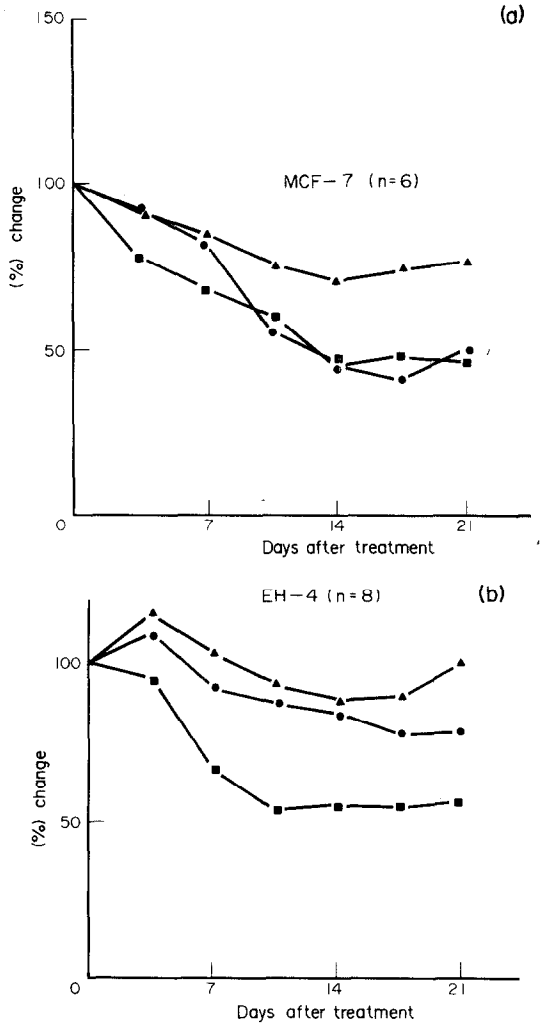

Fig. 2. Antineoplastic effect of erbstatin against MCF-7 (a) and EH-4 (b) in athymic mice. % change: tumor volume compared with control (= 100%). ▲ = 1, ● = 2 and ■ = 4 mg erbstatin per mouse. The standard errors in these experiments were less them 10% of each value.

DISCUSSION

In our study of human tumors in athymic nude mice, erbstatin had antineoplastic activity in one of two mammary tumors and in several esophageal tumor lines. Growth inhibition with MCF-7 and EH-4 tumors was dose-dependent. Erbstatin did not cause extensive necrosis nor fibrosis. There was a discrepancy in growth inhibition by erbstatin between *in vitro* and *in vivo*

Table 2. Effect of erbstatin on EGFR

	EGFR content (K_d)*	
	Before treatment	After treatment
Control	80.5 (0.58)	85.5 (0.61)
Erbstatin (mg per mouse)		
1	80.5 (0.58)	68.8 (0.56)
2	80.5 (0.58)	69.8 (0.62)
4	80.5 (0.58)	74.8 (0.59)

*EGFR content in fmol/mg protein, K_d in nmol.

studies. These results suggest that erbstatin may exhibit an antitumor effect against several kinds of human tumors via a cytostatic action.

Imoto *et al.* [9] reported that erbstatin did not inhibit the binding of EGF to its receptor but did inhibit the internalization of EGFR complexes and that this compound did not inhibit EGF-stimulated phosphotidylinositol turnover in A431 cells. Our *in vivo* study also demonstrated the consistency of EGF binding to its receptor throughout treatment with erbstatin. However, little is known about how the inhibition of tyrosine kinase leads to inhibition of tumor cell growth *in situ*.

No side-effects such as weight loss nor warning signs in organs were observed.

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Effect of Topoisomerase Modulators on Cisplatin Cytotoxicity in Human Ovarian Carcinoma Cells

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The *in vitro* interaction of modulators of topoisomerase I and II with cisplatin in human ovarian carcinoma cells might be synergistic. The interactions were evaluated by median effect analysis of survival data derived from continuous exposure to drug combinations for 10 days in colony-forming assays. The interaction between cisplatin and the topoisomerase I inhibitor camptothecin and the topoisomerase I activator β-lapachone was additive, as was that between cisplatin and the topoisomerase II inhibitor novobiocin. Despite the clinical efficacy of the combination of etoposide (a topoisomerase II inhibitor) and cisplatin, the combination index at 50% cell kill indicated antagonism between these two drugs. Thus, biochemical synergism at the cellular level is not a prerequisite of improved therapeutic efficacy.
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INTRODUCTION

THE USE of cisplatin is frequently limited by the rapid development of resistance. Cisplatin forms intra- and interstrand crosslinks in DNA, and these adducts interfere with replication [1]. DNA topoisomerases alter the topology of DNA molecules [2, 3] and may thus influence one or both of these processes. However, repair replication induced by cisplatin can be enhanced [4], or intrastrand adducts in resistant cells removed more rapidly [5, 6]. The role of topoisomerases, particularly topoisomerase II, in DNA repair has not been fully defined [7]. These enzymes might have a role in DNA repair by making adducts accessible to repair enzymes.

Camptothecin inhibits mammalian topoisomerase I, producing single-strand breaks by blocking the rejoining step of the reaction [8, 9]. β-Lapachone inhibits the repair of potentially lethal damage by activating topoisomerase I [10]. Treatment of HEp-2 cells with β-lapachone or camptothecin produced similar sensitization to X-irradiation, indicating involvement of topoisomerase I in at least some forms of DNA repair. Novobiocin and

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