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In vivo antitumour efficacy of MGI-114 (6-hydroxymethylacylfulvene, HMAF) in various human tumour xenograft models including several lung and gastric tumours

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Abstract

MGI-114 (6-hydroxymethylacylfulvene, HMAF) is a semi-synthetic analogue of the cytotoxic sesquiterpenoid illudins. In the present study, the *in vivo* antitumour efficacy of MGI-114 was examined in a panel of human tumour xenograft models consisting mainly of human lung and gastric tumours, and compared with that of other antitumour drugs such as irinotecan, paclitaxel, cisplatin, doxorubicin, vindesine, etoposide and 5-fluorouracil (5-FU). When different administration schedules were compared, daily administration of MGI-114 was found to be more effective than intermittent administrations. In human tumour xenograft models of nasopharyngeal, breast and colon carcinoma and melanoma, MGI-114 exerted a strong antitumour activity with complete tumour regression being observed. Moreover, in four human lung and three gastric tumour xenograft models, MGI-114 showed a strong antitumour activity with complete tumour regression being observed in some of the models. The antitumour efficacy of MGI-114 was generally higher than or equivalent to that of other antitumour drugs such as irinotecan and paclitaxel. These results support the potential utility of MGI-114 in the treatment of a variety of human solid tumours.

Keywords: MGI-114; 6-Hydroxymethylacylfulvene; *In vivo* antitumour efficacy; Human lung tumours; Human gastric tumours

1. Introduction

Acylfulvene is an analogue of the illudins which are cytotoxic sesquiterpenoids isolated from the fungi *Omphalotus illudens* or the closely related *Lampteromyces japonicus*. Acylfulvene has been reported to inhibit xenograft primary tumour growth and to prolong the life span of tumour-bearing animals [1]. Furthermore, acylfulvene has shown *in vitro* cytotoxicity against various multidrug-resistant tumour cells and was suggested to induce DNA damage in a unique manner that appears to require a functional DNA helicase for efficient repair [1,2].

MGI-114 (6-hydroxymethylacylfulvene, HMAF) is a semi-synthetic analogue of illudins with an improved therapeutic index [3]. In human MV522 lung adenocarcinoma xenograft models, in which the tumour metastasises from local sites to the lung, spleen and lymph node, MGI-114 was shown to inhibit tumour growth and to prolong the life-span of tumour-bearing mice with an efficacy exceeding that of mitomycin C, cisplatin or paclitaxel [4,5]. MGI-114 also exhibited a remarkable *in vivo* antitumour activity against other human tumours, i.e. human MX-1 breast carcinoma and human HT-29 colon carcinoma with partial or complete tumour shrinkage [5]. Although the mechanism of the cytotoxic action of MGI-114 is unknown, it has recently been reported that MGI-114 possibly forms DNA monoadducts after being taken up by cells, induces secondary lesions in cellular DNA, which are probably apoptotic DNA strand breakage, and blocks the cell cycle in S phase [6].

Carcinoma of the lung is one of the leading causes of cancer deaths in the United States [7], and stomach and lung cancers are the leading types of cancer in Japan [8].

In this study, we examined the spectrum of *in vivo* antitumour activity of MGI-114 in a panel of human tumour xenograft models consisting mainly of human

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lung and stomach cancers and compared its efficacy with that of other conventional antitumour drugs.

2. Materials and methods

2.1. Animals

Female nude mice with BALB/c background (BALB/c nu/nu) were purchased from Charles River Japan, Inc. (Tokyo, Japan) and Clea Japan, Inc. (Tokyo, Japan). They were maintained under specific-pathogen-free conditions, and provided with sterile food and water *ad libitum*. Five- to 13-week-old BALB/c nude mice were used in the present study. All experiments were performed in compliance with the regulations of the Animal Experimentation Committee of Dainippon Pharmaceutical Co., Ltd.

2.2. Tumour cells and implantation

NCI-H460 (HTB-177), Calu-6 (HTB-56), NCI-H69 (HTB-119) and A-427 (HTB-53) lung carcinoma cells, Hs746T gastric carcinoma cells (HTB-135), KB nasopharyngeal epidermoid carcinoma cells (CCL-17) and WiDr colon adenocarcinoma cells (CCL-218) were obtained from the American Type Culture Collection (ATCC, Rockville, MD, USA). GT3TKB gastric adenocarcinoma cells (RCB0885) and HGC-27 gastric cancer cells (RCB0500) were obtained from Riken Gene Bank (Ibaragi, Japan). LX-1 lung carcinoma cells and MX-1 breast carcinoma cells were obtained from the Japanese Foundation for Cancer Research (Tokyo, Japan). MKN-45 gastric adenocarcinoma cells and HMV-2 melanoma cells were obtained from the Institute of Medical Sciences, University of Tokyo (Tokyo, Japan).

