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Responsiveness of Human Gastric Tumors Implanted in Nude Mice to Clinically Equivalent Doses of Various Antitumor Agents

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To reproduce clinical effects of various antitumor agents in the human tumor/nude mouse model, we investigated the responsiveness of 11 lines of human gastric tumor xenografts to doses of the agents pharmacokinetically equivalent to the respective clinical doses, which we designated the "rational dose" (RD). We found that the response rates to mitomycin C, 3-[(4-amino-2-methyl-5-pyrimidinyl]methyl-1-[2-chloroethyl]-1-nitrosourea (ACNU), adriamycin, 5-fluorouracil were 18%, and that to vinblastine was 30%; on the other hand, those to vincristine, methotrexate, and cyclophosphamide were poor. In contrast, in our previous study using the maximum tolerated doses, response rates to mitomycin C, ACNU, and vinblastine were as high as 64–82%, and those to adriamycin and 5-fluorouracil were 18%. When these results were compared with the clinical response rates of gastric tumors, as a whole, the results with RD's exhibited much better coincidence with the clinical data in terms of relative therapeutic potency, indicating the validity of the use of clinically equivalent doses instead of maximum tolerated doses in the human tumor model.

Key words: Human gastric tumors — Nude mouse — Response rates — "Rational dose" — Clinical predictability

In our study on experimental chemotherapy against a panel of human gastric tumors implanted in nude mice,[1)] we evaluated the antitumor effects in terms of response rates as in clinical chemotherapy, because it seemed difficult to reasonably evaluate the antitumor effect of a given agent by using a few tumor lines. However, even in such trials, we failed to reproduce the relative effectiveness of various antitumor agents which had been observed in clinical treatment of gastric tumors. These results strongly suggested to us the importance of using a dose of each antitumor agent pharmacologically equivalent to its clinical dose. In the preceding paper,[2)] therefore, we described our attempt to find a dose that could reproduce in the nude mouse the clinically achievable plasma level of each drug, because such a dose, denoted as the "rational dose," was considered to be most suitable for the treatment of human tumor-bearing nude mice.

In the present study, we carried out experimental chemotherapy using the RD as a therapeutic dose against a panel of human gastric tumors implanted in nude mice and examined whether treatment with the RD could reproduce the clinical effectiveness of various antitumor agents in terms of response rate.

MATERIALS AND METHODS

Antitumor Agents VLB[*5] and VCR (Shionogi & Co., Osaka) and MTX (Lederle Japan Ltd., Tokyo) for clinical use were purchased. 5-FU, MMC and ADR were kindly supplied by Kyowa Hakko Kogyo Co., Tokyo, as pure crystals for

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[*5] Abbreviations used: VLB, vinblastine; VCR, vincristine; CPM, cyclophosphamide; 5-FU, 5-fluorouracil; MTX, methotrexate; MMC, mitomycin C; ADR, adriamycin; ACNU, 3-[(4-amino-2-methyl-5-pyrimidinyl]methy-1-[2-chloroethyl]-1-nitrosourea hydrochloride; DDP, cisplatin; MTD, maximum tolerated dose; RD, rational dose.

experimental use. CPM and ACNU were provided by Shionogi & Co. and Sankyo Co., Ltd., Tokyo, respectively, in a form for experimental use.

All drugs were dissolved in sterile 0.85% NaCl solution just before use.

The MTD's of all these drugs were determined as maximum non-lethal doses by single intravenous injection (daily administration for 5 days only in the case of 5-FU and MTX). The test doses were increased at a constant ratio of 1.2.

Human Tumor Xenograft Lines Eleven human gastric tumors established as xenografts in nude mice were used. Their characteristics, including histological types, prior chemotherapy, and growth rates, were presented in the previous paper.[1] NS-3 and NS-8 lines were kindly supplied by Dr. K. Nakatani, Nara Medical College, Nara, and St-4 and St-40, by Dr. T. Kubota, Keio University, Tokyo.

These tumors have been maintained by serial subcutaneous transplantation of 2×2×2 mm cubic fragments in the right subaxillary region of athymic BALB/c-*nu/nu* mice (Clea Japan, Inc., Tokyo) approximately every month.

Mice were housed in ethylene oxide-sterilized filter-capped cages with ^{60}Co-irradiated (3 megarad) food and autoclaved water *ad libitum*. All cages were kept in laminar-air-flow units in our laboratory. Six- to eight-week-old female mice weighing about 25 g were used.

