Tamoxifen inhibits uptake and metabolism of ethanolamine and choline in multidrug-resistant, but not in drug-sensitive, MCF-7 human breast carcinoma cells

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Abstract Tamoxifen (TAM), a widely used agent in the hormonal therapy of breast cancer, is also an antagonist of P-glycoprotein (P-gp), a cell surface protein which confers drug resistance to cells. Here we report that in an estrogen receptor-deficient multidrug-resistant subline of MCF-7 human breast carcinoma cells (MCF-7/MDR), but not in the parent drug-sensitive cells (MCF-7/WT), clinically relevant concentrations (1–5 μM) of TAM inhibited the uptake and phosphorylation of ethanolamine and choline. These inhibitory effects resulted in decreased synthesis of the corresponding phospholipids. In view of the known dependence of P-gp function on phosphatidylethanolamine (PtdEtn), inhibition of PtdEtn synthesis may represent an additional mechanism by which TAM inhibits P-gp-mediated drug efflux.

Key words: Tamoxifen; Phosphatidylethanolamine synthesis; P-glycoprotein; Breast cancer

1. Introduction

Increased expression of P-glycoprotein (P-gp), a membrane glycoprotein of 170 kDa encoded by the multidrug resistance (MDR)-1 gene, in culture cells has been shown to confer both intrinsic and acquired resistance to anti-neoplastic drugs [1–3]. A wide range of compounds have been shown to be transported out of cells by P-gp [4]. It has been suggested that increased transport of P-gp substrates is associated with poorer antagonistic properties, while P-gp antagonists are more poorly transported than the typical substrates [5]. This, in principle, may provide an opportunity to enhance the cytotoxic effects of drugs by using them in combination with a P-gp antagonist.

Tamoxifen (TAM), a triphenylethylene non-steroidal antiestrogen, has been extensively used for treatment of patients with all stages of breast cancer [6–8]. In addition to its growth inhibitory effects, TAM has also been shown to be an antagonist of P-gp [9]. In accordance with the above prediction [5], in a multidrug-resistant subline of MCF-7 human breast carcinoma cells (MCF-7/MDR), but not in the drug-sensitive parent cells (MCF-7/WT), TAM was shown to enhance the cytotoxicity of vinblastine and doxorubicin [10]. However, it is not known whether interaction with P-gp is the only mechanism by which TAM inhibits P-gp-mediated drug efflux.

Recent evidence has indicated that the ATP-dependent function of P-gp requires phosphatidylethanolamine (PtdEtn) [11]. It has also been amply documented that TAM is capable of decreasing both membrane fluidity in model and native membranes [12–14] and drug efflux from liposomes [15]. Based on these observations, it was of interest to examine the effects of TAM on the uptake and metabolism of phospholipid precursors leading to phospholipid synthesis. Here we report that in MCF-7/MDR cells, but not in MCF-7/WT cells, TAM inhibited the uptake and phosphorylation of ethanolamine (Etn) and choline (Cho) and, as a result, the synthesis of corresponding phospholipids.

2. Materials and methods

2.1. Materials

TAM and Dowex-50W [H^+] form were purchased from Sigma; [2-^{14}C]ethanolamine (50 mCi/mmol) and [methyl-^{14}C]choline chloride (55 mCi/mmol) were from Amersham; tissue culture reagents were bought from Gibco BRL.

2.2. Cell culture

The MCF-7 human breast cancer cell lines (drug-sensitive MCF-7/WT cells and multidrug-resistant MCF-7/MDR cells) were generously provided by Dr. Kenneth Cowan (National Cancer Institute, NIH, Bethesda, MD, USA). MCF-7 cells were maintained as described earlier [16].

