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# ANTITUMOR ACTIVITIES OF SEVENTEEN ALKYLATING AGENTS AGAINST HUMAN MAMMARY CARCINOMA (MX-1) IN NUDE MICE

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## ABSTRACT

The antitumor activities of seventeen antitumor alkylating agents have been studied in the xenograft of human mammary carcinoma transplanted in nude mice (MX-1). The drugs employed in this study were; cyclophosphamide, ifosfamide, 1-(2-chloroethyl)-3-cyclohexyl-1-nitrosourea (CCNU), 1-(2-chloroethyl)-3-(4-methylcyclohexyl)-1-nitrosourea (me-CCNU), 2-[3-(2-chloroethyl)-3-nitrosoureido]-2-deoxy- D-glucopyranose (chlorozotocin, or DCNU), 3-[(4-amino-2-methyl-5-pyrimidinyl)methyl]-1-(2-chloroethyl)-1-nitrosourea hydrochloride (ACNU), 1-(2-chloroethyl)-3-(methyl α-D-glucopyranos-6-yl)-1-nitrosourea (MCNU), 1-(2-chloroethyl)-3-(β-D-glucopyranosyl)-1-nitrosourea(GANU), 4-[bis(2-chloroethyl)amino]-L-phenylalanine (L-PAM), chlorambucil, busulfan, Bis(3-methylsulfonyloxypropyl)amine *p*-toluenesulfonate (864-T), N, N′, N″-triethylenimino thiophosphoramide (thio-TEPA), carbazilquinone, dibromomannitol, procarbazine and 5-(3, 3-dimethyl-1-triazeno) imidazole-4-carboxamide (DTIC). Cyclophosphamide, ifosfamide, chlorozotocin, ACNU, MCNU, GANU, 864-T, thio-TEPA, carbazilquinone and DTIC were administered intravenously through a tail vein, and the others were given intraperitoneally. Among these seventeen antitumor alkylating agents, the most active compounds (maximum rate of tumor regression : ≧90%) are cyclophosphamide, ACNU, L-PAM, chlorambucil, thio-TEPA, carbazilquinone and dibromomannitol. Another group of compounds showed moderate activity (maximum rate of tumor regression: 89−50%), including ifosfamide, CCNU, MCNU, GANU, busulfan, 864-T and procarbazine. The remaining three compounds showed less than moderate activity (≦49%) and were therefore considered to be inactive. These results in nude mouse-human tumor xenograft system correspond to clinically observed patterns of chemotherapy sensitivity in patients with breast cancer.

Key words: Experimental chemotherapy; Nude mice; Xenograft; Human mammary carcinoma; Antitumor alkylating agents

## INTRODUCTION

The objective of the screening in cancer chemotherapy is to search for chemical agents useful in cancer treatment. For a primary screening, mouse leukemia (L1210, P388), mouse ascitic tumor (Sarcoma 180, Ehrlich carcinoma) and mouse solid tumor (B16 melanoma, Lewis lung carcinoma) have been mainly used, and from these screening systems active antitumor agents have been selected.[1] In the nude mouse-human tumor xenograft system, most human tumors preserve their morphological and functional characteristics of parent tumors through many transplant generations.[2,3] Therefore, a use of the xenograft system may offer a great potential for further screening of clinically active agents among many drugs

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selected in the screening with conventional mouse tumor models. The antitumor activities of seventeen alkylating agents against the human breast adenocarcinoma designated as MX-1 were investigated. The correlation between antitumor effects of alkylating agents in xenograft and clinical activities of those drugs, and the usefulness of the nude mouse-human tumor xenograft system in the screening of anticancer agents were discussed.

## MATERIALS AND METHODS

*Mice*: Nude mice (nu/nu) with a BALB/c genetic background which had been bred and maintained under specific-pathogen-free conditions were supplied by the Central Institute for Experimental Animals (Kawasaki), and were housed in autoclaved filter cap cages with autoclaved food and bedding. All cages were placed in a laminar-air-flow unit in our laboratory. Six- to seven-week-old male mice weighing about 25 g were used for these experiments.

*Tumor*: The tumor was a mammary carcinoma designated as MX-1, diagnosed histologically as an infiltrating duct cell carcinoma (medullary tubular carcinoma) and established in nude mice in 1974 from a surgical specimen taken from a 29-year-old woman who had not been treated with anticancer agents in the National Cancer Institute (NCI), U.S.A.