Measurement of Tumor Size After the transplantation, the mice were observed and randomized into several experimental groups consisting of 6 animals each after the tumors had reached palpable size. The tumor volume (V) was calculated by means of the equation

$$V = 1/2 \times a \times b^2$$

where a and b are the experimental measurements in mm of length and width, respectively. Each tumor volume was then expressed as relative tumor volume (RV),

$$RV = V_n/V_0$$

where V_n is the tumor volume at day n and V_0 is the initial tumor volume at the time when the treatment was started (day 0).

Chemotherapy When the tumor volume reached 100–300 mm^3, chemotherapy was initiated. The RD's or MTD's determined in advance were used as therapeutic doses. Drugs were given intravenously by daily injection for five days in the case of 5-FU and MTX or by single administration for all other drugs. The dose of each drug (determined as RD in the preceding study[2]) was as follows: MMC, 1.7 (mg/kg); CPM, 65; ADR, 12; VCR, 0.4; VLB, 2.6; 5-FU, 19; MTX, 15. ACNU was given as 3 intermittent injections of 8 mg/kg (0 min), 2 (25 min) and 0.8 (70 min) in one day. Observation was continued for 3–4 weeks.

Evaluation At any given experimental day, T/C (%) was expressed as the average of RV of the

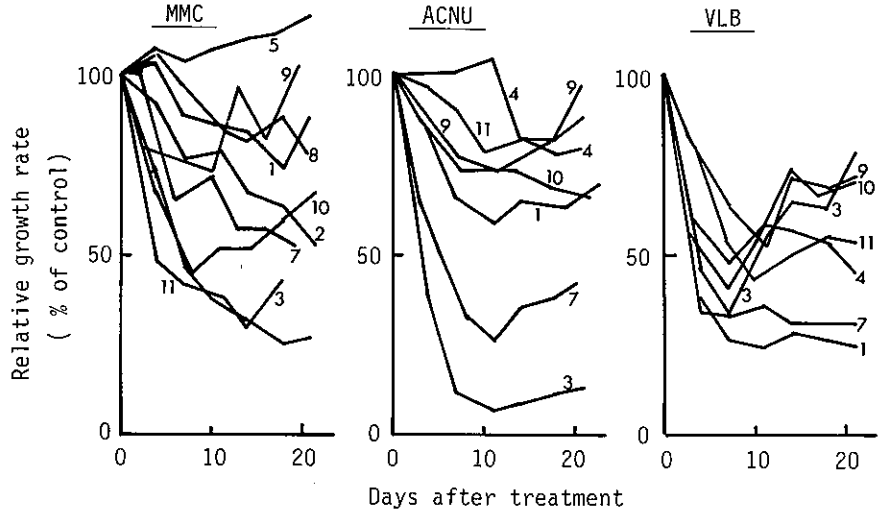

Fig. 1. Responsiveness to "rational doses" of MMC, ACNU, and VLB of a panel of human gastric tumor xenografts. MMC, ACNU, or VLB was intravenously injected at its "rational dose" when the tumor size had reached 100–300 mm^3. Tumor sizes were measured with calipers twice a week, and relative growth rates were obtained according to the procedure described in "Material and Methods." Tumor lines are indicated by numbers as follows: 1, SC-2; 2, SC-4; 3, SC-6; 4, St-4; 5, NS-3, 6, NS-8; 7, 4-1ST; 8, SC-7; 9, SC-9; 10, St-40; 11, St-15.

treated mice with respect to the control. The effectiveness of each drug was evaluated in terms of the T/C(%) value at day 14. Evaluation as "effective" was based on a T/C(%) of 50% or less, with statistical significance as determined by the Mann-Whitney U-test ($P<0.01$, one-sided).

Results

Responses of human gastric tumors to RD's of MMC, ACNU, and VLB are presented in Fig. 1; these drugs demonstrated potent therapeutic effectiveness against most of these tumors when the MTD's of the drugs were employed.[1] Since their RD's were found to be much lower than the MTD's,[2] tumors which did not significantly respond to the MTD of the drug were expected to be unresponsive to its RD as well. Therefore, in Fig. 1, only the responses to the RD's of those tumors that had responded positively to the MTD's are shown.

With MMC, only 2 out of 9 tumor lines examined exhibited a statistically significant decrease in growth rate to 50% or less of the control. In the case of ACNU, 2 out of 7 tumor lines responsive to its MTD also significantly responded to its RD. On the other hand, 3 out of 7 tumor lines were responsive to VLB at its RD, although responsiveness of St-15 was at a relatively low level.

