# ANTITUMOR ACTIVITY OF NEW MORPHOLINO ANTHRACYCLINES

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Antitumor activity of 3′-deamino-3′-morpholino anthracyclines was examined. Morpholino derivatives of 13-deoxocarminomycins, MX2 (**1**), MX (**2**) and MY5 (**3**) shown in Fig. 1, administered iv showed increase in life span (ILS) values over 110% against ip-inoculated P388 leukemia. The ranges of effective doses of these compounds were broader than those of their parent drugs. The morpholino derivatives of doxorubicin and carminomycin, however, were not so effective as their parent drugs. Among these compounds, MX2 administered orally showed nearly the same effects as those obtained by iv administration against P388 leukemia. MX2 administered iv showed 89% ILS against intracerebrally-inoculated L1210 leukemia. The highly lipophilic nature of MX2 could contribute partly to achieving chemotherapeutic responses against intracerebrally-inoculated tumors or by oral administration.

Five morpholino anthracyclines: MX2 (**1**), MX (**2**), MY5 (**3**)[1], 3′-deamino-3′-morpholinocarminomycin (**4**)[2] and 3′-deamino-3′-morpholinoadriamycin (**5**)[2] were prepared by *N*-alkylation of 13-deoxo-10-hydroxycarminomycin (oxaunomycin) (**6**)[3], 13-deoxocarminomycin (**7**)[4], 13-deoxo-11-deoxycarminomycin (**8**)[5], carminomycin (**9**) and doxorubicin (**10**), respectively. The present study investigates the effect of these morpholino anthracyclines on murine tumor models including a brain tumor model, and compares these effects with those of their parent drugs.

## Materials and Methods

### Drugs

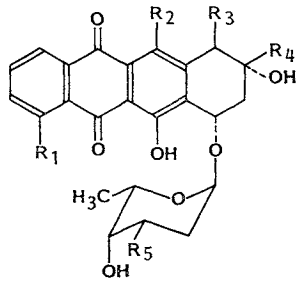
The following compounds were prepared in our laboratory: MX2 (**1**); MX (**2**); MY5 (**3**); 3′-deamino-3′-morpholinocarminomycin (**4**); 3′-deamino-3′-morpholinoadriamycin (**5**); 13-deoxo-10-hydroxycarminomycin (**6**); 13-deoxocarminomycin (**7**); 13-deoxo-11-deoxycarminomycin (**8**) and carminomycin (**9**). Doxorubicin (**10**) formulated for clinical use was the product of Kyowa Hakko Kogyo Co., Ltd., Tokyo, Japan.

### Tumors and Animals

P388 leukemia and L1210 leukemia were obtained from the NCI, NIH, U.S.A., and were maintained by the Cancer Chemotherapy Center, Tokyo, Japan. Mice were supplied by the Shizuoka Laboratory Animal Center, Hamamatsu, Japan. Six to 8 weeks old female $CDF_1$ (BALB/c×DBA/2) mice were used. One-tenth ml of cell suspension in phosphate-buffered saline (PBS) containing $10^6$ P388 leukemia cells was inoculated ip into mice. In another experiment, 5 $\mu$l of cell suspension in PBS containing $10^4$ L1210 leukemia cells was inoculated intracerebrally (ic) into mice. Drugs were administered ip, iv or po at a constant rate of 0.01 ml/g body weight.

From the mean survival times of the treated (T) and the control (C) mice, the increase in life span

Fig. 1. Structure of morpholino anthracyclines and parent drugs.