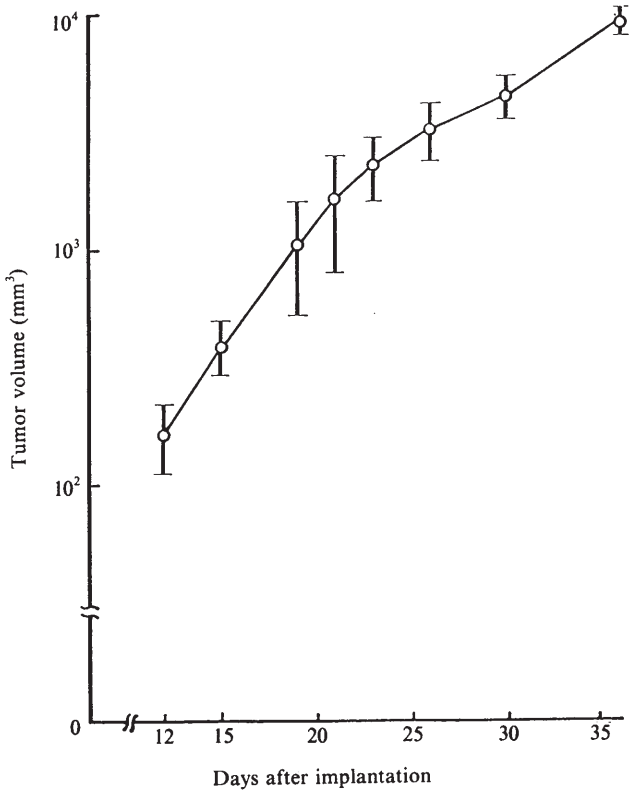


Fig. 1 Growth of human mammary carcinoma (MX-1) in nude mice (nu/nu) with a BALB/c genetic background. Bars, S.D.

The growth curve of MX-1 is shown in Fig. 1. The mass doubling time of MX-1 during the logarithmic growth phase was 3–4 days and the efficiency of transplantation was approximately 95%. The MX-1 tumor was estrogen receptor-negative and the level of progesterone receptor was marginal.

*Chemotherapeutic agents*: The fourteen antitumor alkylating agents that have been already established the clinical activity in various human cancers and three new water-soluble derivatives of nitrosourea developed in Japan which have recently been undergoing clinical trials are investigated in this study. The following seventeen alkylating agents were used; cyclophosphamide, ifosfamide, 1-(2-chloroethyl)-3-cyclohexyl-1-nitrosourea (CCNU), 1-(2-chloroethyl)-3-(4-methylcyclohexyl)-1-nitrosourea (me-CCNU), 2-[3-(2-chloroethyl)-3-nitrosoureido]-2-deoxy-D-glucopyranose (chlorozotocin, or DCNU), 3-[4-amino-2-methyl-5-pyrimidinyl)methyl]-1-(2-chloroethyl)-1-nitrosourea hydrochloride (ACNU), 1-(2-chloroethyl)-3-(methyl α-D-glucopyranose-6-yl)-1-nitrosourea (MCNU), 1-(2-chloroethyl)-3-(β-D-glucopyranosyl)-1-nitrosourea (GANU), 4-[bis(2-chloroethyl)amino]-L-phenylalanine (L-PAM), chlorambucil, busulfan, Bis(3-methylsulfonyloxypropyl)amine *p*-toluenesulfonate (864-T), N, N′, N″-triethylenimino thiophosphoramide (thio-TEPA), carbazilquinone, dibromomannitol, procarbazine, and 5-(3, 3-dimethyl-1-triazeno)-imidazole-4-carboxamide (DTIC).

The makers and supplier of the drugs evaluated in this study were summarized in Table 1.

To evaluate the antitumor activities of the drugs, we employed the predetermined dose lethal to one-tenth of $BDF_1$ mice ($LD_{10}$) as standard therapeutic dose of twelve drugs for nude mice. Furthermore, the dose level of ifosfamide, thio-TEPA, and carbazilquinone were selected on the basis of toxicity study obtained from the literatures, and that of procarbazine and DTIC were selected on the basis of the chemotherapy study with nude mice in Battelle Columbus Laboratories, U.S.A. The employment of $LD_{10}$ of $BDF_1$ mice for the standard therapeutic dose in this study is on account of accumulation of the toxicological data of $BDF_1$