BALB/c nude mice were inoculated with $2.5–5\times10^6$ cells of KB, Hs746T, Calu-6, NCI-H460, A-427, GT3TKB, MKN-45, HMV-2 or WiDr cells, or a 2–3 mm square lobes of NCI-H69, HGC-27, MX-1 or LX-1 tumours. KB, MKN-45, HMV-2 and WiDr tumour cells were inoculated intradermally (i.d.), and the other tumour cells and tumour lobes were inoculated subcutaneously (s.c.).

2.3. Drugs

MGI-114 (6-hydroxymethylacylfulvene, HMAF) (Fig. 1) was supplied by MGI Pharma, Inc. (Minnetonka, MN, USA). Doxorubicin and 5-fluorouracil (5-FU) were purchased from Kyowa Hakko Kogyo Co., Ltd. (Tokyo, Japan). Cisplatin and etoposide were obtained from Nippon Kayaku Co., Ltd (Tokyo, Japan). Paclitaxel, irinotecan and vindesine were obtained from Sigma Co., Ltd (Tokyo, Japan), Yakult Honsha (Tokyo, Japan) and Shionogi Co., Ltd. (Osaka, Japan), respectively.

Varying amounts of MGI-114 were first dissolved in a small volume of absolute ethanol and then diluted either with distilled water in the case of intraperitoneal (i.p.) injection, unless otherwise stated, or with 5% (w/v) glucose solution in the case of intravenous (i.v.) injection. A final solution contained 1% (v/v) ethanol. MGI-114 solutions thus prepared were injected i.p. or i.v. into mice in a volume of 0.1 or 0.125 ml/10 g body weight. Vehicle solutions were prepared as above without MGI-114 and injected i.p. or i.v. to mice.

Cisplatin, doxorubicin, 5-FU, vindesine or irinotecan was dissolved in distilled water and administered i.v. to mice. Paclitaxel was first dissolved in a mixture of equal volumes of ethanol and Cremophor EL (Nacalai Tesque, Kyoto, Japan), further diluted with distilled water, and then administered i.v. to mice. All the above drugs were administered in a volume of 0.1 or 0.15 ml/10 g body weight.

2.4. In vivo *evaluation of drugs*

When the transplanted tumours have grown to approximately 5–12 mm in diameter, animals were divided according to tumour weight into test groups. Administration of MGI-114 or other antitumour drugs started from day 0.

Tumour weight was calculated according to the following formula:

$$\text{Tumour weight (mg)} = L \times W^2/2$$

where L and W represent the length (mm) and the width (mm) of the tumour mass, respectively.

The antitumour activity of MGI-114 and that of other conventional drugs used in this study was assessed 30–32 days after drug administration (end of all experiments) by the cured ratio (CR, number of mice cured/number of mice tested), inhibition rate (IR, %) and survival ratio (number of mice alive/number of mice tested).

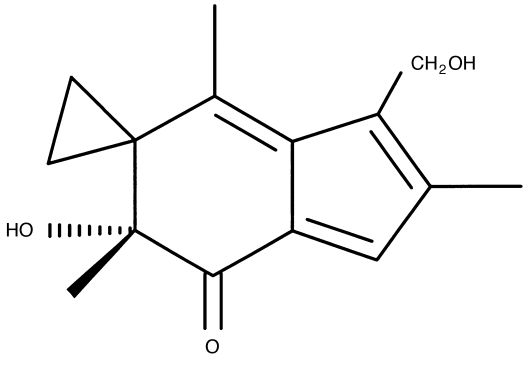

Fig. 1. Structure of MGI-114.

IR of tumour growth was calculated according to the following formula:

$$\text{IR}\ (\%) = \big(1 - \text{average tumour weight in treated group} / \text{average tumour weight in control group}\big) \times 100$$

The efficacy of a drug was considered to be significant when its inhibition rate was greater than 58% with no cytotoxic death of mice.

Generally, when mice lost weight by more than approximately 30% following drug administration, they tended to die. However, depending on the dose and administration schedule of MGI-114, some mice stayed alive even after body weight loss of more than 40%.

2.5. Statistical analysis

Statistical analysis by the Dunnett's multiple comparison test was performed to compare the means between the treated groups and the control groups. Statistically significant differences were identified by the Statistical Analysis System ver.6.12 (SAS Institute Inc.). Two-tailed P values <0.05 were considered to be statistically significant.

3. Results

3.1. Comparison of antitumour activity of MGI-114 according to various administration schedules and routes

In the first series of experiments, we examined the *in vivo* antitumour activity of MGI-114 in KB human nasopharyngeal carcinoma xenograft models and compared its efficacy according to the various administration schedules. KB tumour-transplanted nude mice were injected i.p. with MGI-114 (2.5, 5.0, 7.5 and 10 mg/kg) either daily for 5 consecutive days (Q1d×5) or five times at 7-day intervals (Q7d×5). The time course of MGI-114 antitumour activity is shown in Fig. 2.