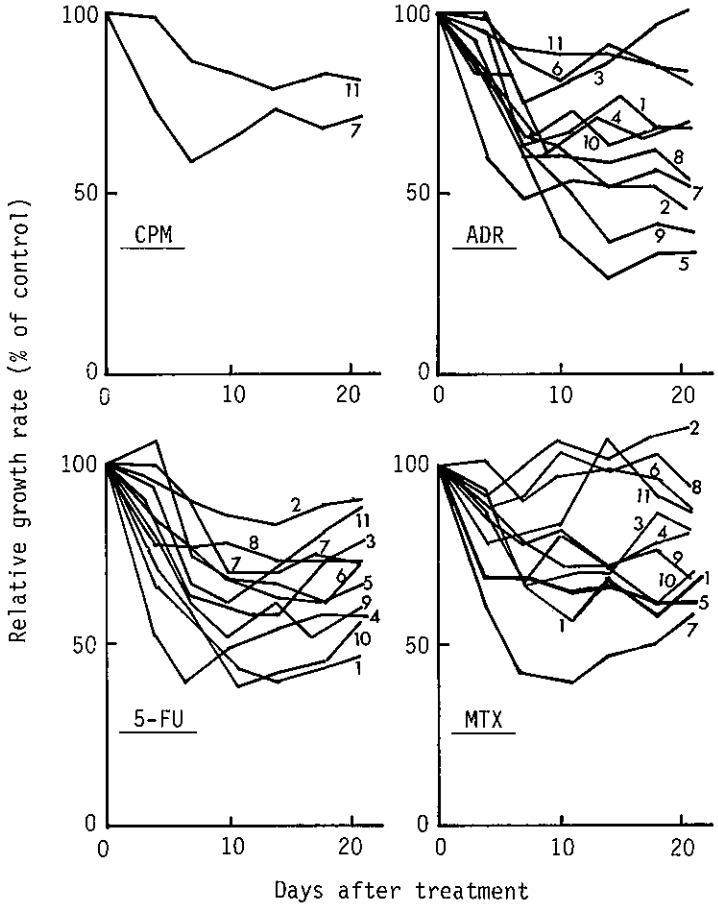

Fig. 2. Responsiveness to "rational doses" of CPM, ADR, 5-FU and MTX of a panel of human gastric tumor xenografts. Experimental conditions and numbering of tumor lines were the same as shown in Fig. 1, except for 5 daily injections of 5-FU and MTX.

In Fig. 2, tumor responses to the other 4 drugs are presented. The tumor lines did not respond well to CPM even at its MTD, and neither of 2 tumor lines that responded to its MTD was responsive to its RD. Since the RD of ADR was estimated to be approximately equivalent to its MTD,[2] responses of all 11 human gastric tumor lines to 12 mg/kg of ADR are shown. Only two of the lines exhibited significant responses. When plasma levels of 5-FU and MTX were compared between man and nude mouse, the level of neither agent in the nude mouse reached those levels found in human patients given the clinical dose, even when the MTD's were injected into the animals.[2] Therefore, in the case of these drugs, it was impossible to observe the responses to their RD's. Alternatively, we were obliged to examine the responses to their MTD's. Two and 1 out of 11 tumor lines showed significant responses to 5-FU and MTX, respectively, although their growth-inhibitory effects were not so remarkable.

With VCR, as previously reported,[1] none of the human gastric tumors responded even to its MTD. Since its RD is estimated to be one-fourth of the MTD,[2] the tumors would be expected to be unresponsive to its RD as well. Therefore, the experiment with the RD of VCR was not done.

Table I. Responses of a Panel of Human Gastric Tumor Xenografts to Rational Doses of Various Antitumor Drugs

Human tumor line	Relative tumor growth rate (T/C%)[a]							
	MMC	CPM	ACNU	ADR	VCR	VLB	5-FU	MTX
SC-2	84	(−)[b]	65	76	(−)	28[c]	40	67
SC-4	66	(−)	(−)	51	(−)	(−)	83	101
SC-6	30	(−)	8	87	(−)	65	58	78
St-4	(−)	(−)	81	72	(−)	56	55	72
NS-3	110	(−)	(−)	27	(−)	(−)	63	71
NS-8	(−)	(−)	(−)	91	(−)	(−)	66	98
4-1ST	57	73	35	51	(−)	32	70	46
SC-7	81	(−)	(−)	58	(−)	(−)	74	98
SC-9	97	(−)	77	37	(−)	72	61	70
St-40	52	(−)	74	63	(−)	74	42	68
St-15	32	79	83	88	(−)	50	72	109

a) Determined as ratio (%) of mean relative tumor volume of the treated group to that of the untreated one at day 14 (see "Materials and Methods" for details).
b) Judged "ineffective" from data on response to MTD.
c) Underlined values are "effective" according to our evaluation criteria: T/C value of 50% or less with a statistical significance by the Mann-Whitney U-test ($P<0.01$, one-sided).