Table 1. List of drugs

| Drug | Maker or Supplier |
|---|---|
| Cyclophosphamide | Shionogi & Co., Tokyo |
| ACNU | Sankyo Co., Tokyo |
| MCNU | Tokyo Tanabe Co., Tokyo |
| GANU | Meiji Seika Co., Tokyo |
| L-PAM | Nippon Wellcome Co., Osaka |
| Chlorambucil | Burroughs Wellcome Co., U.K. |
| Busulfan | Burroughs Wellcome Co., U.K. |
| 864-T | Yoshitomi Pharmaceutical Co., Tokyo |
| Thio-TEPA | Sumitomo Chemical Ind., Osaka |
| Carbazilquinone | Sankyo Co., Tokyo |
| Dibromomannitol | Kyorin Pharmaceutical Co., Tokyo |
| Procarbazine | Nihon Roche Co., Tokyo |
| Ifosfamide | National Cancer Institute, U.S.A. |
| CCNU | National Cancer Institute, U.S.A. |
| Me-CCNU | National Cancer Institute, U.S.A. |
| Chlorozotocin (DCNU) | National Cancer Institute, U.S.A. |
| DTIC | National Cancer Institute, U.S.A. |

mice in our laboratory.

*Treatment*: Cyclophosphamide, ifosfamide, chlorozotocin, ACNU, MCNU, GANU, 864-T, thio-TEPA, carbazilquinone, procarbazine and DTIC were dissolved in physiological saline, and CCNU and me-CCNU were suspended in physiological saline with a few drops of Tween 80 (Tokyo Kasei Co., Tokyo). L-PAM, chlorambucil, busulfan and dibromomannitol were suspended in 0.5% carboxymethylcellulose (CMC) (Wako Pure Chemical Ind., Tokyo). Solutions or suspensions of all drugs were prepared immediately before injection. Drugs were given intravenously (iv) through tail vein of mice (cyclophosphamide, ifosfamide, chlorozotocin, ACNU, MCNU, GANU, 864-T, thio-TEPA, carbazilquinone and DTIC), or intraperitoneally (ip) (CCNU, me-CCNU, L-PAM, chlorambucil, busulfan, dibromomannitol and procarbazine) in a volume of 0.01 ml per g body weight of mice by a single injection. Control animals were given physiological saline or 0.5% CMC by the same route. Thio-TEPA, carbazilquinone, procarbazine and DTIC were administered on a q4d × 3 schedule, and other drugs were given on a single treatment schedule.

*Evaluation of antitumor activity*: In this experiment, a 2-mm tumor fragment was implanted subcutaneously aseptically by means of a trocar into the back of each mouse. Mice were randomized into test groups consisting of three to six mice each and treatment was initiated when the tumor mass reached about 150–200 $mm^3$ in volume, usually 10–14 days after tumor implant. The length, width and height of the subcutaneous tumors and the body weight of tumor-bearing host mice were measured twice a week for three weeks after the initiation of treatment. To evaluate chemotherapeutic effectiveness, a minimum ratio of mean tumor volume of treated mice to that of control mice (T/C) during the experimental period from the initial (day 0) to the final day of treatment was calculated in all groups. However, since the mean tumor volume of each group at the initiation of treatment was generally inconsistent, it was normalized to 1.0 and a relative mean tumor volume of each group was calculated. Then, the maximum rate of tumor regression (chemotherapeutic effect) was assessed from the following formula:

$$[1 - (\frac{Tn}{T0}/\frac{Cn}{C0})] \times 100\ (\%)$$

where Tn/T0 is the relative mean tumor volume of treatment group between the initial (day 0) and the day n from treatment, and Cn/C0 is that of control group. The study was terminated if more half of mice were judged to have died from drug toxicity. Student's *t*-test was used to compare tumor volume in the control and treated groups.

## RESULTS

A single intravenous dose of 220, 110 or 55 mg/kg of cyclophosphamide and 300, 75, 50 or 25 mg/kg of ifosfamide, an analog of cyclophosphamide, were given. The highest dose of cyclophosphamide (220 mg/kg) was found to be highly effective, resulting in complete tumor regression of all mice ($P < 0.001$). In mice treated with ifosfamide, significant regression of the tumor was observed at doses of 50 and 25 mg/kg, and the maximum rate of tumor regression for these two doses were 87% ($P < 0.01$) and 86% ($P < 0.001$), respectively. Three hundred and 75 mg/kg of ifosfamide were toxic to the mice. Among the six nitrosourea derivatives tested in this study, ACNU gave the highest tumor regression. Forty mg/kg of ACNU which is the dose of $LD_{10}$ (iv), induced 92% ($P < 0.001$) tumor regression. Tumor regression rate at the 20

mg/kg was 46%, but no tumor regression was observed at the dose of 10 mg/kg. MCNU had higher antitumor activity comparable to that of CCNU. MCNU at doses of 15 and 7.5 mg/kg induced 73% ($P < 0.01$) and 66% tumor regression, respectively. The animals treated with 30 mg/kg ($LD_{10}$, iv) were presumed to be died from toxicity. Meanwhile, the $LD_{10}$ of GANU (iv) was 6.5 mg/kg and the greatest tumor regression (50%) was seen at this dose.