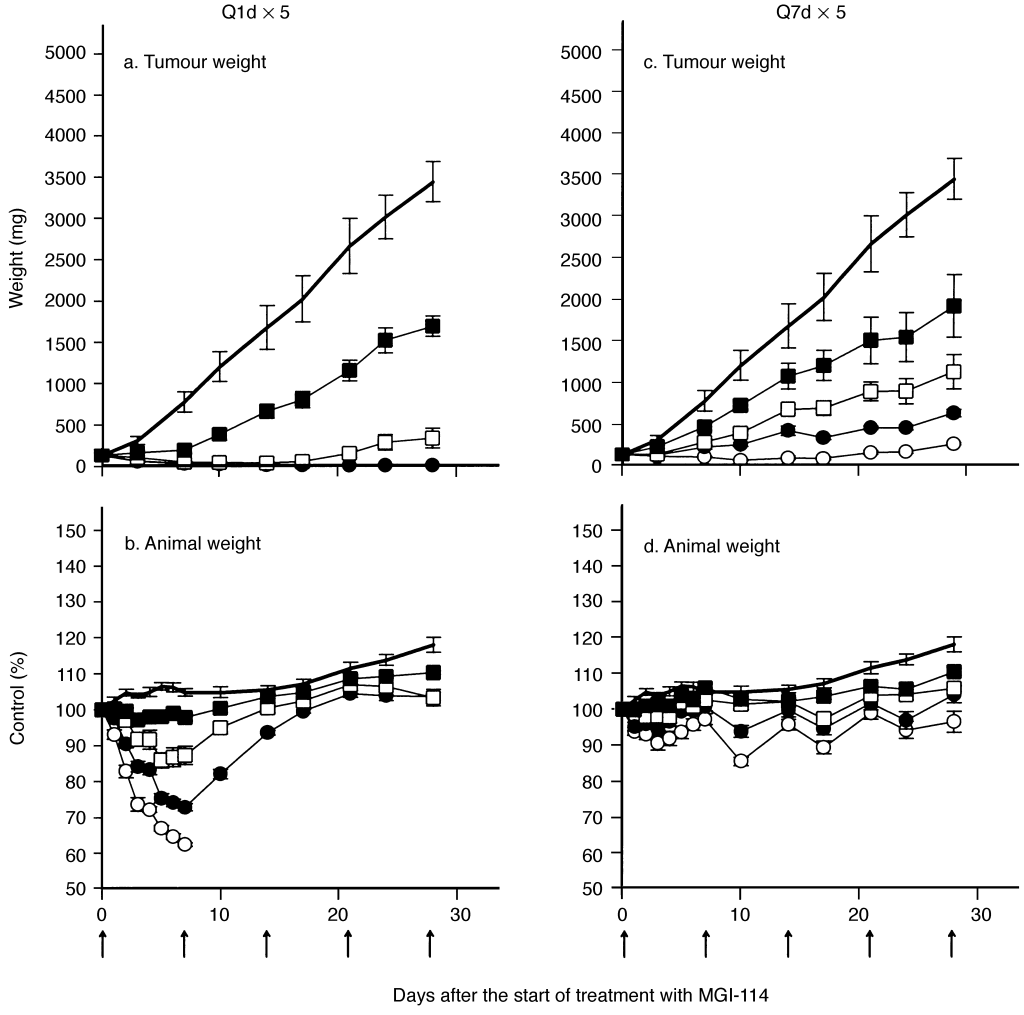

Fig. 2. Growth curves of the human KB nasopharyngeal carcinoma treated with MGI-114, intraperitoneally (i.p.) (no symbol, vehicle control; ■, 2.5 mg/kg; □, 5.0 mg/kg; ●, 7.5 mg/kg; ○, 10.0 mg/kg) (mean ±standard error of the mean, S.E.M.) and the change in body weight relative to body weight at day 0 (mean ±S.E.M.). Q1d×5 = daily for 5 consecutive days and Q7d×5 = five times at 7 day-intervals (see arrows).

MGI-114 (5.0, 7.5 mg/kg, i.p.) administered daily for 5 consecutive days demonstrated a strong antitumour activity evidenced by complete tumour regression. Animals lost weight for a few days after each MGI-114 injection, but rapidly recovered thereafter.

