REVERSAL OF RESISTANCE BY *N*-ACETYLTYRAMINE OR *N*-ACETYL-2-PHENYLETHYLAMINE IN DOXORUBICIN-RESISTANT LEUKEMIA P388 CELLS

Sir:

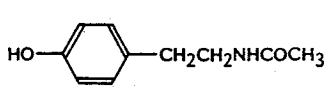
Acquisition of resistance to antitumor agents by cancer cells has been one of the most serious problems in the clinic. We tried to search for an active substance among microbial metabolites which could convert the resistant state of the cells to the sensitive state. Growth of doxorubicin-resistant leukemia P388 cells (P388/ADR) was scarcely affected by the presence of 0.1 μg/ml of doxorubicin On the other hand, sensitive P388 cells (P388/S) could not grow in the same concentration of doxorubicin. So culture filtrates of some *Streptomyces* species which did not show cytotoxicity were tested for the ability to recover doxorubicin sensitivity of P388/ADR cells.

P388/ADR cells obtained from Dr. M. INABA, Cancer Chemotherapy Center, Japanese Foundation for Cancer Research, Tokyo were maintained by serial ip transplantation in DBA mice. Ascitic cells, 7 days after transplantation to CDF_1 mice, were harvested and cultured in RPMI 1640 medium supplemented with 10% fetal bovine serum and 10 μM 2-mercaptoethanol. The cells (5×10^4/ml, 0.2 ml/well) were incubated in the absence or presence of 0.1 μg/ml of doxorubicin and 10 μl of culture filtrates diluted 20 folds for 48 hours at 37°C. Cell growth was determined by counting cell number using a Coulter counter.

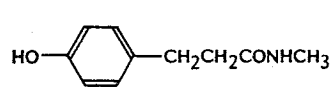
An active substance produced by MH106-AF1 strain was purified from the culture fluid (15 liters) by extraction with BuOAc at pH 8.0, silica gel column chromatography developed by a mixture of $CHCl_3$ and MeOH, Sephadex LH-20 column chromatography developed by MeOH and crystallization from a mixture of MeOH and EtOAc. Then, the purified material (32 mg) was obtained. The structure of this material was predicted to be *N*-acetyltyramine (I) or β-(4-hydroxyphenyl)propionic acid *N*-methylamide (II) by mass (*m/z*, 179 (M^+)), ^1H and ^{13}C NMR spectroscopy. An authentic sample of *N*-acetyltyramine was synthesized by acetylation of tyramine with acetic anhydride solution in MeOH. The product of MH106-AF1 was the same as *N*-acetyltyramine in all respects of TLC, mass, IR and NMR spectroscopy. Thus, the structure of MH106-AF1 substance was determined to be *N*-acetyltyramine (I).

N-Acetyltyramine has low toxicity; iv injection of 405 mg/kg did not cause the death of mice (ICR, female). The effect of *N*-acetyltyramine on cytotoxicity of doxorubicin was shown in Fig. 1. Growth inhibition of *N*-acetyltyramine was 6.0, 5.8, 11.6 and 32.8% at 5, 20, 40 and 200 μg/ml, respectively. IC_{50} of doxorubicin to P388/ADR cells came to 0.13 μg/ml in the presence of *N*-acetyltyramine from 0.48 μg/ml in the absence of this but did not approach the IC_{50} of P388/S (0.033 μg/ml). Thus partial recovery of drug sensitivity was achieved by addition of *N*-acetyltyramine. This enhancement of cytotoxicity of doxorubicin was specific to P388/ADR cells because it was not observed in P388/S cells. In order to find a compound which could recover drug sensitivity of P388/ADR cells completely, some analogs were tested. *N*-Ethylacetamide, acetanilide, *N*-acetylglycine, acetoacetanilide, *N*-benzylacetamide, tyramine, *N*-acetyltyrosine hydrazide and 2-phenylethylamine were not effective, although *N*-acetyl-2-phenylethylamine was partially active as shown in Fig. 2. Because *N*-acetyl-2-phenylethylamine was active in decreasing resistance, the hydroxyl group at *para* position of *N*-acetyltyramine was shown not to be related to the activity. Uptake and efflux of doxorubicin by P388/ADR cells determined by the method described previously[1] was not affected by the presence of *N*-acetyltyramine. So it might be caused by the different mechanism from that of calcium antagonists, calmodulin inhibitors[2], membrane-active agents[3~6] or non-antitumor synthetic anthracycline analogs[7], which had been shown to increase intracellular drug concentration by inhibiting efflux of drug in P388/ADR cells.

We will continue screening for an ideal com-



I



II

Fig. 1. Effect of *N*-acetyltyramine on cytotoxicity of doxorubicin to P388/ADR cells.

P388/S (●) and P388/ADR (○) cells were cultured with various concentrations of doxorubicin and *N*-acetyltyramine. Concentration of *N*-acetyltyramine (μg/ml) is shown in the figure.