The $LD_{10}$ of CCNU was reported to be 48 mg/kg (ip) and a single dose of CCNU at the doses of 50 and 25 mg/kg induced 69 and 60% tumor regression, respectively. Although the toxicity study of me-CCNU in $BDF_1$ mice gave a value of 50 mg/kg for $LD_{10}$ (iv), the animals treated with the doses of 50 and 25 mg/kg were presumed to be died from drug toxicity. Me-CCNU at a dose of 12.5 mg/kg induced a 26% tumor regression. The toxicity study of chlorozotocin gave a value of 15 mg/kg for $LD_{10}$ (iv). The maximum rate of tumor regression of chlorozotocin at the single dose of 7.5 mg/kg given intravenously is 34% as shown in Fig. 2. The dose of 15 mg of chlorozotocin per kg ($LD_{10}$, iv) was toxic to mice.

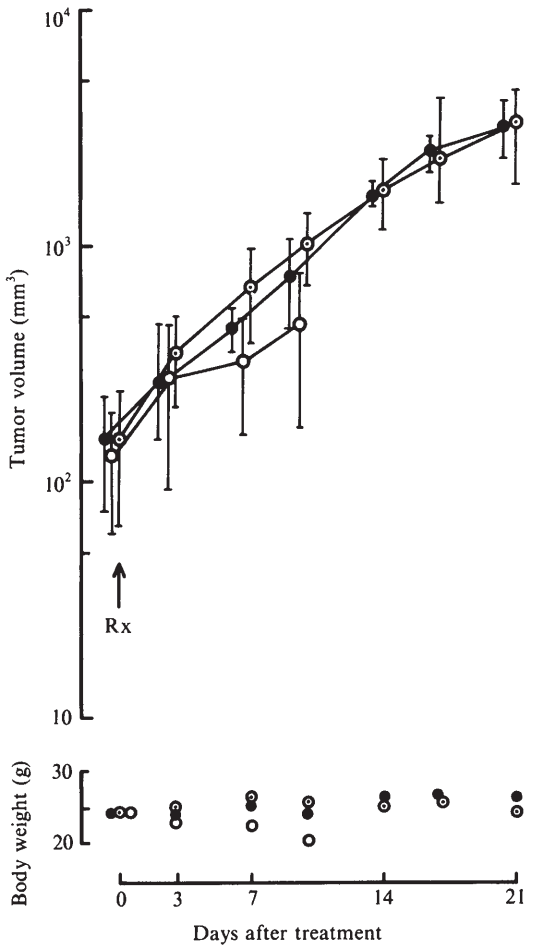


Fig. 2 Chemotherapy of human mammary carcinoma (MX-1) in nude mice to chlorozotocin: ○, 15 mg/kg; ●, 7.5 mg/kg: ◉, controls. Drug was administered only one time intravenously at the time indicated by the arrow. Bars, S.D.

Activity of L-PAM against the MX-1 tumor was demonstrated with a dose of 9 mg/kg ip single treatment, and complete tumor regression was seen, whereas maximum weight loss was 20% and it was presumed to be moderately toxic to mice. A dose of 27 and 18 mg/kg ($LD_{10}$, ip) of L-PAM was toxic. A dose of 28, 14 and 7 mg/kg of chlorambucil were administered ip and the dose of 28 mg/kg ($LD_{10}$, ip) induced 94% ($P < 0.001$) tumor regression as shown in Fig. 3.