**1** $R_1 = R_2 = R_3 = OH$ $R_4 = C_2H_5$ $R_5 =$ 

**2** $R_1 = R_2 = OH$ $R_3 = H$ $R_4 = C_2H_5$ $R_5 =$ 

**3** $R_1 = OH$ $R_2 = R_3 = H$ $R_4 = C_2H_5$ $R_5 =$ 

**4** $R_1 = R_2 = OH$ $R_3 = H$ $R_4 = COCH_3$ $R_5 =$ 

**5** $R_1 = OCH_3$ $R_2 = OH$ $R_3 = H$ $R_4 = COCH_2OH$ $R_5 =$ 

**6** $R_1 = R_2 = R_3 = OH$ $R_4 = C_2H_5$ $R_5 = NH_2$

**7** $R_1 = R_2 = OH$ $R_3 = H$ $R_4 = C_2H_5$ $R_5 = NH_2$

**8** $R_1 = OH$ $R_2 = R_3 = H$ $R_4 = C_2H_5$ $R_5 = NH_2$

**9** $R_1 = R_2 = OH$ $R_3 = H$ $R_4 = COCH_3$ $R_5 = NH_2$

**10** $R_1 = OCH_3$ $R_2 = OH$ $R_3 = H$ $R_4 = COCH_2OH$ $R_5 = NH_2$

(ILS=(T/C−1)×100%) was calculated. Tumor-free survivors were excluded from the calculation.

Determination of MX2 in Brain Tissue

MX2 was intravenously administered to male SD rats. After 2 or 8 hours, the whole brain was excised, and homogenized. MX2 was extracted from the homogenate with $CHCl_3$ - MeOH (4:1) separated by reversed phase HPLC and detected fluorimetrically.

Log P Measurement

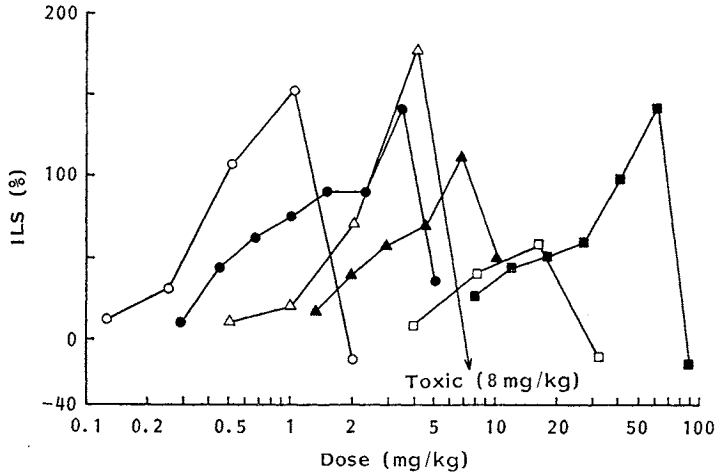
Drugs were partitioned between 1-octanol and 100 mM phosphate buffer (pH 7.4). The concentration of the drug in each phase was determined by reversed phase HPLC equipped with a UV absorption detector. Log P was calculated from the equation; log P=log (concentration in an organic phase/concentration in an aqueous phase).

## Results

### The Antitumor Activity of Morpholino Anthracyclines and their Parent Drugs against P388 Leukemia

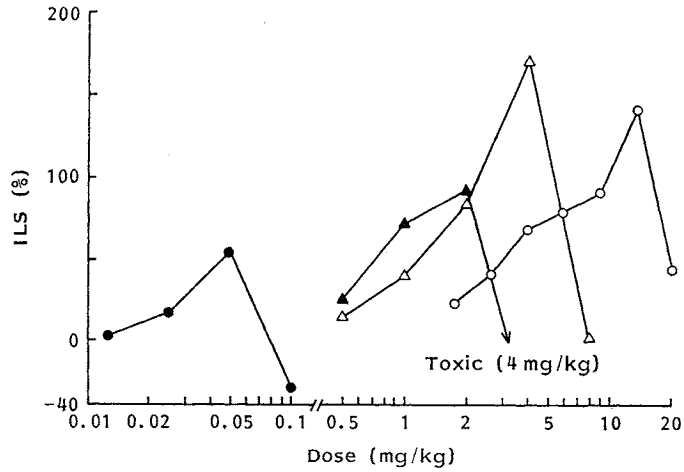
The antitumor activity of the drugs administered iv against ip-inoculated P388 leukemia on days 1 and 5 was examined (Figs. 2 and 3). MX2, MX and MY5 showed 140, 111 and 142% ILS at doses of 3.33, 6.67 and 60 mg/kg, respectively. MX2, MX and MY5 required 1.7 to 3.8 times higher doses

Fig. 2. Antitumor effect of morpholino anthracyclines and their parent drugs.
○ 6, △ 7, □ 8, ● MX2, ▲ MX, ■ MY5.




