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Tamoxifen decreases drug efflux from liposomes: relevance to its ability to reverse multidrug resistance in cancer cells?

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Abstract

Tamoxifen decreased the efflux of the fluorescent marker drug, chloroquine, from phosphatidylcholine liposomes. Tamoxifen is a known structural-mimic of cholesterol, which were both found to be similarly effective in preventing drug release from liposomes. This ability of tamoxifen and cholesterol to decrease drug efflux in a concentration-dependent manner is likely to arise from their known ability to decrease membrane fluidity both in liposomes and also in cancer cells. The possible importance of the ability of tamoxifen to inhibit drug efflux from liposomes in relation to its ability to reverse multidrug resistance in cancer patients caused by the efflux of cytotoxic therapeutic agents, is discussed.

Key words: Tamoxifen; Multidrug resistance; Liposome; Cholesterol; Anticancer action; Membrane fluidity

1. Introduction

Tamoxifen is successfully used in the treatment of breast cancer [1–4] and is now being investigated as a prophylactic agent for this disease [5–9]. In addition, tamoxifen may be of use clinically in the treatment of other cancers, including cancer of the liver [10], pancreas [11] and brain [12,13]. The ability of tamoxifen to cause the production of transforming growth factor β in normal cells, which inhibits the growth of adjacent cancer cells [14], suggests that tamoxifen might find general application in the prevention and treatment of cancer.

Currently there is also considerable interest in the potential clinical use of tamoxifen to reverse multidrug resistance (MDR) [15], which is an important clinical problem. An MDR phenotype can result from the overproduction in resistant cells of a transmembrane P-170 glycoprotein (encoded by the *mdr*-1 gene), which appears to act through an ATP-dependent drug-efflux mechanism to pump the cytotoxic drugs used in cancer chemotherapy (e.g. adriamycin) out of the cell, thus preventing the accumulation of drugs to an effective cytotoxic concentration. Tamoxifen has been shown to reverse P-170 glycoprotein-induced MDR in human and murine leukaemic cells [16] and in P-170 glycoprotein-expressing cell lines [17], and also vinblastine resistance in multidrug-resistant cell lines [18]. In addition, tamoxifen can increase the cytotoxic effects of adriamycin and vinblastine [19]. Furthermore, tamoxifen has been used in combination with vinblastine as a MDR reversal agent in Phase-I clinical trials [20], and high-dose tamoxifen with etoposide is also being studied [21].

The recognized ability of tamoxifen to decrease membrane fluidity [22,23] could directly inhibit the action of the P-170 glycoprotein pump, possibly by altering its conformation [24,25]. However, an underlying basal mechanism for the ability of tamoxifen to prevent drug efflux across the lipid bilayer component of membranes is also possible. Tamoxifen and related compounds decrease membrane fluidity in phospholipid liposomes [23], which are a useful model membrane (lipid bilayer) system [26] for studying possible protein pump-independent mechanisms of the anti-MDR action of tamoxifen. We investigated, therefore, the ability of tamoxifen and the membrane fluidity modulator, cholesterol (structures shown in Fig. 1), to modulate drug efflux from liposomes. This was measured by the efflux of the fluorescent marker drug, chloroquine, from phosphatidylcholine liposomes, which can be readily assayed as a model for the cytotoxic drugs administered during cancer chemotherapy treatments that are effluxed by the cancer cell.

2. Materials and methods

2.1. Chemicals

Tamoxifen, chloroquine disulphate salt and Sephadex G-50 were obtained from Sigma Chemical Co. (Poole, UK); cholesterol was obtained from Judex Laboratory Reagent (Sudbury, UK); and soya bean phosphatidylcholine was obtained from BDH Laboratory Suppliers (Poole, UK).

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2.2. Preparation and purification of liposomes

Liposomes were prepared with and without the introduction of the compounds shown in Fig. 1. Soya bean phosphatidylcholine was dissolved in chloroform to give a final concentration of 10 mg/ml. When included, tamoxifen or cholesterol were added to the chloroform to give the final concentrations stated. The chloroform was evaporated in a rotary evaporator and the residue was sonicated for 40 min with 50 ml of chloroquine in aqueous solution (1 mg/ml) to produce liposomes (small unilamellar vesicles, SUVs, were formed). The liposomes with entrapped chloroquine were separated from the unentrapped chloroquine by gel-filtration with Sephadex G-50.

