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Enhancement of tamoxifen-induced E-cadherin function by Ca^{2+} channel antagonists in human breast cancer MCF7/6 cells

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Abstract

Despite its intensive use in adjuvant breast cancer therapy for more than 30 years, the exact mechanisms of action of tamoxifen have not yet been fully characterized. Tamoxifen was recently shown to restore the E-cadherin function of human breast cancer MCF7/6 cells and to suppress their invasive phenotype. Because tamoxifen interacts with targets implicated in Ca^{2+} homeostasis, we explored the possibility that the restoration of E-cadherin function in MCF7/6 cells induced by this drug could be affected by Ca^{2+} modulators. Two different Ca^{2+} channel antagonists (verapamil and nifedipine) potentiated the effect of tamoxifen on E-cadherin function, as evaluated with a fast cell aggregation assay. These molecules decreased the tamoxifen concentration needed to restore the E-cadherin function from 10^{-6} M to 10^{-7} M. When incubated with a Ca^{2+} channel agonist, Bay K8644 (methyl-1,4-dihydro-2,6-dimethyl-3-nitro-4-(2-trifluoro-methylphenyl)-pyridine-5-carboxylate), the effect of tamoxifen on E-cadherin function was completely abolished. These results demonstrate that the restoration of the E-cadherin function induced by tamoxifen depends, at least in part, on a Ca^{2+} pathway, and support the evidence of an effect of tamoxifen on Ca^{2+}-dependent mechanisms. Our data also suggest that Ca^{2+} channel modulators could make it possible to decrease the dose of tamoxifen administered to patients without reducing the therapeutic effects.

Keywords*:* Tamoxifen; MCF7/6; E-cadherin; Adhesion; Ca^{2+}; channel antagonist

1. INTRODUCTION

Tamoxifen is the first molecule considered as a breast cancer prevention agent. Although this antiestrogen has been successfully used for more than 30 years in adjuvant therapy of advanced breast cancer, its exact mechanisms of action remain unclear (Coezy et al., 1982; Osborne et al., 1983; Wiseman et al., 1993). There is now clinical and experimental evidence suggesting that tamoxifen acts through several mechanisms, some of which are independent of its interactions with estrogen receptors (Antoniotti et al., 1992; Charlier et al., 1994; Jeng et al., 1993). Tamoxifen has been reported to have a therapeutic effect on patients with estrogen receptor negative breast cancer, while it is sometimes ineffective in cancers with estrogen receptor positive cells (Baker et al., 1992; Fisher et al., 1989; Fuqua et al., 1993). The proliferation of MDA-MB435 cells, a cell line which does not express estrogen receptors, is inhibited by tamoxifen (Charlier et al., 1995). The identification of the exact mechanisms of action of tamoxifen is necessary to assure a safe and appropriate administration of the drug both to breast cancer patients as adjuvant therapy, and to high risk women as a preventive agent. Because the recommended pharmacological doses are associated with undesirable side effects, any way that would decrease the required doses of tamoxifen without altering its therapeutic or preventive effects would be most welcome. Recently, we showed that tamoxifen (10^{-6} M) restored the function of the expressed but inactive cell-cell adhesion E-cadherin/catenin complex in MCF7/6 human breast cancer cells (Bracke et al., 1994). E-cadherin/catenin is a tumor suppressor complex whose alterations of function or expression are frequent features of invasive cells. At a similar concentration, tamoxifen suppressed the invasive phenotype of MCF7/6 cells in an in vitro invasion assay. These observations suggest that the therapeutic activity of tamoxifen could be linked, at least in part, to E-cadherin function (Bracke et al., 1994). Ca^{2+} participates in the regulation of the E-cadherin/catenin complex. Furthermore, there are several studies indicating that Ca^{2+} dependent pathways could be involved in the mechanisms of action of tamoxifen (Castoria et al., 1988; Strobl et al., 1994). These data prompted us to test the hypothesis that the restoration of the E-cadherin/catenin complex function induced by tamoxifen could be modulated by Ca^{2+} modulators. This paper reports our experiments with voltage-gated Ca^{2+} channel antagonists, verapamil and nifedipine, and agonist, BayK8644 (methyl-1,4-dihydro-2,6-dimethyl-3-nitro-4-(2-trifluoromethylphenyl)-pyridine-5-carboxylate).

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2. MATERIALS AND METHODS

2.1. Cells

Human breast cancer cells MCF7/6 (Soule et al., 1973) were obtained from Dr H. Rochefort (Unité d'Endocrinologie Cellulaire et Moléculaire, Montpellier, France) and maintained in Dulbecco's modified Eagle's medium/Ham F12 50:50 (Flow, Irvine, Scotland, UK) supplemented with 0.05% glutamine (w/v), 250 IU/ml penicillin, 100 µg/ml streptomycin, and 10% fetal calf serum. Cells were incubated in a 5% CO_2 incubator, under a water-saturated atmosphere. The medium was replaced every 3 days.