P388 leukemia cells ($10^6$/mouse) were inoculated ip on day 0. The drugs were administered iv on days 1 and 5.
Toxic: Drug administration was discontinued because of toxicity.

Fig. 3. Antitumor effect of morpholino anthracyclines and their parent drugs.
○ Doxorubicin, △ carminomycin, ● morpholino doxorubicin, ▲ morpholino carminomycin.




P388 leukemia cells ($10^6$/mouse) were inoculated ip on day 0. The drugs were administered iv on days 1 and 5.
Toxic: Drug administration was discontinued because of toxicity.

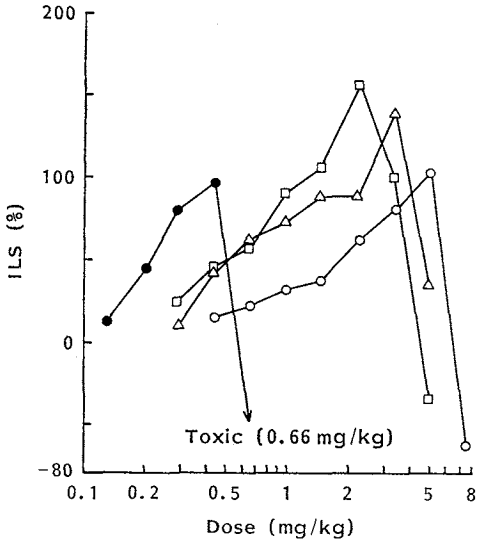
to produce the maximum ILS's as compared to their parent drugs. The effective dose ranges of MX2, MX and MY5 were broader than those of their parent drugs. On the other hand, morpholino carminomycin and morpholino doxorubicin required lower doses to produce maximum ILS's as compared to their parent drugs, and the effective dose ranges were narrower than those of their parent drugs.

The Dosing Schedule Dependence of the Activity of Morpholino Anthracyclines

The antitumor activity of MX2 administered iv against P388 leukemia on day 1, days 1 and 5,

Fig. 4. Dependence of the antitumor effect of MX2 on the administration schedule.

○ Day 1, △ days 1 and 5, □ days 1, 5 and 9, ● days 1~9.

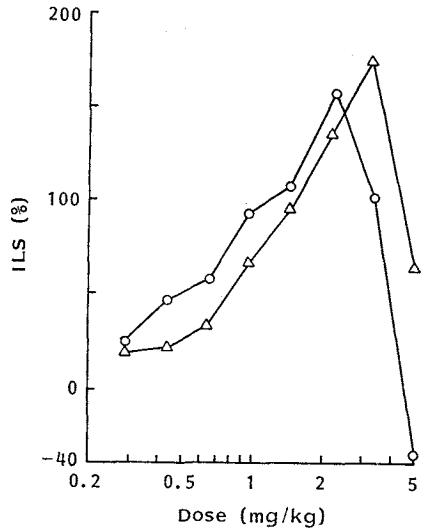



P388 leukemia cells ($10^6$/mouse) were inoculated ip on day 0. MX2 was administered iv.

Toxic: Drug administration was discontinued because of toxicity.

Fig. 5. Antitumor effect of MX2 by oral and intravenous routes.

○ iv, △ po.