Next, we compared the efficacy of MGI-114 administered i.p. according to various schedules in KB tumour xenograft models. Results are shown in Table 1. When time intervals between each MGI-114 administration were compared, five injections of MGI-114 (7.5, 10 mg/

Table 1
In vivo antitumour activity of MGI-114 in KB nasopharyngeal xenograft models: comparison of administration schedules

Schedule[a]	Dose/injection (mg/kg)[b]	Total dose (mg/kg)	Inhibition rate (IR)[c] (%)	Cured ratio (CR)[c]	Survival ratio[c,d]
Q1d×5	10.0	50.0	–[e]	–	0/6
	7.5	37.5	100**	6/6	6/6
	5.0	25.0	88**	2/6	6/6
	2.5	12.5	46**	0/6	6/6
(Q8h×2)×5	5.0	50.0	100**	6/6	6/6
	3.75	37.5	100**	6/6	6/6
	2.5	25.0	74**	0/6	6/6
	1.25	12.5	38**	0/6	6/6
Q3d×5	10.0	50.0	100**	6/6	6/6
	7.5	37.5	92**	1/6	6/6
	5.0	25.0	64**	0/6	6/6
	2.5	12.5	28**	0/6	6/6
Q7d×5	10.0	50.0	94**	0/6	6/6
	7.5	37.5	83**	0/6	6/6
	5.0	25.0	70**	0/6	6/6
	2.5	12.5	45**	0/6	6/6
Q1d×3	12.5	37.5	–	–	0/6
	10	30.0	100**	4/4	4/6
	8.33	25.0	98**	4/6	6/6
	7.5	22.5	99**	5/6	6/6
	5.0	15.0	61**	0/6	6/6
	4.17	12.5	55**	0/6	6/6
	2.5	7.5	33*	0/6	6/6
Q1d×10	5.0	50.0	100**	6/6	6/6
	3.75	37.5	94**	2/6	6/6
	2.5	25.0	60**	0/6	6/6
	1.25	12.5	38*	0/6	6/6

[a] Q1d×3, Q1d×5 or Q1d×10 indicates daily injection for 3, 5 or 10 consecutive days. Q3d×5 or Q7d×5 indicates five injections at 3 or 7 day-intervals. (Q8h×2)×5 indicates injection twice a day at 8 h-intervals for 5 consecutive days.
[b] MGI-114 was injected intraperitoneally (i.p.).
[c] Inhibition rate, cured ratio and survival ratio were determined 31 days after the start of the administration of MGI-114.
[d] Number of mice alive/number of mice tested.
[e] Cytotoxic death.
*$P<0.05$, **$P<0.001$, significantly different from the control group value.

Table 2
In vivo antitumour activity of MGI-114 in KB nasopharyngeal carcinoma xenograft models: Comparison of administration routes

Schedule[a]	Route[b]	Dose/injection (mg/kg)	Inhibition rate (IR)[c] (%)	Cured ratio (CR)[c]	Survival ratio[c,d]
Q1d×5	Intravenous (i.v.)	7.5	100*	6/6	6/6
		5.0	96*	4/6	6/6
		2.5	54*	0/6	6/6
Q1d×5	Intraperitoneal (i.p.)	7.5	100*	6/6	6/6
		5.0	82*	2/6	6/6
		2.5	38*	0/6	6/6

[a] Q1d×5 indicates daily injection for 5 consecutive days.
[b] In this experiment, MGI-114 was prepared as follows: MGI-114 was dissolved in absolute ethanol and further diluted with 5% (w/v) glucose solution in both cases of intravenous (i.v.) and intraperitoneal (i.p.) injections.
[c] Inhibition rate, cured ratio and survival ratio were determined 31 days after the start of the administration of MGI-114.
[d] Number of mice alive/number of mice tested.
*$P<0.001$, significantly different from the control group value.

kg) at 3 day-intervals (Q3d×5) were more effective than 5 injections at 7 day-intervals (Q7d×5), but less effective than daily administration of MGI-114 (2.5, 5.0 and 7.5 mg/kg) for 5 consecutive days (Q1d×5) (Table 1). When administration periods were compared, and in terms of dose per single injection, daily administration of MGI-114 for 10 consecutive days (Q1d×10) showed a higher antitumour efficacy than daily administration for 5 consecutive days (Q1d×5). However, in terms of the total amount of MGI-114 administered, daily administration for 10 days (Q1d×10) was less effective than the daily administration for 5 consecutive days (Q1d×5) (Table 1). As MGI-114 has a short half-life, i.e. approximately 5 min in mice, we examined its antitumour efficacy administered twice a day at 8 h-intervals for 5 consecutive days [(Q8h×2)×5]. In terms of the total amount of drug injected, MGI-114 administered twice a day for 5 consecutive days showed almost the same antitumour efficacy as daily administration for 5 consecutive days (Q1d×5) (Table 1).

These results demonstrate that MGI-114 has a marked antitumour effect in KB tumour xenograft models and that daily administration of MGI-114 is more effective than intermittent administrations such as Q7d×5.

