

Effects of 1-Methyl-3-propyl-7-butylxanthine (MPBX) on Idarubicin-induced Antitumor Activity and Bone Marrow Suppression

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The effects of 1-methyl-3-propyl-7-butylxanthine (MPBX), a xanthine derivative, on idarubicin (IDA)-induced antitumor activity against P388 leukemia cells (P388) and bone marrow suppression were examined. In P388 tumor-bearing mice, the combination of MPBX with IDA increased the antitumor activity of IDA. The IDA concentration in the tumors in the MPBX combination group increased by 2.0-fold compared to the level in the IDA-alone group. On the other hand, as regards IDA-induced bone marrow suppression, the combination of MPBX with IDA reduced the decrease in the bone marrow cell number by 30% compared to that in the IDA-alone group. In addition, the IDA concentration in the bone marrow cells was decreased by the combination of MPBX with IDA. An *in vitro* experiment showed that MPBX facilitated IDA influx and suppressed IDA efflux in P388 cells. In conclusion, the combination of MPBX with IDA increased the antitumor activity and decreased the bone marrow suppression. Therefore, we expect that the combination of MPBX with IDA will be useful for leukemia chemotherapy.

Key words: 1-Methyl-3-propyl-7-butylxanthine — Idarubicin — Antitumor activity — Adverse reaction — Bone marrow suppression

Chemotherapeutic agents play important roles in cancer therapy, but there are many problems, including side effects and the appearance of resistant cells. Studies of biochemical modulation to enhance the antitumor activity and suppress side effects by means of combination treatment with non-antitumor drugs are being carried out and could improve the efficacy of cancer chemotherapy.

In our previous study, we confirmed that caffeine, a xanthine derivative, increases the antitumor activity induced by doxorubicin (DOX) against Ehrlich ascites carcinoma cells through suppression of DOX efflux.^{1–3} Furthermore, it was confirmed that this inhibitory effect on DOX efflux is correlated with the structure of xanthine derivatives.⁴ 1-Methyl-3-propyl-7-butylxanthine (MPBX, Fig. 1) significantly suppresses DOX efflux and increases antitumor activity induced by DOX.^{5,6} On the other hand, idarubicin (IDA), a newly synthesized anthracycline used in the treatment of acute myeloblastic leukemia,^{7–10} can cause suppression of bone marrow cells and death in clinical use. Because IDA-induced antitumor activity is exerted on leukemia cells in the bone marrow and blood, it is impossible to separate the antitumor activity and side effects, so the use of IDA is limited. Most biochemical modulators increase the antitumor activity with a concomitant increase in side toxicity. But we confirmed that MPBX decreases the cardiotoxicity induced by DOX in

addition to enhancing the antitumor activity of DOX.¹¹ Thus, the combination of MPBX with IDA might allow the separation of antitumor activity and bone marrow suppression.

In this study, we examined the effects of MPBX on the antitumor activity and bone marrow suppression induced by IDA. The results indicate that combination treatment with IDA and MPBX could be useful for the treatment of leukemia.

MATERIALS AND METHODS

Reagents IDA injection, 5 mg/vial (Idamycin), was a gift from Pharmacia K. K., Tokyo. MPBX was synthesized in our laboratory.¹² The other chemicals used in this study were of the highest purity available.

Animals Male BDF₁ mice (5 weeks old and weighing 20–25 g) were obtained from Japan SLC (Hamamatsu). The animals were housed in a room maintained at 25±1°C with 55±5% relative humidity, and were given free access to regular chow pellets and water.

Effects of MPBX on the antitumor activity and bone marrow suppression induced by IDA P388 leukemia cells (5×10⁵ cells/animal) were transplanted onto the backs of BDF₁ mice, and then IDA (0.25, 0.5 or 1.0 mg/kg/day for 4 days) was intraperitoneally administered to groups consisting of seven mice, at 8, 10, 12 and 14 days after tumor inoculation. MPBX (10 mg/kg/day for 4 days) was intraperitoneally injected at 9, 11, 13 and 15 days

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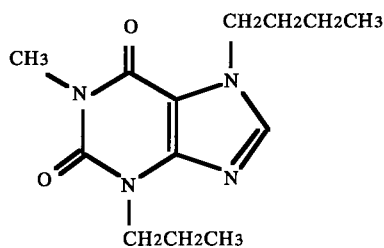


Fig. 1. Structure of 1-methyl-3-propyl-7-butylxanthine (MPBX).

