Reversal of Multidrug Resistance by RU 486

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Abstract

P-Glycoproteins represent a family of drug efflux proteins that convey multidrug resistance to cells in which they are expressed. This phenomenon can lower the efficacy of drugs used in chemotherapy. The steroid progesterone has been shown to bind P-glycoproteins and inhibit their drug efflux. We report that the antiprogestin RU 486 can reverse multidrug resistance in cells overexpressing the mouse mdrl gene. Using flow cytometry to measure inhibition of P-glycoprotein-dependent efflux of rhodamine 123, RU 486 was found to be considerably more effective than progesterone and one-half as effective as verapamil. The results suggest a valuable new use for RU 486.

Introduction

The beneficial effects of chemotherapy can be compromised by cellular mechanisms that allow neoplastic tissue to evade the toxicity of drugs (1, 2). In some cases, pleiotropic resistance to a variety of unrelated drugs has been observed, and this phenomenon has been called multidrug resistance (3, 4). MDR	extsuperscript{2} may be gained through several mechanisms. One such mechanism is a reduction in drug accumulation due to increased rates of drug efflux from cells expressing P-glycoproteins (5—8). P-glycoproteins represent a family of transport proteins capable of causing an ATP-dependent efflux of a wide variety of compounds across the plasma membrane. The drugs involved in this form of MDR share little structural similarity but are most often small and hydrophobic and thought to enter cells by diffusion across the cell membrane (9—14). This category includes such diverse compounds as Vinca alkaloids (vinblastine and vincristine), antracyclins (daunomycin), colchicine, taxol, and puromycin. Resistance to hydrophobic peptides, such as gramamicin, has also been observed (15). A variety of hydrophobic compounds have also been identified that have a potent capacity to inhibit drug transport (16). Recently, we and others have found that certain steroids are transported by P-glycoproteins (17—19). The mouse mdrl P-glycoprotein causes a reduced accumulation of corticosteroids containing an hydroxyl group at the 11-position of the steroid molecule. An additional hydroxyl group at the 17-position greatly enhances this capacity. Thus, cortisol, dexamethasone, aldosterone, and other similar steroids appear to be substrates for transport. In contrast, steroids such as progesterone and cortexolone, which lack the 11-hydroxyl group, are not transported (19). However, progesterone has been shown to bind to the mouse and human P-glycoproteins and to inhibit their function, even though progesterone is not transported (20—22).

If RU 486 can inhibit P-glycoprotein function, it should be able to reverse the drug-resistant phenotype of the murine thymoma cell line S7CD-5. The S7CD-5 cell line was derived from a steroid-sensitive mouse line, WTTB (26). It expresses the mdrl gene and is resistant to a variety of drugs including colchicine, puromycin, daunomycin, and...
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Fig. 1. Effects of RU 486 and puromycin on the viability of S7CD-5 cells. Separate cultures of S7CD-5 cells initially containing $4 \times 10^6$ cells/ml were incubated with: •, no additions; O, 5 μM RU 486; Δ, 20 μM puromycin; or Δ, 5 μM RU 486 plus 20 μM puromycin. At the times indicated, samples were removed, and the concentration of viable cells was evaluated using a trypan blue exclusion test. The values represent the mean of two determinations for each time point.

Fig. 2A. Reversal of puromycin resistance in S7CD-5 cells by RU 486 and verapamil. Separate sets of S7CD-5 cultures initially containing $5 \times 10^6$ cells/ml were incubated in the indicated concentrations of puromycin for 5 days. The cultures contained: O, no additions; •, 5 μM RU 486; Δ, 5 μM verapamil. Another set of cultures containing the parental W7TB line without additions (O) is shown for comparison. At the end of the incubation period, the turbidities of the cultures ($A_{600\text{ nm}}$) were measured and normalized to values from the cultures without puromycin. Each value represents the mean of two determinations. These data are representative of three separate experiments.

Fig. 2B. Reversal of daunomycin resistance in S7CD-5 cells by RU 486 and verapamil. Separate sets of S7CD-5 cultures, containing RU 486 or verapamil, were set up and incubated in the indicated concentrations of daunomycin in a manner similar to the experiment shown in A. A set of cultures containing W7TB cells was again used for comparison. These data are representative of two separate experiments.

Fig. 3. Concentration dependence of drug resistance reversal by RU 486 and verapamil. Separate sets of S7CD-5 cultures, initially containing $5 \times 10^6$ cells/ml, were incubated in the indicated concentrations of either RU 486 (•, Δ) or verapamil (O, Δ). Two sets of cells were prepared for each drug, either with (Δ, Δ) or without (O, O) 20 μM puromycin. After 5 days, the cultures were analyzed as described for Fig. 2. These data are representative of results obtained in three separate experiments.

dexamethasone (19). The resistance to dexamethasone is due to reduced intracellular accumulation of steroid, even though the glucocorticoid receptors in S7CD-5 are present at normal levels and are fully functional. The drug resistance in this cell line is effectively reversed by 5 μM verapamil, an established inhibitor of P-glycoprotein function (16, 28, 29). Fig. 1 illustrates the effect of growing S7CD-5 cells in the presence of RU 486 (5 μM), puromycin (20 μM), or a combination of both drugs. Neither drug alone has a significant effect on the viability or proliferation of the cells. The combination, on the other hand, causes a complete loss of viability at times greater than 42 h. The result with RU 486 alone is not unexpected since it normally has little or no agonist activity for the glucocorticoid receptor (30). Thus, the results shown in Fig. 1 are consistent with the possibility that RU 486 acts at another target and promotes the accumulation of puromycin in the cells.