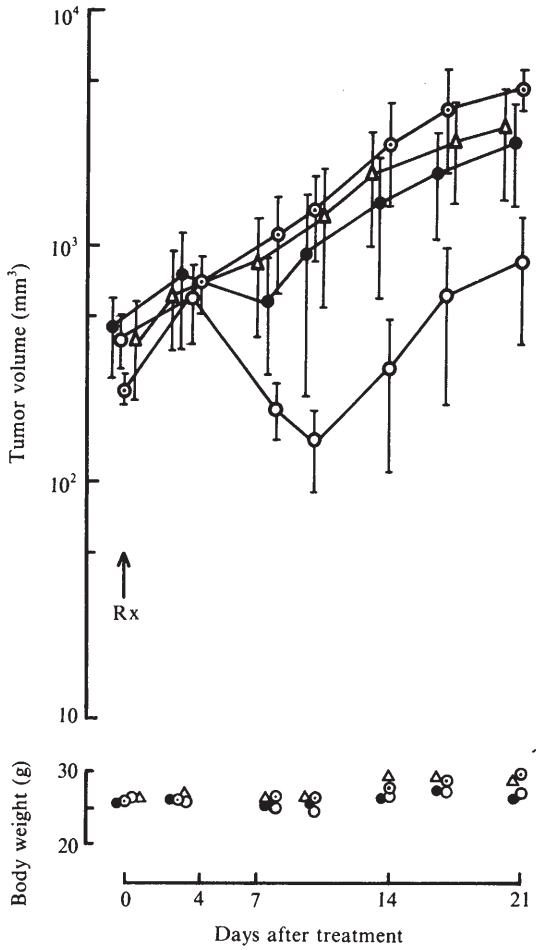


Fig. 3 Chemotherapy of human mammary carcinoma (MX-1) in nude mice to chlorambucil: ○, 28 mg/kg; ●, 14 mg/kg; △, 7. mg/kg: ◉, controls. Drug was administered only one time intravenously at the time indicated by the arrow. Bars, S.D.

Among methanesulfonate group of alkylating agents, the highest dose of 68 mg/kg of busulfan ($LD_{10}$, ip) was effective in suppressing the tumor growth and 69% of growth inhibition was induced, whereas no significant retardation of tumor growth was seen at a dose of 34 mg/kg as shown in Fig. 4. A dose of 53 mg/kg 864-T induced 83% tumor regression ($P < 0.01$). Doses of 210 ($LD_{10}$, iv) and 105 mg/kg iv injection were toxic, and all animals died at 7 and 17 days respectively after initiation of treatment.

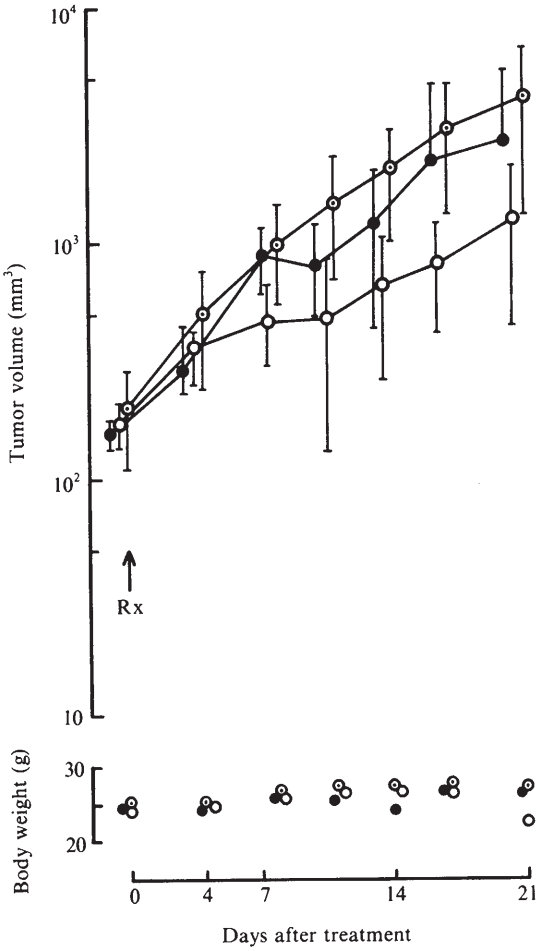


Fig. 4 Chemotherapy of human mammary carcinoma (MX-1) in nude mice to busulfan: ○, 68 mg/kg; ●, 34 mg/kg: ◉, controls. Drug was administered only one time intraperitoneally at the time indicated by the arrow. Bars, S.D.

Thio-TEPA and carbazilquinone, ethyleneimine group, were tested on a q4d × 3 schedule. Doses of 10 and 5 mg/kg per injection of thio-TEPA were very effective in tumor regression of MX-1, and more than 99% ($P < 0.05$) tumor regressions were induced. Carbazilquinone was tested at doses of 2 and 1 mg/kg per injection on a q4d × 3 schedule. Acute toxicity was observed at a dose of 2 mg/kg per injection, whereas a 1 mg/kg per injection of carbazilquinone produced a 100% regression of tumor growth ($P < 0.05$) as shown in Fig. 5.

