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SHORT COMMUNICATIONS

Interactions among membrane transport systems: anthracyclines, calcium antagonists and anti-estrogens

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One mode of resistance to anthracyclines, and certain other natural and synthetic products, involves an energy-dependent outward transport system which limits drug accumulation to sub-lethal levels [1–5]. This transport system can be inhibited, and drug responsiveness thereby promoted, by a variety of agents of unrelated structure including other anthracyclines [5], acridines [6], calcium antagonists [7–11], calmodulin inhibitors [7, 12–14], and anti-estrogens [15]. In this report, we describe transport studies carried out with verapamil, nitrendipine, and tamoxifen to explore the circumvention of daunorubicin resistance by these different drugs.

The P388 murine leukemia cell line, and P388/ADR* [16], an adriamycin-resistant sub-line, were grown in culture using Fischer's medium (GIBCO, Grand Island, NY) supplemented with 10% horse serum.

Verapamil[N-*methyl*-^{14}C] (75 Ci/mmole), nitrendipine-[5-*methyl*-^{3}H] (72.5 Ci/mmole), and tamoxifen[N-*methyl*-^{3}H] (82 Ci/mmole) were purchased from the New England Nuclear Corp., Boston MA. [^{14}C]Daunorubicin (30 Ci/mole) was obtained from the Division of Cancer Treatment, NCI. Purity of all labeled substrates was tested by TLC systems suggested by the suppliers; all were >98% pure. Non-radioactive verapamil was obtained from the Knoll Pharmaceutical Co., Whippany, NJ; nitrendipine was from Miles Laboratories, West Haven, CT.

Cell suspensions (7 mg/ml wet weight) in HEPES-buffered growth medium were incubated with labeled substrates (1 μM concentration) plus specified additions at 0° or 37°. In some experiments, cells were loaded for 30 min with labeled substrates (1 μM) and then suspended in fresh medium, and the rate of loss of label was measured. In all studies, cells were collected by centrifugation (30 sec, 250 *g*) after timed intervals and cellular radioactivity was measured. All data are presented in terms of the apparent distribution ratio: [cellular]/[extracellular] drug concentration. Further details of methodology are given in Ref. 17.

Kinetics of drug accumulation by P388 and P388/ADR cells at 0° and at 37° are shown in Fig. 1. Accumulation of all but NTR were, to some extent, temperature-sensitive. There were no significant differences in steady-state intracellular levels of NTR and TAM in P388 versus P388/ADR cells; accumulation of DNR and VER by P388/ADR was 20 and 60%, respectively, of the levels attained by P388 cells.

When P388/ADR cells were incubated with 1 μM labeled DNR, the steady-state intracellular DNR level was doubled by the presence of 3 μM verapamil, 10 μM tamoxifen, 20 μM nitrendipine or 50 μM non-labeled DNR. The corresponding intracellular drug levels (derived from data shown in Fig. 1) are: VER, 15 μM; TAM, 370 μM; NTR, 180 μM; and DNR, 300 μM. These numbers may not reflect the relative affinities of these different agents for an outward transport system, since the concentrations of the free intracellular drugs are not known.

DNR exodus was temperature-sensitive in P388/ADR, with >80% of the initial pool lost within 3 min at 37° (Fig. 2). In contrast, drug exodus from P388 was almost temperature-insensitive, with <20% of the initial pool lost at 37° in 30 min. A study of DNR exodus in the presence of extracellular non-labeled drugs (3 μM VER, 10 μM TAM, 20 μM NTR, or 50 μM DNR) indicated that these additions slowed the initial rate of DNR exodus to 50% of control values. When the levels of the antagonists were increased, exodus of labeled DNR was slowed further.