2.3. Determination of cellular uptake, phosphorylation, and incorporation into phospholipids of [^{14}C]Etn and [^{14}C]Cho in MCF-7 cells

MCF-7/MDR and MCF-7/WT cells were similarly grown in 12-well tissue culture dishes to 90–100% confluency, so the surface area covered by these cells was practically the same in each experiment. Cells were incubated with TAM (1–5 μM) for 45 min in choline-free Richer's Iscove's mediated Eagle medium, followed (with no washing step being included) by incubation of cells for 0–3 h in the presence of 50 μM [^{14}C]Etn (2×10^6 dpm/well) or 50 μM [^{14}C]Cho (2×10^6 dpm/well). At the end of incubation, the incubation medium was aspirated, then cells were gently but rapidly (within 20 s) washed with 5 ml medium followed by the addition of ice-cold methanol (2 ml) to the wells. Then, cells were scraped into methanol, and methanol extracts were rapidly transferred to 2 ml of chloroform. The water-soluble metabolites of [^{14}C]Etn and [^{14}C]Cho were fractionated on Dowex-50W [H^+]-packed columns (Bio-Rad; Econo columns, 1 ml bed volume) as described by Cook and Wakelam [17] with some modifications as follows. The initial flow-through (4.5 ml) along with a following 3.5- to 5-ml water wash contained glycerophosphoethanolamine and glycerophosphocholine, respectively. Ethanolamine phosphate (EtnP) and choline phosphate (ChoP) were eluted by 15 and 20 ml water, respectively. Finally, Etn and Cho were eluted by 12 and 20 ml of 1 M HCl, respectively. The metabolites of [^{14}C]Etn and [^{14}C]Cho were identified and phospholipids were separated as indicated previously [18].

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Abbreviations TAM, tamoxifen; Etn, ethanolamine; EtnP, ethanolamine phosphate; Cho, choline; ChoP, choline phosphate; PtdEtn, phosphatidylethanolamine; PtdCho, phosphatidylcholine; P-gp, P-glycoprotein.

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3. Results

Addition of TAM (1–5 μM) to MCF-7/WT cells failed to significantly modify the uptake of [^{14}C]Etn (Fig. 1A). At each time point examined, MCF-7/MDR cells (Fig. 1B) contained about 50% less [^{14}C]Etn compared to the drug-sensitive cells. In addition, in the MDR cells TAM decreased the cellular content of [^{14}C]Etn in a dose-dependent manner (Fig. 1B).

In MCF-7/WT cells, the major [^{14}C]Etn-labeled component was [^{14}C]EtnP (Fig. 1C), indicating the presence of a very active Cho/Etn kinase. In these cells, TAM slightly, but consistently, enhanced the cellular level of [^{14}C]EtnP (Fig. 1C); the mechanism and possible significance of this TAM effect is presently unknown. In TAM-treated MCF-7/WT cells, increased formation of [^{14}C]EtnP did not lead to increased ^{14}C-labeling of PtdEtn (Fig. 1E). In MCF-7/MDR cells, TAM only slightly decreased the formation of [^{14}C]EtnP (Fig. 1D), while 2.5–5 μM concentrations of TAM greatly reduced the formation of [^{14}C]PtdEtn (Fig. 1F). Presently we are investigating the possibilities that the larger inhibitory effects of TAM on [^{14}C]PtdEtn formation are due to multiple inhibitory effects of TAM, or reflect the existence of a specific TAM-sensitive cellular pool of Etn which is primarily involved in PtdEtn synthesis. Here, we still should draw attention to the finding that despite the relatively large (~2.5-fold) differences in the formation of [^{14}C]EtnP in the two cell lines (Fig. 1C and D), cellular PtdEtn pools in the drug-sensitive (Fig. 1E) and MDR (Fig. 1F) lines were labeled by [^{14}C]Etn roughly to the same extents.