Dibromomannitol in epoxide group was tested at a dose of 900, 450 and 225 mg/kg. A dose of 900 mg/kg ($LD_{10}$, ip) of dibromomannitol showed 100% ($P < 0.001$) tumor regression. Doses of 450 and 225 mg/kg of this drug showed 91 ($P < 0.001$) and 81% ($P < 0.001$) tumor regression, respectively.

Procarbazine and DTIC were tested on a q4d × 3 schedule. Treatment with 400 mg/kg per injection of procarbzine showed significant tumor regression, but all animals died at 3 weeks after the treatment due to drug toxicity. At a dose of 200 mg/kg per injection of procarbazine,

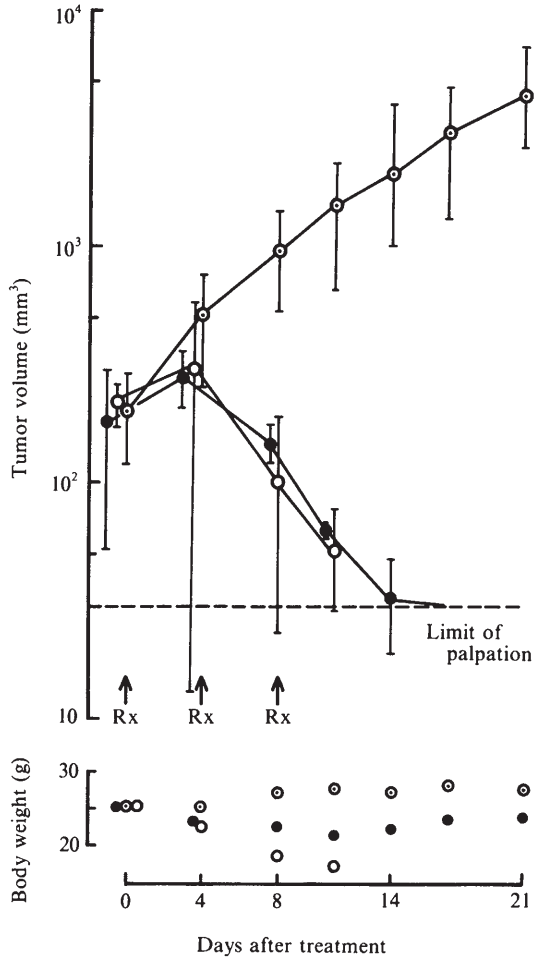


Fig. 5 Chemotherapy of human mammary carcinoma (MX-1) in nude mice to carbazilquinone: ○, 2 mg/kg per injection; ●, 1 mg/kg per injection; ◉, controls. Drug was administered on a q4d × 3 schedule intravenously at the times indicated by the arrows. Bars, S.D.

54% tumor regression was induced. Sixty-three mg/kg per injection of DTIC was inactive, whereas a treatment with higher dose, 125 mg/kg per injection was highly toxic; all three mice died after the second or the third injection.

The maximum weight loss of the mice during the observation period was zero to 26% (864-T, 53 mg/kg) in the groups without any death due to drug toxicity compared with 11 to 36% in the groups which included deaths apparently due to drug toxicity.

The antitumor activities of seventeen alkylating agents against human mammary carcinoma transplanted in nude mice (MX-1) are summarized in Table 2. It is evident that the most active compounds (maximum rate of tumor regression: ≧90%) are cyclophosphamide, ACNU, L-PAM, chlorambucil, thio-TEPA, carbazilquinone and dibromomannitol. Another group of compounds showed moderate activity (maximum rate of tumor regression: 89–50%), including ifosfamide, CCNU, MCNU, GANU, busulfan, 864-T and procarbazine.

The remaining three compounds showed less than moderate activity (≦49%) and were therefore considered to be inactive.

Table 2. Comparison of antitumor activity of alkylating agents against human mammary carcinoma in nude mice (MX-1)