after inoculation. The animals were killed by cervical dislocation on the 16th day after inoculation. Their tumors, hearts, livers and thighbones were rapidly removed and weighed. Tissue samples were homogenized in 10 volumes (w/v) of 10 mM phosphate buffer (pH 7.8). Each suspension was mixed for 60 s with 5 volumes (v/v) of chloroform-methanol (4:1, v/v), and then centrifuged (1200g, 15 min). The concentration of IDA in the organic phase was determined with a fluorescence spectrophotometer (excitation, 470 nm; emission, 585 nm). The thighbones were broken in phosphate buffer (10 mM, pH 7.8), and then sonicated to release bone marrow cells. The numbers of bone marrow cells in the cell suspensions were determined with a blood cell counter.

Effect of MPBX on the IDA concentrations in tissues *in vivo* P388 leukemia cells (5×10^5 cells/animal) were transplanted onto the backs of BDF₁ mice, and then IDA (2.0 mg/kg) was intraperitoneally administered to groups consisting of three mice at 16 days after inoculation. The animals were killed by cervical dislocation at 1, 4, 8 or 24 h after IDA administration. Their tumors, thighbones, hearts and livers were rapidly removed and weighed. The IDA concentrations in the tissues were determined as described above.

Effect of MPBX on the IDA concentration in P388 leukemia cells *in vitro* P388 leukemia cells (1×10^6 cells/animal) were intraperitoneally transplanted into male DBA/2 mice. Ascites fluid was collected on the 7th day after transplantation. The leukemia cells were washed twice and then resuspended in RPMI 1640 medium containing 10% fetal bovine serum.

To examine the effect of MPBX on IDA efflux from P388 leukemia cells, 1×10^7 cells/ml and 10 μg/ml IDA were preincubated in the medium at 37°C for 30 min. The concentration of IDA used was based upon our previous reports.¹⁻⁶ After incubation, the medium was cooled on ice and then centrifuged at 150g for 3 min. The cells were washed and resuspended in medium containing 10% fetal bovine serum. The cell suspension (1×10^7 cells/ml) was incubated at 37°C for 180 min in the presence or absence of 1–100 μM MPBX. After incubation, the medium was cooled on ice and then centrifuged at 150g for 3 min. The

cells were washed and resuspended in ice-cold phosphate buffer (10 mM, pH 7.8), and the IDA concentration was determined as previously described.

To examine the IDA influx into P388 leukemia cells, cells (1×10^7 cells/ml) were incubated with 5.0 μg/ml IDA at 37°C in the presence or absence of 1–100 μM MPBX.

Statistical analysis Statistical analysis was performed using Student's *t* test and ANOVA.

RESULTS

Effect of MPBX on the IDA-induced decrease in tumor weight

The effects of MPBX on the IDA-induced changes in tumors are shown in Figs. 2 and 3. The tumor weight in the IDA 0.5 mg/kg group was decreased to 67% ($P < 0.001$) compared with that in the control group. The tumor weight in the IDA 0.25 mg/kg group was not decreased significantly, but the combination of MPBX with IDA reduced it to 59% ($P < 0.01$, tumor weight in MPBX combination group $\times 100$ /that in the control group) compared with the control level (Fig. 3).

The IDA concentration in the tumors (Fig. 3) of the IDA 0.25 mg/kg group was 0.28 ± 0.03 ng/mg protein (23.5% of that in the IDA 0.5 mg/kg group). The combination of MPBX with IDA significantly increased the IDA concentration in the tumors by 2.0-fold (0.566 ± 0.034 ng/mg protein, $P < 0.01$) compared to that in the IDA 0.25 mg/kg group.

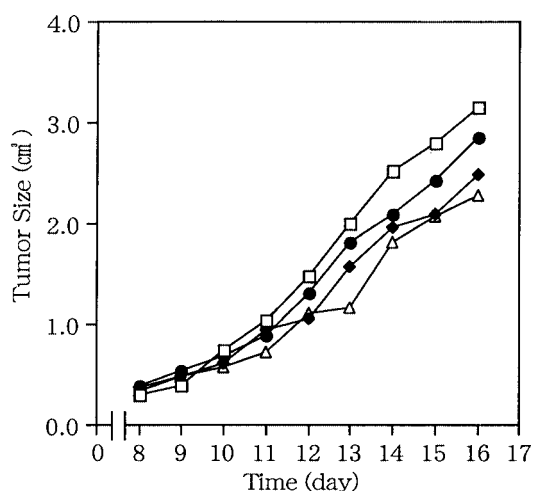


Fig. 2. Changes in tumor growth induced by administration of MPBX with IDA. During the treatment, two perpendicular tumor diameters (a, long diameter; b, short diameter) were determined with calipers. The tumor size was calculated as $a \times b^2 / 2$ and expressed in cm^3 . Each point represents the mean of seven mice. □ control, ◆ IDA 0.5 mg/kg, ● IDA 0.25 mg/kg, △ IDA 0.25 mg/kg+MPBX.