In the next experiment, we compared the antitumour activity of MGI-114 in KB tumour xenograft models according to the different administration routes. As shown in Table 2, MGI-114 administered both i.v. and i.p. produced approximately the same level of antitumour activity.

3.2. Antitumour activity of MGI-114 in three human tumour xenograft models

The *in vivo* antitumour activity of MGI-114 was evaluated in three additional human tumour xenograft models, the MX-1 breast carcinoma, HMV-2 melanoma and WiDr colon adenocarcinoma. MGI-114 (2.5, 5.0 and 7.5 mg/kg, i.p.) was administered once a day for 5 consecutive days (Q1d×5). As shown in Table 3, MGI-114 produced a marked antitumour activity with complete tumour regression observed in some mice against all three tumours.

3.3. Antitumour activity of MGI-114 against human lung tumours

The *in vivo* antitumour activity of MGI-114 was also evaluated against five human lung tumour xenograft models and its efficacy was compared with that of other conventional antitumour drugs. Among the lung tumours tested in this experiment were the non-small cell lung cancers (NSCLC) NCI-H460, Calu-6 and A-427 and the small-cell lung cancers (SCLC) NCI-H69 and LX-1. Conventional antitumour drugs used in this experiment were irinotecan, paclitaxel, cisplatin, doxorubicin, vindesine and etoposide. These drugs are clinically used for chemotherapy of lung cancers. MGI-114 was administered i.v. or i.p. Q1d×5 at a dose of 2.5–7.5 mg/kg. Doses and administration schedules for conventional antitumour drugs were determined according to data from the literature [9–14] so as to have a maximum antitumour response for each drug. Results are shown in Table 4. MGI-114 showed a significant antitumour activity (i.e. inhibition rate greater than 58% with no cytotoxic death of mice) against four lung tumours (i.e. NCI-H460, Calu-6, NCI-H69 and A-427) with complete tumour regression being observed in some mice in all four tumour xenograft models. However, MGI-114 antitumour activity against the LX-1 lung tumour was not significant. Paclitaxel showed a high antitumour activity against two out of three tumours with complete tumour regression in NCI-H69 xenograft models. Irinotecan also showed a high antitumour activity against two out of three tumours, however, no complete tumour regression was observed

Table 3
In vivo antitumour activity of MGI-114 in human tumour xenograft models

Cell lines	Schedule[a]	Dose/injection (mg/kg)[b]	Inhibition rate (IR)[c] (%)	Cured ratio (CR)[c]	Survival ratio[c,d]
MX-1 breast carcinoma	Q1d×5	7.5	100**	7/7	7/7
		5.0	95**	2/7	7/7
		2.5	53**	0/7	7/7
HMV-2 melanoma	Q1d×5	7.5	100**	7/7	7/7
		5.0	100**	7/7	7/7
		2.5	50*	1/7	7/7
WiDr colon carcinoma	Q1d×5	7.5	99**	3/7	7/7
		5.0	66*	0/7	7/7
		2.5	21	0/7	7/7

[a] Q1d×5 indicates daily injection for 5 consecutive days.
[b] MGI-114 was injected intraperitoneally (i.p.).
[c] Inhibition rate, cured ratio and survival ratio were determined 31–32 days after the start of the administration of MGI-114.
[d] Number of mice alive/number of mice tested.
*$P<0.05$; **$P<0.001$, significantly different from the control group value.

except in one mouse in the Calu-6 xenograft models. Cisplatin, in contrast, showed a high activity against one out of three tumours tested, i.e. against Calu-6 tumours where complete tumour regression was observed in some mice, but cytotoxic death was also noted. Doxorubicin showed a significant activity against two tumours tested, with no complete tumour regression. Vindesine showed a significant antitumour activity against two tumours tested with complete tumour regression in the Calu-6 xenograft models. Etoposide did not show any antitumour activity against the NCI-H69 tumours. The antitumour activity of MGI-114 was then compared with that of other conventional antitumour drugs used in the present study. Results of this comparison are summarised in Table 5. Overall, MGI-114 generally had an antitumour efficacy higher than that of other antitumour drugs in the human lung xenograft models examined.