Incubation of MCF-7/WT cells with [^{14}C]Cho resulted in significant labeling of cellular pools of [^{14}C]Cho (Fig. 2A), [^{14}C]ChoP (Fig. 2C) and [^{14}C]PtdCho (Fig. 2E). Again, the cellular ChoP pool contained about 10- to 12-fold more ^{14}C-label than the cellular pools of Cho and PtdCho. These data indicated that in these cells, Cho is efficiently phosphorylated

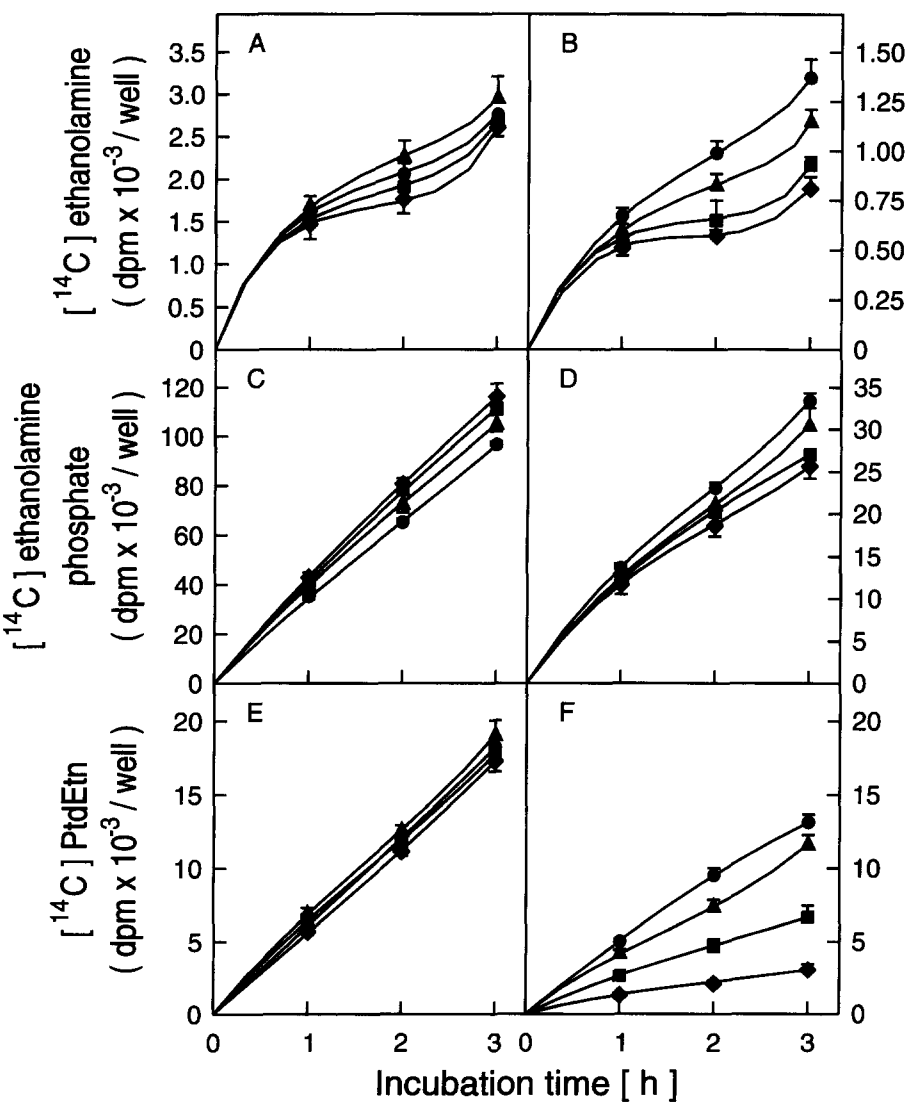

Fig. 1. Uptake and metabolism of [^{14}C]Etn in MCF-7/WT and MCF-7/MDR cells. MCF-7/WT (A, C, E) and MCF-7/MDR (B, D, F) cells, grown in 12-well culture dishes up to 90–100% confluency, were untreated (●), or were treated with 1 μM (▲), 2.5 μM (■), or 5 μM (♦) TAM for 45 min. At this time point, each well received (without removing TAM) [^{14}C]Etn, as described in section 2, and incubations continued up to 3 h. Incorporation of exogenous [^{14}C]Etn into the cellular pool of [^{14}C]Etn (A,B), [^{14}C]EtnP (C,D) and [^{14}C]PtdEtn (E,F) was determined as described in section 2. Each point represents the mean ± S.E.M. of six independent incubations. Similar results were obtained in two other experiments, each performed in triplicate, using the 3 h time point.