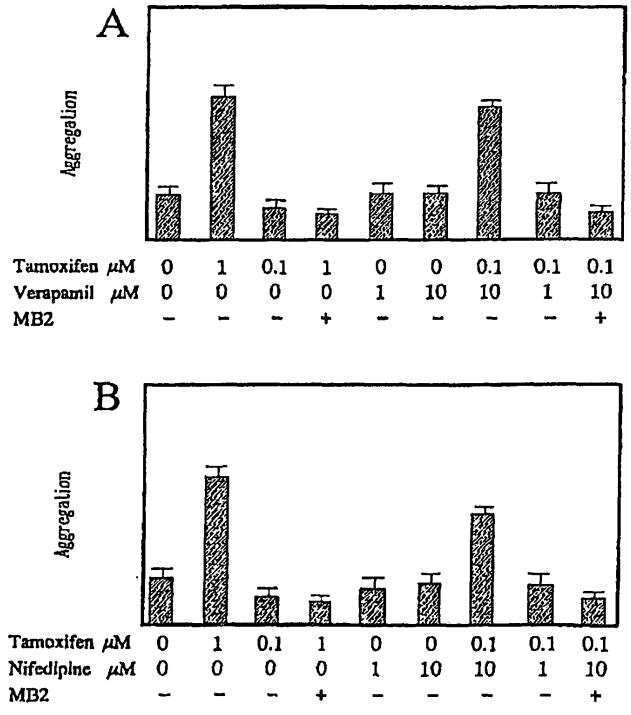
2.2. Chemicals and antibodies

Tamoxifen was kindly provided by Besins-Iscovesco (Paris, France). Verapamil and nifedipine were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Stock solutions of tamoxifen and nifedipine were prepared in ethanol, while stock solutions of verapamil were maintained in culture medium and kept in the dark. Bay K8644 was purchased from ICN (Costa Mesa, CA, USA) and a stock solution was prepared in dimethyl sulfoxyde (DMSO) and kept frozen. The monoclonal anti-E-cadherin antibody MB2 was a gift from Dr. Y. Shimoyama (Pathology Division, National Cancer Research Institute, Tokyo, Japan).

2.3. Fast aggregation assay

The fast aggregation assay was described previously (Bracke et al., 1994). Briefly, a cell suspension prepared under E-cadherin and Ca^{2+} saving conditions was incubated widi various concentrations of tamoxifen alone or in combination with various concentrations of Ca^{2+} channel antagonists. The number of particles in the suspension was measured with a Coulter Counter ZM (Coulter Counter Electronics, Luton, UK) at the start of the incubation (N_0) and after 30 min (N_{30}). All the experiments were done at least three times, in duplicate, and all the tests were simultaneously done in the presence of the MB2 antibody in control experiments.

Fig. 1: *Fast MCF7/6 cell aggregation expressed as 1 — N_{30}/N_0, where N_0 is the initial number of particles in suspension and N_{10} is the number after 30 min. Data for co-incubation of the cells with tamoxifen and verapamil (A) or nifedipine (B), or incubation with the Ca^{2+} channel antagonists alone are presented. The induction was significant with verapamil ($P < 0.01$) and nifedipine ($P< 0.0S$) according to the Student's t-test. The incubation of MCF7/6 cells with tamoxifen 0.1 µM in combination with verapamil 10 µM restored the results of the fast aggregation assay to the same value as the aggregation obtained with 1 µM of tamoxifen alone. Simultaneous incubation with anti-cadherin monoclonal MB2 antibody abolished the fast aggregation. Each experiment was performed in duplicate and was reproduced at least three times.*



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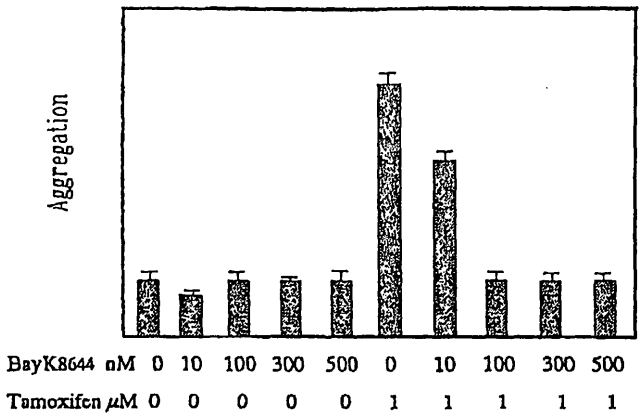
3. RESULTS