Table 4
In vivo antitumour activity of MGI-114 and other conventional antitumour drugs in human lung tumours

Cell lines	Antitumour drugs	Schedule[a]	Dose/injection (mg/kg)[b]	Inhibition rate (IR)[c] (%)	Cured ratio (CR)[c]	Survival ratio[c,d]
NCI-H460	MGI-114	Q1d×5	7.5	100**	7/7	7/7
			5.0	100**	5/7	7/7
			2.5	67**	0/7	7/7
	Irinotecan	Q4d×4	60	55**	0/7	7/7
			30	50**	0/7	7/7
	Paclitaxel	Q4d×3	22	41**	0/7	7/7
			11	26*	0/7	7/7
	Cisplatin	Q1d	12	49**	0/7	7/7
		Q1d× 5	2.5	47**	0/7	7/7
	Doxorubicin	Q1d	16	71**	0/3	3/7
		Q4d× 3	7.5	65**	0/7	7/7
			5	42**	0/7	7/7
	Vindesine	Q7d× 4	3	61**	0/7	7/7
Calu-6	MGI-114	Q1d× 5	7.5	100**	4/7	7/7
			5.0	93**	0/7	7/7
			2.5	70**	0/7	7/7
	Irinotecan	Q4d× 4	60	94**	0/7	7/7
			30	93**	1/7	7/7
	Paclitaxel	Q4d× 3	22	89**	0/7	7/7
			11	41*	0/7	7/7
	Cisplatin	Q1d	12	88**	0/6	6/7
			8	74**	0/7	7/7
		Q1d× 5	5	99**	3/5	5/7
			2.5	84**	0/7	7/7
	Doxorubicin	Q1d	16	85*	0/4	4/7
			12	47*	0/7	7/7
		Q4d×3	7.5	80**	0/7	7/7
			5	75**	0/7	7/7
	Vindesine	Q7d× 4	3	100**	6/7	7/7
NCI-H69	MGI-114	Q1d×5	7.5	90**	1/7	7/7
			5.0	80**	0/7	7/7
			2.5	58*	0/7	7/7
	Irinotecan	Q4d× 4	100	94**	0/6	6/6
			60	86**	0/6	6/6
	Paclitaxel	Q4d×3	33	100**	5/6	6/6
			22	92**	2/7	7/7
	Cisplatin	Q1d	12	51	0/6	6/6
	Etoposide	Q1d×5	12.5	0	0/6	6/6
A-427	MGI-114	Q1d×5	7.5	85*	2/6	6/6
LX-1	MGI-114	Q1d×5	7.5	22	0/6	6/7
			5.0	15	0/7	7/7
			2.5	15	0/7	7/7

[a] Q1d indicates a single injection. Q1d×5 indicates daily injection for 5 consecutive days. Q4d×3 or Q4d×4 indicates three or four injections at 4 day-intervals. Q7d×4 or Q7d×7 indicates four injections at 7 day-intervals.

[b] MGI-114 was injected either intravenously (i.v.) (NCI-H460, NCI-H69, A-427) or intraperitoneally (i.p.) (Calu-6, LX-1). Other antitumour drugs were injected i.v.

[c] Inhibition rate, cured ratio and survival ratio were determined 30–32 days after the start of the administration of MGI-114 or other antitumour drugs.

[d] Number of mice alive/number of mice tested.

*$P<0.05$; **$P<0.001$, significantly different from the control group value.

3.4. Antitumour activity of MGI-114 against human gastric tumours

The *in vivo* antitumour activity of MGI-114 was also evaluated against four human gastric tumour xenografts and its efficacy was compared with that of the other reference antitumour drugs. MGI-114 was administered i.v. or i.p. Q1d×5 at a dose of 2.5–7.5 mg/kg. Doses and administration schedules for the reference antitumour drugs were determined according to data from the literature [9–14] so as to have the maximum antitumour response for each drug. Results are shown in Table 6. MGI-114 showed a significant antitumour activity against three gastric tumours, i.e. Hs746T, GT3TKB and HGC-27 with complete tumour regression being observed for some mice in Hs746T and GT3TKB tumour xenograft models. However, MGI-114 did not show a significant antitumour activity against the MKN-45 tumours. In Hs746T tumour xenograft models, MGI-114 and the other antitumour drugs tested, except 5-FU, showed a marked antitumour activity and produced a complete tumour regression in some mice. In GT3TKB tumour xenograft models, MGI-114, irinotecan and paclitaxel showed a significant antitumour activity. In HGC-27 tumour xenograft models, MGI-114, irinotecan and paclitaxel showed a significant antitumour activity with no complete tumour regression. A comparison of MGI-114 antitumour activity with that of other conventional antitumour drugs is listed in Table 5. Overall, the efficacy of MGI-114 was higher than or almost equivalent to that of other conventional antitumour drugs in the human gastric xenograft models GT3TKB and Hs746T. It showed a lower activity in the HGC-27 tumours.