The effect of the combination of MPBX with IDA 1.0 mg/kg is shown in Fig. 4. The tumor weight in the IDA 1.0 mg/kg group decreased to 34% ($P<0.05$) compared with the control level. With the combination of MPBX with IDA, the decrease in tumor weight was enhanced by 1.2-fold ($P<0.01$ vs. control group) compared to that in the IDA 1.0 mg/kg group. The IDA concentration in the tumors of the IDA 1.0 mg/kg group was 5.76 ± 0.13 ng/mg protein, a 4.8-fold increase over the level in the IDA 0.5 mg/kg group. Furthermore, the combination of MPBX with 1.0 mg/kg IDA increased the IDA level by 1.4-fold.

Effects of MPBX on the IDA-induced changes in the numbers of bone marrow cells and blood cells in P388 tumor-bearing mice The effect of MPBX on the IDA-induced changes in the numbers of bone marrow cells in

P388 tumor-bearing mice is shown in Fig. 5. The number of bone marrow cells in the IDA 1.0 mg/kg group was decreased by 66% ($P<0.001$) compared with the control level. This decrease with the combination of MPBX and 1.0 mg/kg IDA was only 28%, significantly less ($P<0.01$) than the IDA-induced reduction of bone marrow cells. The number of leukocytes showed similar changes.

There were no effects on the numbers of bone marrow cells and blood cells in the IDA 0.5 and 0.25 mg/kg groups (data not shown).

Effects of MPBX on the IDA concentrations in tissues *in vivo* The effects of MPBX on the time courses of the IDA concentrations in tumors and bone marrow in P388 tumor-bearing mice are shown in Fig. 6. The IDA concentration in the tumors at 24 h after administration of IDA

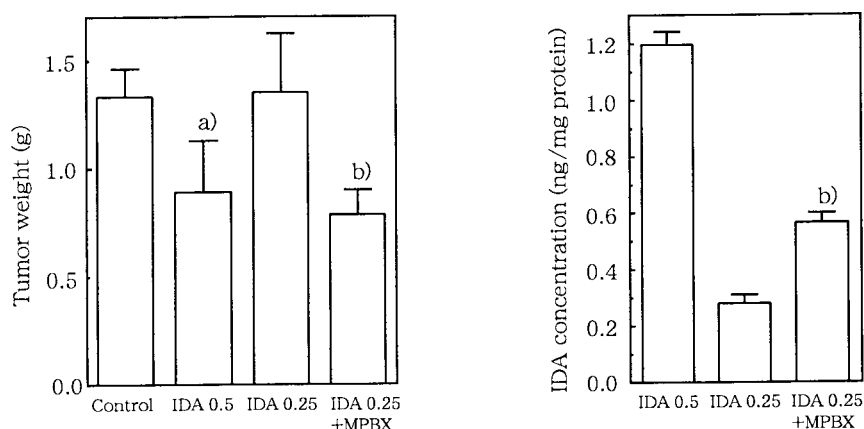


Fig. 3. Effects of MPBX on the change in the tumor weight induced by IDA and the IDA concentration in the tumors of mice (IDA: 0.25 or 0.5 mg/kg). Each column represents the mean \pm SD for seven mice. Significant difference from the level of the control group is indicated by a), $P<0.001$. Significant difference from the level of IDA group is indicated by b), $P<0.01$.