Data shown in Figs. 1 and 2 suggest that the effects of TAM and NTR on anthracycline accumulation by P388/ADR cells do not derive from competition for a common exodus system. But the impaired accumulation and enhanced efflux of VER in P388/ADR cells are consistent with the presence of an outward drug transport system in this cell line. Additional experiments were carried out to assess interactions between TAM, NTR, VER and DNR transport. Addition of a 100-fold excess of DNR, VER,

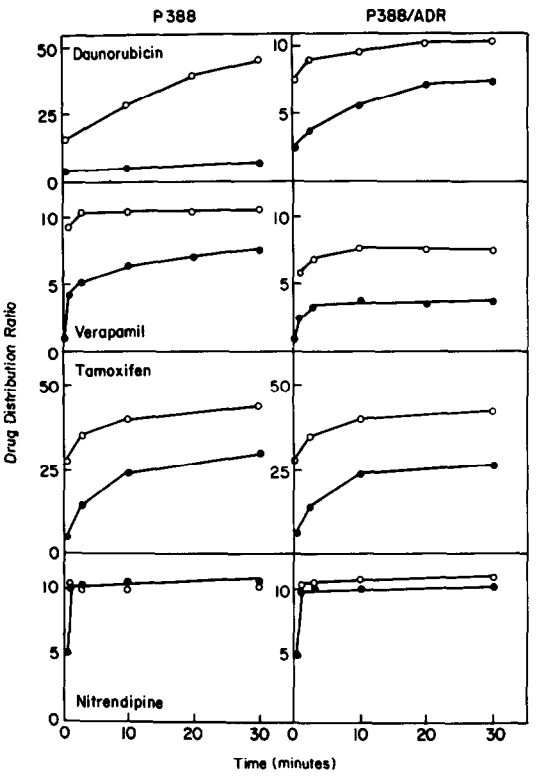

Fig. 1. Distribution ratios of daunorubicin, verapamil, tamoxifen and nitrendipine in P388 (left) and P388/ADR (right) cells as a function of time. Studies were carried out at 37° (○) or at 0° (●). Drug concentrations = 1 μM. Data from typical studies are shown; the variation encountered in replicate analyses was less than ±10% of the values shown.

* Abbreviations: ADR, adriamycin; DNR, daunorubicin; HEPES, 4-(2-hydroxyethyl)-1-piperazine ethanesulfonic acid; NTR, nitrendipine; TAM, tamoxifen; and VER, verapamil.

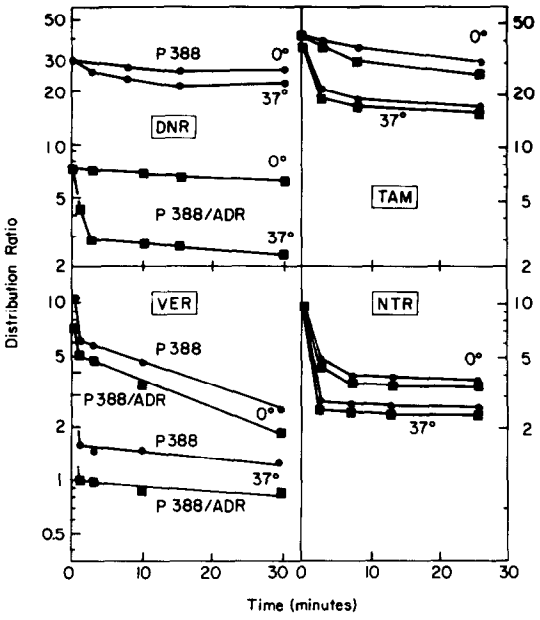

Fig. 2. Exodus of daunorubicin (DNR), verapamil (VER), tamoxifen (TAM) and nitrendipine (NTR) from P388 (●) and P388/ADR (■) cells as a function of time and temperature. Cells were initially loaded with drugs for 30 min at 37° (drug concentrations = 1 μM) and then suspended in fresh medium at the specified temperature. Results from typical experiments are shown; replicates differed from these values by less than ±10%.

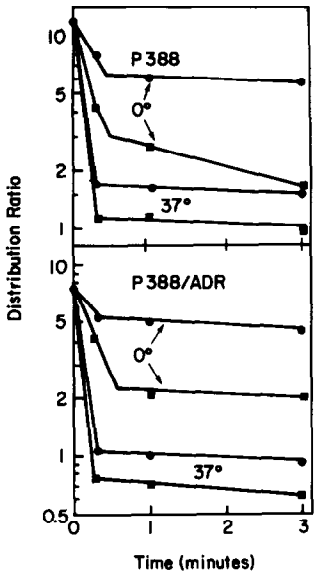

Fig. 3. Time course of verapamil exodus at 37° from P388 or P388/ADR cells loaded as specified in the legend to Fig. 2. Key: (●) growth medium; and (■) growth medium +10 μM tamoxifen.