4. Discussion

In this study, we demonstrated that MGI-114 has a strong *in vivo* antitumour activity against a wide spectrum of human tumours. The *in vivo* efficacy of MGI-114 has previously been reported in human xenograft models of MX-1, HT-29 and MV-522 tumours [4,5]. In particular, MGI-114 has been reported to have a strong antitumour activity against MV-522 tumours against which many conventional antitumour drugs, except mitomycin C and paclitaxel, have failed to show significant efficacy [4,5]. Moreover, MGI-114 has recently been shown to induce tumour regression, including complete regression, in MV522/mdr1 xenograft models [15]. MV522/mdr1 cells are an mdr1/gp170-positive clone of MV522 generated by transfecting an expression vector containing the cDNA encoding for the human gp170 protein, which has been shown to be resistant to mitomycin C and paclitaxel *in vivo* [15]. In the present study, we have confirmed and extended previous observations on the *in vivo* antitumour efficacy of MGI-114 using various human xenograft models. In human lung

Table 5
Comparison of the *in vivo* antitumour activity of MGI-114 with that of conventional antitumour drugs in lung and gastric tumour xenograft models

Types	Cell lines	Antitumour activity of MGI-114			
		Lower than	Slightly lower than or equivalent to	Slightly higher than or equivalent to	Higher than
Lung tumour	Calu-6		Vindesine		Irinotecan Paclitaxel Cisplatin Doxorubicin
	NCI-H460				Irinotecan Paclitaxel Cisplatin Doxorubicin Vindesine
	NCI-H69	Paclitaxel	Irinotecan		Cisplatin Etoposide
Gastric tumour	GT3TKB			Paclitaxel	Irinotecan Cisplatin Doxorubicin 5-Fluorouracil
	Hs746T			Irinotecan Doxorubicin	Paclitaxel Cisplatin 5-Fluorouracil
	HGC-27	Irinotecan Paclitaxel			

tumour xenograft models, we have shown that MGI-114 has a strong antitumour efficacy with complete tumour regression being observed (Table 4). It is known that NSCLC are highly resistant to the available antitumour agents. However, in the panel of five human lung tumours examined this study, three tumours were NSCLC and MGI-114 showed significant antitumour activity, including complete tumour regression, against these NSCLC. It has previously been reported that in a panel of six human lung tumour xenografts, paclitaxel was more effective and had a wider spectrum of antitumour activity than cisplatin [16]. In agreement with this finding, we have found that in most of the human lung tumour xenografts, paclitaxel and irinotecan exhibited a strong antitumour activity higher than or almost equivalent to that of cisplatin or doxorubicin (Table 4). Moreover, we have demonstrated that the overall efficacy of MGI-114 is generally higher than that of all other conventional antitumour drugs used in the present study (Table 5). We have also shown that MGI-114 has a strong antitumour activity against gastric tumours with complete regression in some human gastric

Table 6
In vivo antitumour activity of MGI-114 and other conventional antitumour drugs in human gastric tumours

Cell lines	Antitumour drugs	Schedule[a]	Dose/injection (mg/kg)[b]	Inhibition rate (IR)[c] (%)	Cured ratio (CR)[c]	Survival ratio[c,d]
Hs746T	MGI-114	Q1d×5	7.5	100†	7/7	7/7
			5.0	100†	7/7	7/7
			2.5	96†	3/7	7/7
	Irinotecan	Q4d×4	60	100†	7/7	7/7
			30	99†	5/7	7/7
	Paclitaxel	Q4d× 3	22	81†	2/7	7/7
			11	73*	2/7	7/7
	Cisplatin	Q1d	12	58*	1/7	7/7
		Q1d× 5	2.5	81*	0/7	7/7
	Doxorubicin	Q1d	16	98†	1/6	6/7
			12	95†	2/7	7/7
		Q4d×3	7.5	100†	7/7	7/7
			5	95†	3/7	7/7
	5-Fluorouracil	Q1d× 5	40	–[e]	–	0/7
			20	2	0/7	7/7
GT3TKB	MGI-114	Q1d×5	7.5	89†	1/7	7/7
			5.0	60*	0/7	7/7
			2.5	45	0/7	7/7
	Irinotecan	Q4d× 4	60	58*	0/6	6/7
			30	29	0/7	7/7
	Paclitaxel	Q4d×3	22	76*	1/7	7/7
			11	16	0/7	7/7
	Cisplatin	Q1d	12	−2	0/7	7/7
		Q1d× 5	2.5	53	0/7	7/7
	Doxorubicin	Q1d	16	55	0/7	7/7
	5-Fluorouracil	Q1d×5	20	42	0/6	6/6
HGC-27	MGI-114	Q1d×5	7.5	68*	0/7	7/7
			5.0	52*	0/7	7/7
			2.5	57*	0/7	7/7
	Irinotecan	Q4d×4	120	93*	0/2	2/6
			60	87*	0/7	7/7
	Paclitaxel	Q4d×3	55	97†	0/4	4/6
			44	98†	0/5	5/6
			22	92*	1/7	7/7
MKN-45	MGI-114	Q1d×5	7.5	38	0/6	6/6
			5.0	18	0/7	7/7
			2.5	38	1/7	7/7

[a] Q1d indicates a single injection. Q1d×5 indicates daily injection for 5 consecutive days. Q4d×3 or Q4d×4 indicates three or four injections at 4 day-intervals.