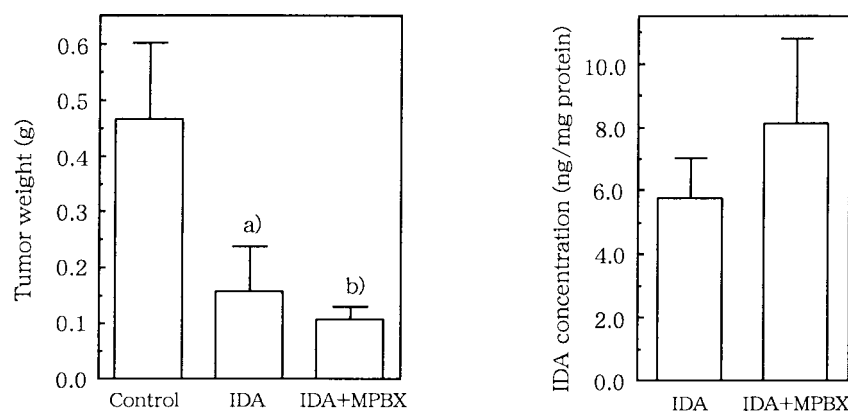


Fig. 4. Effects of MPBX on the change in the tumor weight induced by IDA and the IDA concentration in the tumors of mice (IDA: 1.0 mg/kg). Each column represents the mean \pm SD for five mice. Significant differences from the level of control group are indicated by a), $P<0.05$ and b), $P<0.01$.

in the MPBX combination group was 1.4-fold higher ($P<0.01$) than that in the IDA-alone group. On the other hand, the IDA concentrations in the bone marrow at 1 and 4 h after the administration of IDA in the MPBX combination group were decreased to 24% and 49% of the IDA-alone level, respectively.

Effects of MPBX on the IDA transport across the P388 leukemia cell membrane *in vitro* The effects of MPBX on IDA influx and efflux from P388 leukemia cells are shown in Fig. 7. In the MPBX 10 μM and 100 μM groups, IDA influx was facilitated by 25% and 47% ($P<0.001$) compared with the IDA-alone group after 60 min incubation,

respectively. However, there was no effect in the MPBX 1 μM group. On the other hand, at 180 min, the combination of MPBX 10 μM and 100 μM had inhibited the IDA efflux by 7.7% and 12.7% ($P<0.05$), respectively.

DISCUSSION

In our previous study, we reported that caffeine, a xanthine derivative, is useful as a biochemical modulator. Caffeine enhances the antitumor activity of DOX with an increase in the DOX concentration due to inhibition of DOX efflux from Ehrlich ascites carcinoma cells.¹⁻³ Although caffeine, trimethylxanthine, inhibits DOX efflux from the tumor cells, not all xanthine derivatives have an inhibitory effect. The inhibitory action of xanthine derivatives may be related to the structure at the 7- and 3-positions of xanthine.⁴ We confirmed that MPBX, derived from caffeine, has a strong inhibitory effect on DOX efflux from Ehrlich ascites carcinoma and P388 leukemia cells *in vitro*, and enhances the antitumor activity of DOX *in vivo*.^{5,6} Furthermore, it was found that MPBX reduces the cardiotoxicity of DOX.⁶

IDA tends to accumulate in proliferative cells as well as tumor cells, so it is supposed that the accumulation of IDA in bone marrow cells during continuous administration may cause severe myelotoxicity. The enhancement of the antitumor activity of DOX in combination with MPBX is due to an increase of the DOX concentration in the tumor without an increase in DOX toxicity.⁵ Namely, it is possible to deliver IDA to tumor cells without accumulation in bone marrow cells by means of coadministration of MPBX, which changes the pharmacokinetics of IDA. In

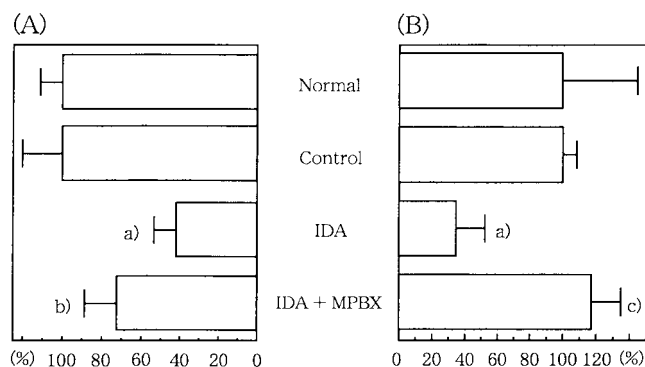


Fig. 5. Effects of MPBX on the changes in the numbers of bone marrow cells (A) and leukocytes (B) induced by IDA (1.0 mg/kg). Each column represents the mean \pm SD for five mice. Significant difference from the level of control group is indicated by a), $P<0.001$. Significant differences from the level of IDA-alone group are indicated by b), $P<0.01$ and c), $P<0.001$.