| Drug | Route of treatment | Dose of drug (mg/kg) | Relative mean tumor volume[a] Tn/T0[b] | Cn/C0[c] | Maximum rate of tumor regression[d] (%) | Maximum loss of weight (%) |
|---|---|---|---|---|---|---|
| 1) β-chloroethylamine group | | | | | | |
| Cyclophosphamide | iv | 220 | 0*** | 17.14 | 100 | 2 |
| | | 110 | 0.32*** | 17.14 | 98 | 0 |
| | | 55 | 0.62** | 4.59 | 96 | 0 |
| Ifosfamide | iv | 300 | — | — | toxic | 13 |
| | | 75 | — | — | toxic | 22 |
| | | 50 | 1.93** | 15.77 | 87 | 0 |
| | | 25 | 2.07*** | 15.77 | 86 | 0 |
| CCNU | ip | 75 | — | — | toxic | 17 |
| | | 50 | 9.31 | 29.41 | 69 | 12 |
| | | 25 | 11.81 | 29.41 | 60 | 4 |
| Me-CCNU | ip | 50 | — | — | toxic | 25 |
| | | 25 | — | — | toxic | 33 |
| | | 12.5 | 3.58 | 4.82 | 26 | 3 |
| | | 6.3 | 5.80 | 4.82 | progression | 4 |
| Chlorozotocin(DCNU) | iv | 15 | — | — | toxic | 20 |
| | | 7.5 | 2.93 | 4.39 | 34 | 4 |
| ACNU | iv | 60 | — | — | toxic | 30 |
| | | 40 | 0.75*** | 8.72 | 92 | 12 |
| | | 20 | 2.88 | 5.34 | 46 | 8 |
| | | 10 | 4.30 | 4.24 | progression | 0 |
| MCNU | iv | 30 | — | — | toxic | 29 |
| | | 15 | 8.55** | 31.43 | 73 | 7 |
| | | 7.5 | 2.91 | 8.53 | 66 | 13 |
| GANU | iv | 10 | — | — | toxic | 19 |
| | | 6.5 | 4.59 | 9.14 | 50 | 8 |
| | | 2.5 | 1.73 | 1.85 | 7 | 0 |
| L-PAM | ip | 27 | — | — | toxic | 30 |
| | | 18 | — | — | toxic | 30 |
| | | 9 | 0** | 18.90 | 100 | 20 |
| Chlorambucil | ip | 28 | 0.36*** | 5.98 | 94 | 4 |
| | | 14 | 1.32 | 4.65 | 72 | 0 |
| | | 7 | 6.95 | 15.31 | 55 | 0 |
| 2) Methanesulfonate group | | | | | | |
| Busulfan | ip | 68 | 4.73 | 14.93 | 69 | 12 |
| | | 34 | 5.17 | 7.24 | 29 | 4 |
| 864-T | iv | 210 | — | — | toxic | 15 |
| | | 105 | — | — | toxic | 11 |

(to be continued)

Table 2. continued

| | | | | | | |
|---|---|---|---|---|---|---|
| | | 53 | 1.63** | 9.07 | 83 | 26 |
| 3) Ethyleneimine group | | | | | | |
| Thio-TEPA | iv × 3 | 10 | 0* | 21.4 | 100 | 13 |
| | | 5 | 0.09* | 14.93 | 99 | 15 |
| Carbazilquinone | iv × 3 | 2 | — | — | toxic | 36 |
| | | 1 | 0.03* | 14.93 | 100 | 20 |
| 4) Epoxide group | | | | | | |
| Dibromomannitol | ip | 900 | 0*** | 10.80 | 100 | 5 |
| | | 450 | 1.02*** | 10.80 | 91 | 0 |
| | | 225 | 2.81*** | 14.27 | 81 | 0 |
| 5) Others | | | | | | |
| Procarbazine | ip × 3 | 400 | — | — | toxic | 34 |
| | | 200 | 13.18 | 28.64 | 54 | 11 |
| DTIC | iv × 3 | 125 | — | — | toxic | 12 |
| | | 63 | 2.28 | 2.68 | 15 | 17 |

a) Values are at the time of the maximum tumor regression during the observation period and they are rounded to three decimal places.
b) Tn/T0: relative mean tumor volume of treatment group between day 0 and day n after initial treatment.
c) Cn/C0: that of control group.
d) Maximum rate of tumor regression is calculated from the following formula:

$$[1 - (\frac{Tn}{T0}/\frac{Cn}{C0})] \times 100\ (\%)$$

and values are rounded to the nearest whole number.
Significantly different at $P < 0.001$ (***), $P < 0.01$ (**) and $P < 0.05$ (*), compared with control group.

## DISCUSSION

The majority of new anticancer agents were discovered by their antitumor activities in rodent tumor test systems such as L1210, P388, B16 melanoma and Lewis lung carcinoma.[1] However, it has been difficult to predict the perfect antitumor activity against various human cancers by these models.[4] The human tumor-xenograft system has been expected to have a potential test for the proper selection of anticancer agents in treatment of individual human tumors.[5] However, whether the effect of antitumor agents on the human tumor-xenograft system correlates with clinical response of individual tumor is a question that has not yet clarified.