[b] MGI-114 was injected either intravenously (i.v.) (Hs746T, GT3TKB, HGC-27) or intraperitoneally (i.p.) (MKN-45). Other antitumour drugs were injected i.v.

[c] Inhibition rate, cured ratio and survival ratio were determined 30–32 days after the start of the administration of MGI-114 or other antitumour drugs.

[d] Number of mice alive/number of mice tested.

[e] Cytotoxic death.

*$P<0.05$, †$P<0.001$, significantly different from the control group value.

tumour xenograft models (Table 6). Moreover, the efficacy of MGI-114 against gastric tumours was comparable to that of the other antitumour drugs used in the present study (Table 5). However, the *in vivo* antitumour activity of MGI-114 against LX-1 and MKN-45 was low (Tables 4 and 6). To our knowledge, there is no report on the mechanism of tumour resistance to MGI-114, the reason for the low efficacy of MGI-114 against LX-1 and MKN-45 is not clear. However, according to Woynarowska and colleagues [17], tumour cell lines with a low sensitivity to MGI-114 have a low ability to uptake MGI-114 into the cells compared with tumour cell lines with a high sensitivity to MGI-114. It may therefore be interesting to examine the ability of LX-1 and MKN-45 cells to uptake MGI-114.

Administration schedules of MGI-114 were also examined in detail in this study (Table 1). We have found that daily administration of MGI-114 for 5 consecutive days (Q1d×5) was more effective than intermittent administrations (Q3d×5 or Q7d×5). Moreover, in terms of the total amount of MGI-114 injected, administration of MGI-114 twice a day at 8 h-interval for 5 consecutive days [(Q8h×2)×5] had a similar efficacy as the administration of MGI-114 once a day for 5 consecutive days (Q1d×5). This last observation may be associated with the *in vivo* antitumour effect of MGI-114. Pharmacokinetic study of MGI-114 was carried out and the following results were obtained (data not shown): (a) the plasma half-life of MGI-114 is short, i.e. approximately 5 min, (b) plasma concentration of MGI-114 after i.v. injection of MGI-114 is dependent on the dose injected, i.e. C_{5min} (concentrations of MGI-114 5 min after its i.v. injection). This plasma concentration is 448 and 7867 ng/ml for administration doses of 1 and 12.9 mg/kg, respectively. (c) $AUC_{0-\infty}$ (area under the plasma concentration–time) after i.v. injection of MGI-114 is dependent on the dose of MGI-114, i.e. 8924 and 136,420 ng min/ml for administration doses of 1 and 12.9 mg/kg, respectively. From these parameters, it can be suggested that, the total dose of MGI-114 injected per day (or over 5 days) is the same, C_{5min} in the group treated twice a day is approximately half of that in the group treated once a day, whereas AUC in the group treated twice a day is almost the same as that in the group treated once a day for 5 days. This indicates that the *in vivo* antitumour activity of MGI-114 is likely to be dependent on the AUC, but not on the concentration of MGI-114 in the blood-circulation. AUC-dependency of MGI-114 antitumour activity is further suggested by an *in vitro* cytotoxic study. Nakai and colleagues [18] showed that various conventional antitumour drugs can be grouped into two types with respect to cytotoxic action *in vitro*. These types are cell cycle phase non-specific (type I) and specific (type II). The cytotoxic activity of type I drugs which includes alkylating agents and antitumour antibiotics is AUC-dependent. However, the cytotoxic activity of type II drugs which includes antimetabolites and vinca alkaloids is time-dependent. Our results show that the cytotoxic action of MGI-114 is AUC-dependent and that MGI-114 belongs to the type I group (data not shown). Covalent binding of radiolabelled equivalents of illudin S and acylfulvene to DNA in tumour cell lines has been previously published [2,19] and covalent binding of radiolabelled equivalents of MGI-114 to DNA, RNA and proteins in tumour cells has recently been demonstrated [20]. These findings indicate that molecules of this class have alkylating activity.

In the present study, we compared the efficacy of MGI-114 given either i.p. or i.v. in the KB xenograft model and found that there were no major differences in efficacy between these two routes of administration, although more complete tumour regressions were observed at the 5 mg/kg dose level with the i.v. administration (Table 2). This result is comparable to a previous report [5] utilising MX-1 xenograft models in which a somewhat greater efficacy was observed with i.v. versus i.p. administration.

In conclusion, the present study clearly demonstrates a high antitumour activity of MGI-114 in several human tumour xenograft models including lung, gastric, breast, colon and nasopharyngeal tumours and melanoma. MGI-114 is currently in phase II clinical trials in the United States and represent a promising new chemotherapy for the treatment of a wide spectrum of human cancers, including lung and gastric tumours.

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