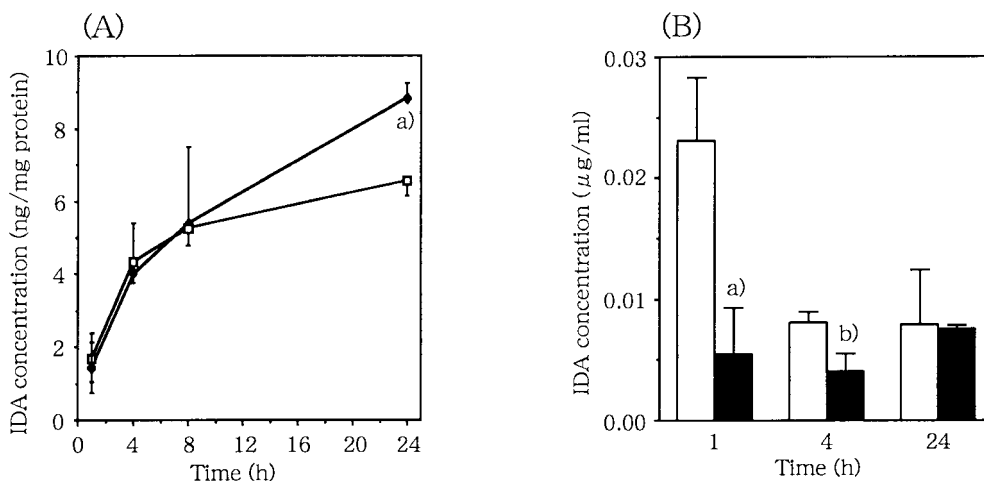


Fig. 6. Effects of MPBX on the changes in the IDA concentration in the tumors (A) and bone marrow (B) (IDA: 2.0 mg/kg). Each point and column represent the mean \pm SD for three mice. Significant differences from the level of IDA-alone group are indicated by a), $P<0.05$ and b), $P<0.01$. A and B: \square IDA, \blacksquare IDA+MPBX.

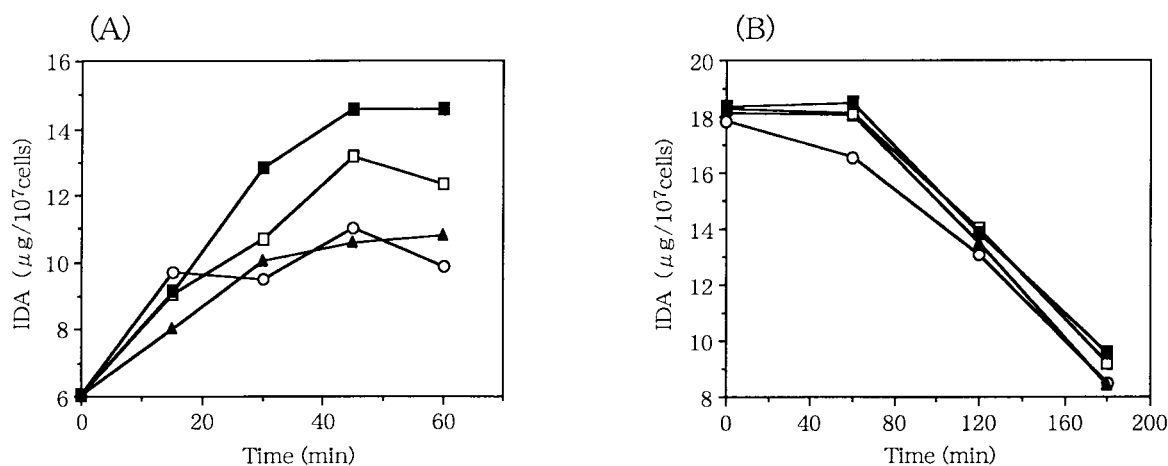


Fig. 7. Effects of MPBX on the IDA influx (A) and efflux (B) in P388 leukemia cells. Each point represents the mean value for four samples. Each SD is less than 10%. (A) ○ IDA 5 $\mu\text{g}/\text{ml}$, ▲ IDA+MPBX 1 μM , □ IDA+MPBX 10 μM , ■ IDA+MPBX 100 μM . (B) ○ IDA 10 $\mu\text{g}/\text{ml}$, ▲ IDA+MPBX 1 μM , □ IDA+MPBX 10 μM , ■ IDA+MPBX 100 μM .

this study, we examined the effects of MPBX on IDA-induced antitumor activity against P388 leukemia cells and bone marrow suppression.