2.3. Chloroquine efflux studies

The efflux of chloroquine, in the absence and presence of a range of concentrations of tamoxifen and cholesterol, was determined by measuring the release of chloroquine from liposomes. Thus 3 ml of the purified liposomes in a sealed dialysis bag was placed in 25 ml glycine buffer (pH 9.2) at 25°C and at the times stated 3 ml was removed from the buffer and the released chloroquine was determined by fluorescence. The sample was then returned to the buffer to keep the volume constant. The fluorescence values were measured using a Perkin-Elmer spectrofluorimeter: chloroquine exhibits a fluorescence emission maximum at 383 nm (excitation maximum at 330 nm). The amount of chloroquine entrapped was measured by mixing and centrifuging a 2 ml sample of the purified liposome suspension with an equal volume of chloroform at 3,000 rpm for 10 min in a bench centrifuge. The chloroquine present in 1 ml of the aqueous chloroquine-containing layer was determined by the fluorescence technique described above.

3. Results

Fig. 2 shows that tamoxifen, over the concentration range used of 0–50 μM, decreased the efflux of chloroquine from the liposomes (amount released was expressed as a % of the amount entrapped), which was measured over a time–course of 6 h: similar results were obtained for cholesterol over the same concentration range and time–course (Fig. 3). The effect of both tamoxifen and cholesterol on chloroquine efflux (see Fig. 4) was clearly concentration dependent, and tamoxifen and cholesterol appear to have a similar effect in decreasing the efflux of chloroquine. These results also show (see Figs. 2–4) that for both tamoxifen and cholesterol, 10 μM of each compound is considerably more effective than 5 μM at decreasing chloroquine efflux, and, in the case of cholesterol, very little further effect is observed at higher concentrations: tamoxifen concentrations of >10 μM did produce some further increases in effect (see Fig. 2) but at a concentration of approximately 25 μM both compounds had reached the same maximum level of reduction in chloroquine efflux (see Fig. 4).

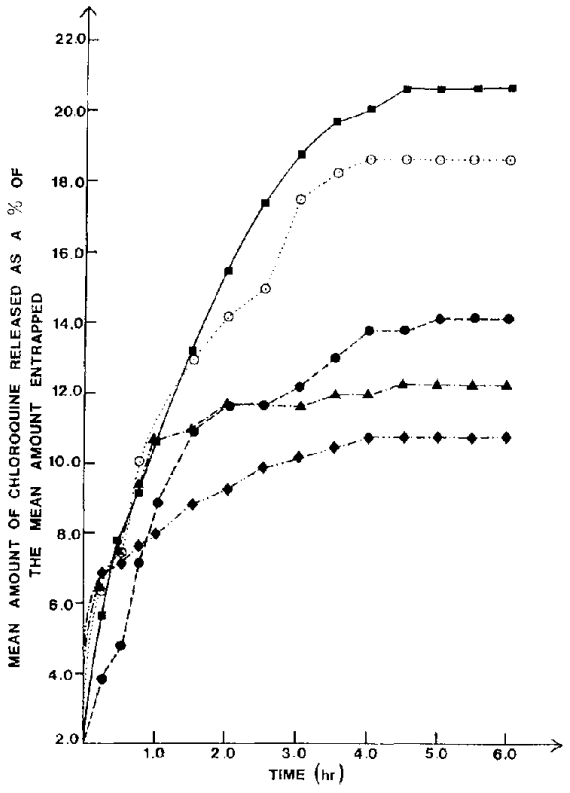
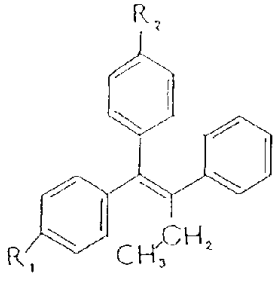
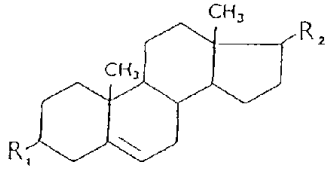


Fig. 2. Time–course of the effect of (■) 0 μM tamoxifen (control), (○) 5 μM tamoxifen, (●) 10 μM tamoxifen, (▲) 25 μM tamoxifen and (◆) 50 μM tamoxifen on the mean amount of chloroquine release from liposomes as a % of the mean amount of chloroquine entrapped. Results are quoted as the mean of the measurements from two separate experiments.