by Cho kinase, and that formation of ChoP is not the rate-limiting step in the synthesis of PtdCho. Treatment of MCF-7/WT cells for 1–3 h with TAM (1–5 μM) failed to significantly modify the uptake (Fig. 2A), phosphorylation (Fig. 2C), or incorporation of [^{14}C]Cho into PtdCho (Fig. 2E).

MCF-7/MDR cells exhibited dramatically lower rates of [^{14}C]Cho uptake (Fig. 2B), [^{14}C]ChoP formation (Fig. 2D), or [^{14}C]PtdCho synthesis (Fig. 2F), compared to MCF-7/WT cells. In addition, at each time period (1–3 h), TAM inhibited the accumulation of [^{14}C]Cho (Fig. 2B), [^{14}C]ChoP (Fig. 2D) and [^{14}C]PtdCho (Fig. 2F) to varying degrees. After incubation for 3 h, 5 μM TAM inhibited all these processes by 50% or more. As it was observed in the case of PtdEtn formation, ^{14}C-labeling of PtdCho was inhibited to the greatest extent by TAM. It remains to be determined whether this reflects additional inhibition of PtdCho synthesis at a step beyond Cho uptake, or it is due to the utilization of a specific TAM-sensitive cellular pool of Cho for PtdCho synthesis.

4. Discussion

We showed that 1–5 μM concentrations of TAM, which appear to be physiologically relevant [19–21], significantly inhibited the labeling of both PtdEtn and PtdCho by ^{14}C-labeled precursors in MCF-7/MDR cells, but not in the parent drug-sensitive cells. Since these cells contain relatively high levels of EtnP and ChoP, and because these intermediates normally do not limit phospholipid synthesis [22,23], initially TAM may not significantly affect the net synthesis of PtdEtn and PtdCho. However, phospholipid synthesis will eventually become dependent on the supply of precursors from the medium, and this is when the inhibitory effects of TAM on the uptake of precursors are expected to lead to lower rates of phospholipid synthesis.

It is presently not clear why TAM affected the metabolism of Etn and Cho only in the MCF-7/MDR cells. Furthermore, in view of the finding that different mechanisms are involved