Among seventeen alkylating agents evaluated in this study, seven drugs which are cyclophosphamide, carbazilquinone, thio-TEPA, L-PAM, chlorambucil, dibromomannitol and ACNU showed more than 90% tumor regression against the MX-1 tumor. Cyclophosphamide, carbazilquinone, thio-TEPA, L-PAM and chlorambucil are significantly active against breast cancer and the overall response for these five drugs are 34,[6] 33,[7] 30,[6] 23,[6] and 20%,[6] respectively as shown in Table 3, and the clinical activities of dibromomannitol and ACNU have not yet evaluated. Another group, including ifosfamide, CCNU, procarbazine, 864-T, MCNU, busulfan and GANU showed moderate activity (maximum rate of tumor regression; 89–50%). The clinical activities of 864-T, MCNU, busulfan and GANU have not yet been evaluated, while ifosfamide is active with an overall response rate of 40%,[8] and CCNU and procarbazine are inactive[6] against breast cancer. Chlorozotocin,[9] me-CCNU[6] and DTIC[6] are inactive in clinical study as observed in this study.

Table 3. Comparison of activity spectrum of alkylating agents in xenograft (MX-1) and in human breast cancer.

| Drug | Maximum rate of tumor regression (%) | | Clinical effect[a] (%) | |
|---|---|---|---|---|
| Cyclophosphamide | 100*** | ++[b] | 34 | ++[c] |
| Carbazilquinone | 100* | ++ | 33 | ++ |
| Thio-TEPA | 100* | ++ | 30 | ++ |
| L-PAM | 100** | ++ | 23 | + |
| Chlorambucil | 94*** | ++ | 20 | + |
| Ifosfamide | 87** | + | 40 | ++ |
| CCNU | 69 | + | 12 | − |
| Procarbazine | 54 | + | 5 | − |
| Chlorozotocin (DCNU) | 34 | − | 4 | − |
| Me-CCNU | 26 | − | 6 | − |
| DTIC | 15 | − | 7 | − |
| Dibromomannitol | 100*** | ++ | | |
| ACNU | 92*** | ++ | | |
| 864-T | 83** | + | | |
| MCNU | 73** | + | | |
| Busulfan | 69 | + | | |
| GANU | 50 | + | | |

***$p < 0.001$, **$p < 0.01$, *$p < 0.05$

a) Datas from Carter (1974) *et al.*

b) (++) ≧ 90%, (+) 89–50%, (−) ≦ 49%

c) (++) ≧ 30%, (+) 29–20%, (−) ≦ 19%

On the basis of these observations, the response of alkylating agents against the MX-1 tumor is correlated well with clinical effectiveness. The correlation of antitumor effect between xenograft and clinical efficacy in antitumor antibiotics and antimetabolites are studying now.

In this study, the doses of $LD_{10}$ in $BDF_1$ mice, dose lethal to one-tenth of $BDF_1$ mice for twelve drugs were employed as standard therapeutic dose to nude mice. Even though it is not yet concluded whether the $LD_{10}$ and $LD_{50}$ for nude mice are higher than those for conventional mouse, our study[10] of nitrosourea already published suggested that the utilization of the $LD_{10}$ in $BDF_1$ mice is appropriate, and in this study the rationality of our suggestion was confirmed.

The human tumor xenograft in nude mice system is of great potential value in chemotherapy studies as described already, and for a progress of this system as a screening tool it is necessary to clarify whether tumor cell kinetics in nude mice is similar to those in the human tumor, but little is known about the growth characteristics in nude mice. Mattern and collaborators[11] reported that the xenografts in nude mice grew faster than the original tumors did in the patients and in their first passage in nude mice an increase in the percentage of S-phase cells was reported. Sharkey and collaborators[12] reported the greater mitotic index and an increase of mitotic activity in xenografted tumor than that of the original human tumors.

On the basis of the above considerations, there seems to be the limitation in direct

application of results derived from treatment of nude mice grown human tumors to human chemotherapy and the difficulty in evaluation of inactive drugs in xenografts. In spite of these limitations and difficulties, the nude mouse-human tumor xenograft system will greatly extend the application in the field of cancer chemotherapy study.

In this report, we were able to demonstrate the correlation between antitumor effects of alkylating agents against the MX-1 tumor and clinical activities of those drugs, and the usefulness of the nude mouse-human tumor xenograft system in the screening of anticancer agents. In the future, we want to utilize the *in vitro* human tumor stem cell assay (the human tumor clonogenic assay) developed by Hamburger and Salmon[13] in addition to human tumor xenograft system, and these *in vivo* (human tumor xenograft system) and *in vitro* (human tumor stem cell assay) sensitivity tests will improve predictability of drug sensitivity against human cancers cooperatively.

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