TAMOXIFEN R_1=H, R_2=$OCH_2CH_2N(CH_3)_2$



CHOLESTEROL R_1=OH, R_2=$CH(CH_3)(CH_2)_3CH(CH_3)_2$

Fig. 1. Structures of the triphenylethylene anticancer drug, tamoxifen, and the membrane sterol, cholesterol.

4. Discussion

Tamoxifen and cholesterol were both found to decrease the efflux of chloroquine from phosphatidylcholine liposomes. Furthermore, over the concentra-

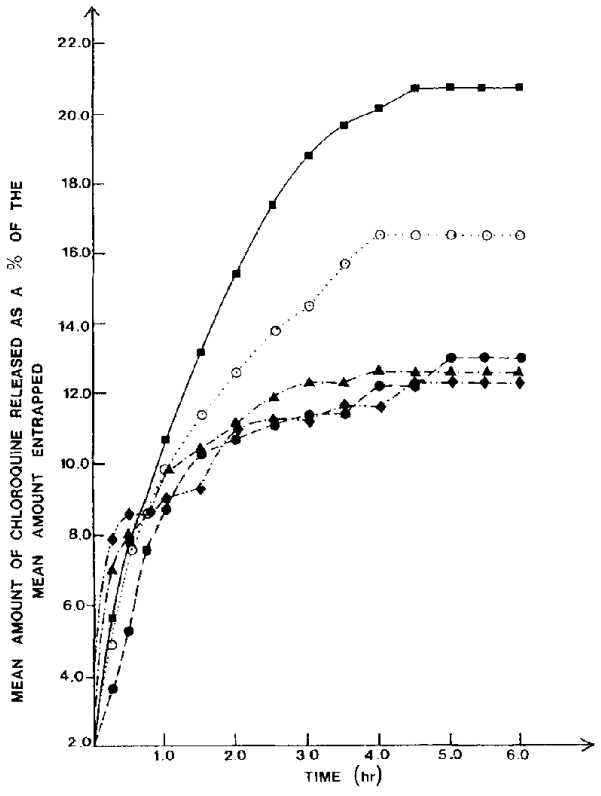


Fig. 3. Time-course of the effect of (■) 0 μM cholesterol (control), (○) 5 μM cholesterol, (●) 10 μM cholesterol, (▲) 25 μM cholesterol and (◆) 50 μM cholesterol on the mean amount of chloroquine release from liposomes as a % of the mean amount of chloroquine entrapped. Results quoted are the mean of measurements from two separate experiments.

tion range tested, both compounds were similarly effective at preventing chloroquine release. This suggests that tamoxifen and cholesterol (see Fig. 1) are acting in a similar manner to decrease drug efflux, probably through a mechanism involving decreased membranes fluidity. Tamoxifen has been shown to decrease membrane fluidity in phospholipid liposomes [23] and in human cancer cells [22], and it has been suggested that tamoxifen decreases membrane fluidity through a similar mechanism to that of cholesterol, which it structurally mimics [23,27]. The well-documented ability of cholesterol to stabilize membranes through decreased membrane fluidity is thought to involve an interaction between the rigid hydrophobic ring structure of cholesterol and the saturated, mono-unsaturated, and to a much lesser extent the poly-unsaturated, fatty acid side chains of phospholipids [28,29].