Fig. 2A compares the relative abilities of verapamil and RU 486 to alter the resistance of S7CD-5 to puromycin. The two drugs have a very similar effect. Each lowers the puromycin resistance approximately 17-fold to a level nearly equal to that seen with the sensitive parental cell line. Fig. 2B demonstrates that RU 486 and verapamil also have the capacity to completely reverse the resistance to daunomycin seen in the S7CD-5 cells. RU 486 and verapamil have the additional capacity to reverse colchicine resistance in S7CD-5 cells and in cells expressing the mdr3 gene (data not shown). This similarity in behavior between RU 486 and verapamil is particularly significant since verapamil has been found to be a potent inhibitor of P-glycoprotein function.

To determine the relative concentrations at which RU 486 and verapamil begin to reverse drug resistance, the experiment shown in Fig. 3 was carried out. Fig. 3 shows the effects of growing S7CD-5 cells in increasing concentrations of either verapamil or RU 486 in the presence or absence of 20 μM puromycin. Without puromycin, verapamil and RU 486 have only modest effects on the proliferation of cells and no visible effect on viability. In the presence of puromycin, verapamil causes a sharp decrease in proliferation at concentrations above 0.5 μM. RU 486 exhibits a very similar profile, only slightly displaced to higher concentrations. Microscopic inspection of the cultures revealed that all of the cells were dead in the presence of 20 μM puromycin and verapamil or RU 486 at concentrations above 2 μM.
If RU 486 and verapamil act in a similar fashion, then the two drugs should have comparable capacities to inhibit the rate of drug efflux from S7CD-5 cells. This possibility can be tested directly since previous studies have shown that P-glycoproteins transport the fluorescent drug rhodamine 123 (31, 32). Rhodamine fluorescence can be used to measure its relative intracellular concentration. Fig. 4A presents a comparison by flow cytometry of the capacities of RU 486 and verapamil to inhibit the efflux of rhodamine 123. In the absence of inhibitory drugs, 50% of the rhodamine 123 was transported out of the cells within 3.4 min, and RU 486, at 1 μM, had no effect upon this rate. In the presence of 5 and 10 μM RU 486, the rates were significantly slower; 50% of the rhodamine 123 was transported in 6 and 10.3 min, respectively. The RFER can be calculated (see “Materials and Methods”) by dividing the time needed to transport 50% of the rhodamine 123 (without drug) by the comparable time with drug. Thus, at 5 μM RU 486, the RFER was 57% of the untreated cells, and the value for 10 μM RU 486 was 33% of untreated cells. At 5 μM verapamil, the RFER was 29%. Using this comparison, 5 μM RU 486 appears to be one-half as effective as 5 μM verapamil (57 versus 29%) at inhibiting rhodamine 123 efflux from S7CD-5 cells. Fig. 4B compares the ability of RU 486 to inhibit rhodamine efflux with that of dexamethasone and progesterone. In this experiment, 10 μM dexamethasone had no effect on the efflux rate. As expected from previous reports (20–22), 10 μM progesterone had a measurable effect, but it was considerably smaller than the inhibition caused by 5 μM RU 486. In this instance, the RFER was 61% for progesterone and 45% for RU 486. In another similar experiment comparing 10 μM RU 486 and 10 μM progesterone, these inhibitors gave RFERs of 40 and 80%, respectively (data not shown). Thus, RU 486 was at least two times as effective as progesterone at inhibiting rhodamine 123 efflux.

These studies demonstrate that RU 486 has the capacity to efficiently inhibit drug efflux promoted by the mouse mdr1 P-glycoprotein and to reverse the multidrug-resistant phenotype conveyed by expression of this protein. RU 486 can achieve this effect at relatively low doses, just above 1 μM (Fig. 3). Studies carried out in humans have demonstrated that RU 486 serum concentrations above 1 μM are readily achievable (33). Thus, given the similarity between the mouse and human P-glycoproteins, the results indicate that RU 486 could potentially be used as a chemosensitizing agent. This is particularly true since high concentrations of RU 486 have been found not to have serious side effects. Recently, a series of hydrophobic compounds, including quinoline and cyclosporin derivatives and dihydropyridine analogues, were reported to be potent inhibitors of MDR function which act at submicromolar concentrations (34–36). The results with RU 486 suggest that there may be steroid derivatives with a similar capacity to inhibit drug transport by P-glycoproteins.

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References


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