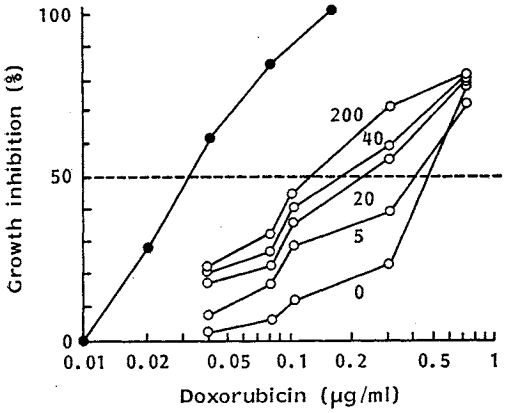
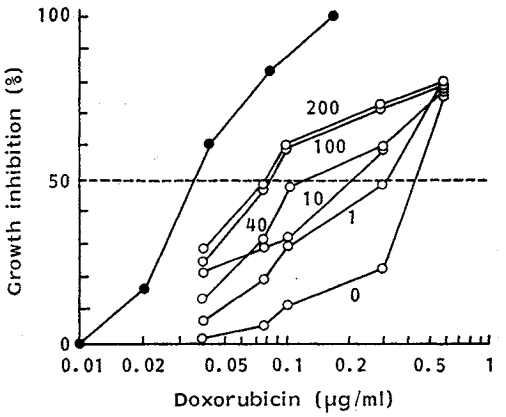


Fig. 2. Effect of *N*-acetyl-2-phenylethylamine on cytotoxicity of doxorubicin to P388/ADR cells.

P388/S (●) and P388/ADR (○) cells were cultured with various concentrations of doxorubicin and *N*-acetyl-2-phenylethylamine. Concentration of *N*-acetyl-2-phenylethylamine (μg/ml) is shown in the figure.



pound which has the activity to recover completely drug sensitivity without cytotoxicity or any clinically harmful effect.

Acknowledgments

We would like to thank Dr. MAKOTO INABA for the kind supply of P388/ADR cells.

This research was partially supported by the Grants-in-Aid for Cancer Research from the Ministry of Education, Science and Culture, Japan and by the Contract No. NO1-CM-47593 of the Division of Cancer Treatment, National Cancer Institute, U.S.A.

SETSUKO KUNIMOTO
CHIN-ZHI XU
HIROSHI NAGANAWA
MASA HAMADA
TORU MASUDA
TOMIO TAKEUCHI
HAMAO UMEZAWA

Institute of Microbial Chemistry,
3-14-23 Kamiosaki, Shinagawa-ku,
Tokyo 141, Japan

(Received May 22, 1987)

References

1) KUNIMOTO, S.; K. MIURA, K. UMEZAWA, C.-Z. XU, T. MASUDA, T. TAKEUCHI & H. UMEZAWA: Cellular uptake and efflux and cytostatic activity of 4'-*O*-tetrahydropyranyladriamycin in adriamycin-sensitive and resistant tumor cell lines. J. Antibiotics 37: 1697~1702, 1984
2) TSURUO, T.; H. IIDA, S. TSUKAGOSHI & Y. SAKURAI: Increased accumulation of vincristine and adriamycin in drug-resistant P388 tumor cells following incubation with calcium antagonists and calmodulin inhibitors. Cancer Res. 42: 4730~4733, 1982
3) RIEHM, H. & J. BIEDLER: Potentiation of drug effect by Tween 80 in Chinese hamster cells resistant to actinomycin D and daunomycin. Cancer Res. 32: 1195~1200, 1972
4) RAMU, A.; Z. FUKS, S. GATT & D. GLAUBIGER: Reversal of acquired resistance to doxorubicin in P388 murine leukemia cells by perhexiline maleate. Cancer Res. 44: 144~148, 1984
5) RAMU, A.; D. GLAUBIGER & Z. FUKS: Reversal of acquired resistance to doxorubicin in P388 murine leukemia cells by tamoxifen and other triparanol analogues. Cancer Res. 44: 4392~4395, 1984
6) IKEZAKI, K.; T. YAMAGUCHI, C. MIYAZAKI, H. UEDA, T. KISHIYE, Y. TAHARA, H. KOYAMA, T. TAKAHASHI, H. FUKAWA, S. KOMIYAMA & M. KUWANO: Potentiation of anticancer agents by new synthetic isoprenoids. I. Inhibition of the growth of cultured mammalian cells. J. Natl. Cancer Inst. 73: 895~901, 1984
7) INABA, M.; K. NAGASHIMA, Y. SAKURAI, M. FUKUI & Y. YANAGI: Reversal of multidrug resistance by non-antitumor anthracycline analogs. Gann 75: 1049~1052, 1984