This study indicates that the ability of tamoxifen to decrease drug efflux from liposomes, probably as a result of decreased membrane fluidity, is likely to contribute to the ability of tamoxifen to reverse MDR in cancer cells [16–21,24,25] through a similar basal mechanism for the decreased removal of cytotoxic drugs from the cancer cell. This is in addition to the ability of tamoxifen to inactivate the P-170 glycoprotein efflux pump that has evolved in cancer cells as a particular cause of MDR. Inactivation of this superimposed efflux pump-dependent mechanism as for the basal mechanism reported here is proposed to be through decreased membrane fluidity [19,22,24,25]. The ability of cholesterol to decrease drug efflux to a similar extent to tamoxifen suggests that increased cholesterol content in the plasma membrane of cancer cells and the associated decrease in membrane fluidity could also be beneficial in preventing the cancer cell from developing MDR. Cholesterol is likely, therefore, to decrease the basal rate of efflux of an administered cytotoxic drug through the lipid bilayer and to contribute to the inactivation of the superimposed P-170 glycoprotein-dependent mechanism, both as a result of the decreased membrane fluidity. The administration of other drugs and compounds (in addition to tamoxifen) that are known fluidity modulators, e.g. synthetic oestrogens [30], is thus likely to prove a useful approach in overcoming the serious clinical problem of MDR.

This approach could, therefore, be readily extended to investigate other drugs of interest. The liposome system thus provides a rapid and convenient test for identifying the ability of a range of compounds to decrease the underlying basal rate of drug efflux through a phospholipid membrane system in the absence, in the first instance, of the protein efflux pump component of cancer cells displaying this form of MDR. Liposomal systems may,

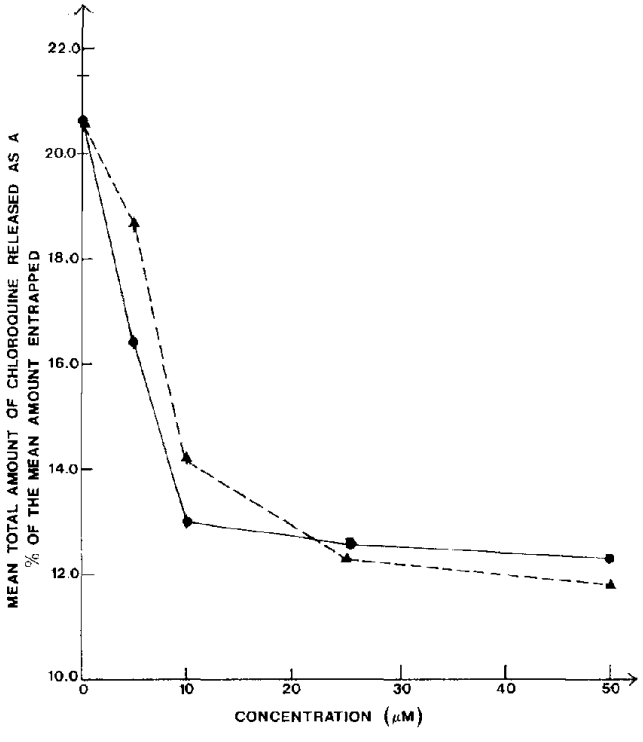


Fig. 4. Concentration-dependent effect of (▲) tamoxifen and (●) cholesterol on the mean amount of chloroquine release from liposomes as a % of the mean amount of chloroquine entrapped. Results quoted are the mean of measurements from two separate experiments.

therefore, facilitate the development of improved agents for MDR reversal in cancer cells by providing an aid to the identification of appropriately modified versions of tamoxifen and related fluidity modulators that have undergone re-design by computer-predicted molecular modelling for improved efficacy.