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NTR or TAM to media containing 1 μM concentrations of labeled NTR or TAM did not affect steady-state accumulation of radioactivity. Furthermore, exodus of label from cells loaded with radioactive NTR or TAM was not affected by the presence of 100 μM (non-labeled) DNR, TAM, NTR or VER in media while efflux of label was measured. While VER did not affect kinetics of NTR transport, TAM antagonized VER accumulation in both P388 and P388/ADR. Moreover, the presence of 10 μM TAM promoted exodus of verapamil from both P388 and P388/ADR cells at 0° or at 37° (Fig. 3).

Data described here are in agreement with reports on inhibition of DNR exodus, in drug-resistant cell lines, by calcium antagonists and tamoxifen [7–15]. In the case of verapamil, data shown here and reported previously [18] suggest competition between this agent and DNR for an outward drug transport system in P388/ADR cells. Neither tamoxifen nor nitrendipine appears to be a substrate for this outward transport system. While NTR and DNR did not affect VER transport, an interaction between TAM and VER was observed. Exodus of verapamil was *promoted* by tamoxifen in both P388 and P388/ADR. In contrast, exodus of DNR was *inhibited* by TAM, but only in P388/ADR.

We conclude that no common exodus system explains the effects of VER, TAM and NTR on anthracycline transport in P388/ADR cells. Evidence presented here indicates that these drugs exert their effects by different classes of membrane interactions.

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REFERENCES

1. M. Inaba, H. Kobayashi, Y. Sakurai and R. K. Johnson, *Cancer Res.* **39**, 2200 (1979).
2. D. Kessel, V. Botterill and I. Wodinsky, *Cancer Res.* **28**, 938 (1968).
3. K. Danø, *Acta path. microbiol. scand.* Suppl. **256**, 11 (1976).
4. T. Skovsgaard, *Cancer Res.* **38**, 1785 (1978).
5. T. Skovsgaard, *Cancer Res.* **40**, 1077 (1980).
6. D. Kessel and C. Wheeler, *Biochem. Pharmac.* **33**, 991 (1984).
7. T. Tsuruo, H. Iida, M. Yamashiro, S. Tsukagoshi and Y. Sakurai, *Biochem. Pharmac.* **31**, 3138 (1982).
8. T. Tsuruo, H. Iida, S. Tsukagoshi and Y. Sakurai, *Cancer Res.* **42**, 4730 (1982).
9. S. Yanovich and L. Preston, *Cancer Res.* **44**, 1743 (1984).
10. A. M. Rogan, T. C. Hamilton, R. C. Young, R. W. Klecker Jr. and R. F. Ozols, *Science* **224**, 994 (1984).
11. A. Ramu, R. Spanier, H. Rahamimoff and Z. Fuks, *Br. J. Cancer* **50**, 501 (1984).
12. R. Ganapathi and D. Grabowski, *Cancer Res.* **43**, 3696 (1983).
13. R. Ganapathi, D. Grabowski, R. Turinic and R. Valenzuela, *Eur. J. Cancer clin. Oncol.* **20**, 799 (1984).
14. R. Ganapathi, D. Grabowski, W. Rouse and F. Riegler, *Cancer Res.* **44**, 5056 (1984).
15. A. Ramu, D. Glaubiger and Z. Fuks, *Cancer Res.* **44**, 4392 (1984).
16. R. K. Johnson, M. P. Chitnis, W. M. Embrey and E. B. Gregory, *Cancer Treat. Rep.* **61**, 1553 (1982).
17. D. Kessel and C. Wilberding, *Cancer Res.* **45**, 1687 (1985).
18. D. Kessel and C. Wilberding, *Biochem. Pharmac.* **33**, 1157 (1984).