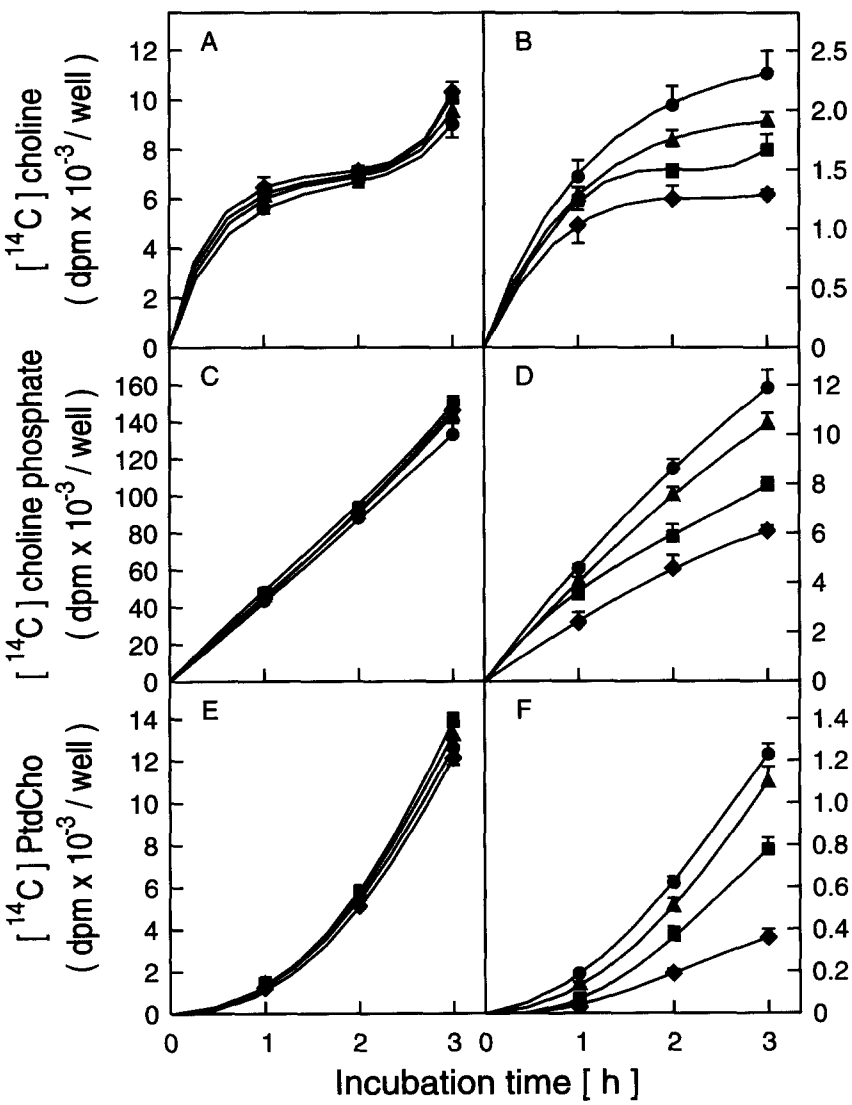

Fig. 2. Uptake and metabolism of [^{14}C]Cho in MCF-7/WT and MCF-7/MDR cells. MCF-7/WT (A, C, E) and MCF-7/MDR (B, D, F) cells, grown in 12-well culture dishes up to 90–100% confluency, were untreated (●), or were treated with 1 μM (▲), 2.5 μM (■), or 5 μM (♦) TAM for 45 min. This was followed by the addition of [^{14}C]Cho as described in section 2, and incubations continued up to 3 h. Incorporation of exogenous [^{14}C]Cho into the cellular pool of [^{14}C]Cho (A, B), [^{14}C]ChoP (C, D), and [^{14}C]PtdCho (E, F) was determined as described in section 2. Each point represents the mean ± S.E.M. of eight independent incubations.

in the uptake of Etn and Cho [24], it is not clear how TAM can affect both mechanisms. Nevertheless, the MDR phenotype in MCF-7 cells is clearly associated with significant changes in the metabolism of phospholipids. For example, we previously reported that TAM [25] and several known activators of phospholipase D [16] specifically stimulated the hydrolysis of PtdEtn in MCF-7/MDR, but not in MCF-7/WT cells. Collectively, these data suggest that the effects of TAM on phospholipid synthesis and degradation somehow reflect its binding to P-gp. However, until further experiments prove this possibility, we also have to consider the possibility that the effects of TAM on phospholipid metabolism do not involve P-gp.

Finally, it is worth noting that in the same MCF-7/MDR cell line that was used in this work, similar concentrations of TAM which inhibited phospholipid labeling/synthesis (this work) were also shown to enhance the cytotoxic effects of vinblastine and doxorubicin [10]. These enhancing effects of TAM apparently reflected its binding to P-gp, resulting in the inhibition of efflux of vinblastine and doxorubicin [10]. Nevertheless, the question remains whether this is the sole mechanism by which TAM can decrease the functional activity of P-gp. In view of recent findings that PtdEtn is required for the activity of P-gp [11], stimulation of PtdEtn hydrolysis [16] and inhibition of PtdEtn synthesis (this work) may provide additional mechanisms by which TAM can inhibit P-gp-mediated efflux of cytotoxic drugs. Considering the importance of this subject, further experiments are warranted to examine this possibility.

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