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References

[1] Jordan, V.C. (1992) J. Natl. Cancer Inst. 84, 231–234.
[2] Riley, D., Baum, M., MacIntyre, J., Berstock, D., McKinna, A., Jackson, I., Sainsbury, J.R.C., Wilson, A., Wheeler, T. and Dobbie, J. (1992) Eur. J. Cancer 28A, 904–907.
[3] Cummings, F.J., Gray, R., Tormy, D.C., Davis, T.E., Volks, H., Harris, J., Falkson, G. and Bennett, J.M. (1993) J. Clin. Oncol. 11, 29–35.
[4] Baum, M., Houghton, J., Riley, D., MacIntyre, J., Berstock, D., McKinna, A., Jackson, I., Sainsbury, J.R.C., Wilson, A. and Wheeler, T., (1992) Acta Oncolog. 31, 251–258.
[5] Bush, T.L. and Helzlsouer, K.J. (1993) Epidemiol. Rev. 15, 233–243.
[6] Powles, T.J. (1992) Lancet 340, 1145–1147.
[7] Chlebowski, R.J., Butler, J., Nelson, A. and Lillington, L. (1993) Cancer 72, 1032–1037.
[8] Jordan, V.C. (1993) Br. J. Pharmacol. 110, 507–518.
[9] Jordan, V.C. (1993) Br. Med. J. 307, 1371–1372.
[10] Farinati, F., Demaria, N., Fornasier, A., Salvangini, M., Fagiuoli, S., Chiaramonte, M. and Naccarto, R. (1992) Dig. Dis. Sci. 37, 659–662.
[11] Taylor, O.M., Benson, E.A. and McMahon, M.J. (1993) Br. J. Surg. 80, 384–386.
[12] Vertosick, F.T., Selker, R.G., Pollack, I.F. and Arena, V. (1992) Neurosurgery 30, 897–903.
[13] Couldwell, W.T., Weiss, M.H., Degiorgio, C.M., Weiner, L.P., Hinton, D.R., Ehresmsan, G.R., Conti, P.S., Apuzzo, M.L.J., Kornblith, P. and Detriboler, N. (1993) Neurosurgery 32, 485–490.
[14] Butta, A., Maclennan, K., Flanders, K.C., Sacks, N.P.M., Smith, I., McKinna, A., Dowsett, M., Wakefield, L.M., Sporn, M.B., Baum, M. and Colletta, A.A. (1992) Cancer Res. 52, 4261–4264.
[15] Pasman, P.C. and Schouten, H.C. (1993) Netherlands J. Med. 42, 218–231.
[16] Berman, E., Adams, A., Duigou-Osterndorf, R., Godfrey, L., Clarkson, B. and Andreeff, M. (1991) Blood 77, 818–825.
[17] Stuart, N.S.A., Philip, P., Harris, A.L., Tonkin, K., Houlbrook, S., Kirk, J., Lien, E.A. and Carmichael, J. (1992) Br. J. Cancer 66, 833–839.
[18] Kirk, J., Houlbrook, S., Stuart, N.S.A., Stratford, I.J., Harris, A.L. and Carmichael, J. (1993) Eur. J. Cancer 29A, 1152–1156.
[19] Leonessa, F., Jacobson, M., Boyle, B., Lippman, J., McGarvey, M. and Clark, R. (1994) Cancer Res. 54, 441–447.
[20] Trump, D.L., Smith, D.C., Ellis, P.G., Rogers, M.P., Schold, S.C., Winer, E.P., Panella, T.J., Jordan, V.C. and Fine, R.L. (1992) J. Natl. Cancer Inst. 84, 1811–1815.
[21] Millward, M.J., Lien, E.A., Robinson, A. and Cantwell, B.M.J. (1994) Oncology 51, 79–83.
[22] Clarke, R., van den Berg, H.W. and Murphy, F.R. (1990) J. Natl. Cancer Inst. 82, 1702–1705.
[23] Wiseman, H., Quinn, P. and Halliwell, B. (1993) FEBS Lett. 330, 53–56.
[24] Wiseman, H. (1994) Trends Pharmacol. Sci. 15, 983–989.
[25] Wiseman, H. (1994) in: Tamoxifen: Molecular Basis of Use in Cancer Treatment and Prevention, Wiley, Chichester, in press.
[26] New, R.R.C. (1992) in: Liposomes: A Practical Approach (New, R.R.C. ed.) pp. 1–161, IRL, Oxford.
[27] Wiseman, H., Cannon, M., Arnstein, H.R.V. and Barlow, D.J. (1992) Biochim. Biophys. Acta 1138, 197–202.
[28] Gutteridge, J.M.C. (1978) Res. Commun. Chem. Pathol. Pharmacol. 22, 563–572.
[29] Gurr, M.I. and Harwood, J.L. (1991) in: Lipid Biochemistry, 4th ed. Chapman and Hall, London.
[30] Wiseman, H. and Quinn, P. (1994) Free Rad. Res. (in press).