Table II. Comparison of Experimental and Clinical Response Rates of Gastric Tumors to Various Antitumor Agents

Antitumor agent	Experimental response rate (%)		Clinical[3,4] response rate (%)
	MTD	RD	
MMC	82 (9/11)	18 (2/11)	31 (70/227)
CPM	18 (2/11)	0 (0/11)	7 (5/72)
ACNU	64 (7/11)	18 (2/11)	11 (4/37)
ADR	18 (2/11)	18 (2/11)	18 (38/208)
VCR	0 (0/11)	0 (0/11)	0 (0/11)
VLB	64 (7/11)	30 (3/11)	—
5-FU	18 (2/11)	18 (2/11)	23 (122/527)
MTX	9 (1/11)	9 (1/11)	10 (3/30)

Efficacy of each drug at its RD against each tumor line was expressed as T/C(%) of growth rate on day 14, and evaluation of effectiveness according to our criteria was also made (Table I). From these data, effectiveness of various antitumor agents examined at their RD was obtained as a response rate. Those values are shown together with response rate to the MTD,[1] and both are compared with clinical response rate cited from other reports (Table II).[3,4] It is clear that response rates to RD's are in much better accordance with the clinical response rates than are the experimental response rates to MTD's.

Discussion

In clinical trials on gastric tumors, relatively high response rates were observed with MMC, 5-FU, and ADR (Table II). As a matter of fact, these agents are the ones most frequently used for clinical treatment of gastric tumors. On the other hand, when a panel of human tumors implanted in nude mice were treated with various antitumor agents at their MTD's, MMC, ACNU, and VLB exhibited extremely high response rates of 82, 64 and 64%, respectively. Other drugs such as ADR and 5-FU showed relatively low effectiveness. Such results are obviously not in good accordance with the clinical data.

However, in the present study using the dose pharmacokinetically equivalent to the clinical dose, i.e., the RD, MMC, ADR, 5-FU, ACNU, and VLB demonstrated almost equal therapeutic effectiveness. In terms of relative therapeutic efficacy, these results, except for VLB, seem to show much better coincidence with the clinical ones. For VLB, we could not find any report presenting clinical data for gastric tumors. Accordingly, it is difficult to evaluate this result; it might predict some clinical activity of this agent against gastric tumors, or the result may simply be due to an overestimated RD. In contrast, none of the tumors exhibited any significant response to VCR even at its MTD.

As previously noted for 5-FU and MTX, their RD's were estimated to be somewhat greater than their MTD's, suggesting the possibility of underestimation of their clinical effects in this model. Practically, the effect of both agents had to be evaluated at their MTD's. Two and 1 out of 11 tumor lines significantly responded to 5-FU and MTX, respectively, although the inhibitory effects were relatively low. These results indicate that the degree of underestimation might be not so great.

With respect to the accuracy of RD and number of tumor lines used for evaluation of response rate, the present experimental conditions are not necessarily sufficient. Nevertheless, we have clearly proved that use of the RD as a clinically equivalent dose, as compared with the MTD, reproduces well in the human tumor/nude mouse model the clinically observed relative effectiveness of several antitumor agents.

In considering the practical application of the RD, it seems most desirable to use it in the sensitivity testing of individual clinical tumors using the nude mouse model, where we can compare the sensitivities to various antitumor agents on the standard of achievable plasma levels of each drug in man, but not in the nude mouse. We think it probable that use of the RD's of various drugs will provide a more accurate prediction of the relative sensitivities of a given tumor *in situ* to various antitumor agents tested.

Most important is how we can apply the concept underlying RD to the evaluation of new antitumor compounds. In the preclinical study of new candidate compounds, therapeutic effectiveness is examined using various murine and human tumor models. Such drugs often exhibit potent activities against some of these tumors. However, if the clinical MTD of such a compound is significantly smaller than the effective dose in the mouse from a pharmacokinetic point of view, the drug would not be expected to be clinically effective despite a positive response in the mouse. In other words, to reasonably predict clinical effects of new compounds, we need to have not only therapeutic results but also knowledge of their pharmacokinetics.

In predicting RD's of new compounds in the development stage, further progress in kinetic analysis of cell-killing action of the drugs and prediction of their human pharmacokinetic parameters by the method of animal scale-up[5,6] is absolutely necessary. Our current efforts are directed toward such kinetic studies.

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