In an *in vivo* experiment, the tumor weight in the IDA 0.5 mg/kg group decreased significantly, whereas the tumor weight in the IDA 0.25 mg/kg group did not decrease. However, the combination of MPBX with IDA (0.25 mg/kg) reduced it to 59% ($P < 0.01$) compared with the control level. The IDA concentration in the MPBX combination group was 2.0-fold higher than that in the IDA 0.25 mg/kg group, but did not reach that in the IDA (0.5 mg/kg)-alone group. In the IDA 0.25 mg/kg group, a reduction in the tumor weight was not observed, so we supposed that the combination with MPBX caused the IDA level to exceed the threshold concentration for antitumor activity. In the IDA 1.0 mg/kg experiment, however, the combination with MPBX enhanced the antitumor activity as well as increasing the IDA concentration in the tumors. In these experiments, we fixed the time, not the tumor weight, after transplantation of the tumor. Since the tumor weights (Figs. 3 and 4) were different, it is possible that IDA concentration in the tumor is influenced by the tumor growth rate. However, whether this is the case or not, it remains important that MPBX enhances IDA-induced antitumor activity.

With regard to biochemical modulation by the combination of another drug with an antitumor agent to enhance the antitumor activity, it is important to determine whether side effects are also increased. Considering the effectiveness of MPBX as a biochemical modulator, we examined the effect of MPBX on bone marrow suppression induced by IDA. In the IDA 1.0 mg/kg group, the number of bone marrow cells decreased, whereas the combination of

MPBX with IDA significantly reduced the IDA-induced decrease of bone marrow cells. Furthermore, a similar effect was seen on the number of leukocytes. Our results indicated that the combination of MPBX with IDA might reduce the bone marrow suppression. In addition, other pharmacological activities of MPBX are weaker than those of other xanthine derivatives.¹²⁻¹⁸) Therefore, the combination with MPBX may allow separation of the IDA-induced antitumor activity and the adverse reaction.

The IDA concentrations in the bone marrow at 1 and 4 h after administration in the MPBX combination group were decreased to 24% and 49% compared with that in the IDA-alone group, respectively. On the other hand, the IDA concentration in the tumors at 24 h after administration in the MPBX combined group was 1.4-fold higher than that in the IDA-alone group. These results suggested that the enhancement of the antitumor activity and the reduction of bone marrow suppression were due to the combination with MPBX, causing an increased IDA concentration in the tumors and a decrease in the bone marrow. In addition, MPBX had no effects on the IDA concentrations in the heart and liver, indicating that MPBX does not increase the cardiotoxicity induced by anthracycline.

In our previous study, MPBX was confirmed to inhibit DOX efflux from Ehrlich ascites carcinoma cells *in vitro* and to enhance the antitumor activity of DOX, with an increase in the DOX concentration in the tumor.⁵) In addition, MPBX tended to facilitate DOX influx into P388 leukemia cells.⁶) The IDA uptake into the cells was rapid, because IDA is lipophilic compared to DOX. Therefore, the IDA uptake into the cells was suggested to depend mainly on simple diffusion.^{19,20}) However, since IDA is an anthracycline derivative like DOX, we examined the

effects of MPBX on the IDA influx and efflux in P388 leukemia cells.

After 60 min incubation, MPBX had facilitated IDA influx and the IDA level in the MPBX group was significantly higher than that in the IDA-alone group. This effect was dependent on the MPBX concentration. After 180 min incubation, MPBX at 10 μ M or more caused IDA efflux inhibition. These results indicated that MPBX increases the IDA concentration in the tumor by facilitating IDA influx and inhibiting IDA efflux, thereby enhancing IDA-induced antitumor activity. We speculate that MPBX may modulate the IDA level by acting on a nucleoside transporter. The nucleoside transport system is thought to con-

tribute to the uptake of anthracycline into HL60 cells.^{21–23} Nucleoside transport involves many transporter isoforms,^{24, 25} and differences in their expression in various cell lines may result in cell type-specific differences of IDA uptake characteristics.

In conclusion, the combination of MPBX with IDA not only increased the antitumor activity of IDA, but also decreased the IDA-induced adverse reaction, particularly bone marrow suppression. We suggest that MPBX may be clinically useful as a biochemical modulator of IDA.

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