P388 leukemia cells ($10^6$/mouse) were inoculated ip on day 0. MX2 was administered on days 1, 5 and 9.

days 1, 5 and 9 or days 1 to 9 was examined (Fig. 4). MX2 induced remarkable prolongation of the life span in parallel with the extension of the effective dose range either by days 1 and 5 or days 1, 5 and 9 administration though it induced a less prolonged life span accompanied with a less extended effective dose range by day 1 or days 1 to 9 administration. MX and MY5 were also more effective by days 1 and 5 or days 1, 5 and 9 administration than the other schedules (data not shown). Therefore the drugs were administered on days 1, 5 and 9 in the following experiments.

## The Antitumor Activity of po Administered Morpholino Anthracycline

The antitumor activity of MX2 administered po against P388 leukemia on days 1, 5 and 9 was examined (Fig. 5). The maximum tolerated dose (3.33 mg/kg) of MX2 by po administration was equal to that by iv administration, and a maximum ILS of 175% was obtained. MX and MY5 were also effective by po administration (data not shown).

## The Antitumor Activity of Morpholino Anthracyclines against ic-Inoculated L1210 Leukemia

Since MX2 was effective by po administration and its log P value was as much as 2.84, the effect of MX2 on a brain tumor model was tested, expecting that MX2 would potentially pass blood-brain barrier. MX2 showed maximum ILS of 89% at 3 mg/kg by days 1, 5 and 9 iv administration. However the parent compound 13-deoxo-10-hydroxycarminomycin showed marginal activity in the dose range of 0.25 to 2 mg/kg (Table 1).

Table 1. Antitumor activity of MX2 and **6** against ic-inoculated L1210 leukemia.

| Drug | Dose (mg/kg) | MST±SD (days) | ILS (%) | Drug | Dose (mg/kg) | MST±SD (days) | ILS (%) |
|---|---|---|---|---|---|---|---|
| Control | | 8.1±0.69 | | Control | | 7.9±1.07 | |
| MX2 | 0.375 | 9.7±0.82 | 20 | **6** | 0.25 | 9.0±0.00 | 14 |
| | 0.75 | 12.5±1.64 | 55 | | 0.5 | 10.2±0.75 | 29 |
| | 1.5 | 15.0±0.00 | 86 | | 1 | 10.2±0.75 | 29 |
| | 3 | 15.2±2.93 | 89 | | 2 | 8.3±0.52 | 5 |

L1210 leukemia cells ($10^4$/mouse) were inoculated ic on day 0.
The drugs were administered on days 1, 5 and 9.

### Concentration of MX2 in Brain Tissue

The concentrations of MX2 in brain tissue 2 and 8 hours after iv administration of 2 mg/kg MX2 were 0.25 and 0.02 $\mu$g/g tissue, respectively.

### Log P

Log P values of MX2, doxorubicin and aclarubicin were 2.84, −0.032 and 3.20, respectively.

## Discussion

It has been known that the antitumor activity of anthracycline derivatives alters dramatically by cyclo-alkylation at N-3′ position in its daunosamine residue into morpholino derivatives[6]. In our results, it is clear that the antitumor potency of the derivatives of 13-deoxocarminomycin is reduced by the alkylation so that high doses are required to produce the activity similar to that of parent compounds, however, the effective dose ranges are broadened. Administration of the morpholino derivatives on days 1, 5 and 9 induced the most effective effect against P388 leukemia among the administration schedule tested. Therefore days 1, 5 and 9 administration was adopted in the later experiments. MX2 shows nearly equal activity by po administration as that obtained by iv administration, suggesting that MX2 could readily be absorbed into the gastro-intestinal tract. In addition, MX2 administered systemically through the iv route exerts an antitumor effect on ic-inoculated L1210 leukemia, indicating that this compound passes the blood-brain barrier. Existence of MX2 in the cerebrum after iv injection of MX2 was confirmed by HPLC. The lipophilicity of MX2 might contribute to the absorption and the penetration of the drug, however, it was not the sole determinant because morpholino doxorubicin, cyanomorpholino doxorubicin[7] and aclarubicin which have comparable log P values were not active against ic-inoculated L1210 leukemia (data not shown).

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