To evaluate the effects of voltage-gated Ca^{2+} channel antagonists on tamoxifen induction of E-cadherin/catenin function, we used the fast aggregation assay previously described (Bracke et al., 1994). As shown in Fig. 1, tamoxifen at 1 μM - but not at 0.1 μM - stimulated the aggregation of MCF7/6 cells. Anti-E-cadherin monoclonal antibody MB2 suppressed the induced aggregation, suggesting that tamoxifen restores the E-cadherin function. Addition of the voltage-gated Ca^{2+} channel antagonists, verapamil and nifedipine, to MCF7/6 cells in the presence of 0.1 μM tamoxifen led to a partial or complete restoration of the aggregation. In the presence of verapamil, tamoxifen was able to restore the aggregation function at a concentration of 10^{-7} M, which was 10 times lower than the minimal one needed when the antiestrogen was used alone. This effect was less pronounced with nifedipine than with verapamil. The effect observed with verapamil and nifedipine was dose dependent, reaching a plateau at 10 μM, and was inhibited by the presence of monoclonal antibody MB2 (Fig. 1). When added alone at various concentrations, the Ca^{2+} channel antagonists had no effect on MCF7/6 aggregation (Fig. 1).

Cell aggregation induced by tamoxifen and Ca^{2+} channel antagonists increased rapidly, with a maximum activity observed after 10 min (data not shown).

We also tested a Ca^{2+} channel agonist, Bay K8644, in the same fast aggregation assay. Bay K8644 is a dihydropyridine which opens the Ca^{2+} channel. The drug completely abolished the tamoxifen induction of E-cadherin/catenin complex function (Fig. 2). The effect of Bay K8644 was dose dependent and started at a concentration of 100 nM. Used alone, this drug had no effect on the aggregation of MCF7/6 cells.

Fig. 2: *Fast MCF7/6 cell aggregation expressed as 1 - N_{30}/N_0 in the presence of the Ca^{2+} channel agonist Bay K8644 alone or in combination with tamoxifen. Bay K8644 abolished the effect of the optimal concentration of tamoxifen. This effect was dose dependent and started at a concentration of 100 nM ($P < 0.01$). Each experiment was performed in duplicate and was reproduced at least three times.*



4. DISCUSSION

The mechanisms of action of tamoxifen as a therapeutic and potentially preventive agent in human breast cancer are still under intense investigations. Recendy, we have demonstrated that tamoxifen restores, at pharmacological concentrations, the function of the invasion suppressor complex E-cadherin/catenin in human breast cancer cells MCF7/6 (Bracke et al., 1994). This activity could participate in the therapeutic benefit associated with tamoxifen uptake. The possibility that Ca^{2+}-dependent pathways could be involved was considered because (a) a recent study indicated that voltage-gated Ca^{2+} channel antagonists potentiated the antiproliferative effects of tamoxifen (Gupta et al., 1994), (b) tamoxifen can interact with several Ca^{2+} control elements including calmodulin (Strobl et al., 1994), and (c) E-cadherin/catenin functions are Ca^{2+} dependent. In this work, we have demonstrated that the voltage-gated Ca^{2+} channel antagonist, verapamil, and to a lesser extent, nifedipine, potentiated the effects of tamoxifen on E-cadherin/catenin functions. The modulation of cytoplasmic Ca^{2+} concentration by these drugs could affect E-cadherin/catenin complex function, since Ca^{2+} seems to play a role in stabilizing interactions between successive cadherin domains (Shapiro et al., 1995). Indeed, verapamil caused a 10-fold decrease in the concentration of tamoxifen needed to restore the aggregation of MCF7/6 cells. Our study provides the first evidence that voltage-gated Ca^{2+} channel antagonists are able to significantly reduce the dose of tamoxifen without altering its pharmacological action, i.e. the restoration of a tumor suppressor gene function. Our finding

could have important clinical implications. The combination of tamoxifen with Ca^{2+} homeostasis modulators could indeed permit the dose of tamoxifen required to reach a protective effect to be decreased and thereby reduce the incidence of its side effects.

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References

Antoniotti, S., P. Maggiora, C. Dati and M. De Bortoli. 1992. Tamoxifen up-regulates cerb-B2 expression in oestrogen-responsive breast cancer cells in vitro, Eur. J. Cancer 28, 318.

Baker, V.A., J.R. Puddefoot, S. Marsigliante, S. Baker, A.W. Goode and G.P. Vinson, 1992, Oestrogen receptors isoforms, their distribution and relation to progesterone receptor level in breast cancer samples, Br. J. Cancer 66, 1083.

Bracke, M.E., C. Charlier, E.A. Bruyneel, C. Labit, M.M. Mareel and V. Castronovo, 1994, Tamoxifen restores the E-cadherin function in human breast cancer MCF7/6 cells and suppresses their invasive phenotype. Cancer Res. 54, 4607.

Castoria, G., A. Migliaccio, E. Nola and F. Auricchio, 1988, In vitro interaction of oestradiol receptor with Ca-calmodulin, Mol. Endocrinol. 2, 167.

Charlier, C, C. Colin, M.P. Merville, J. Gielen. R. Lambotte, G. Plom-teux and V. Castronovo, 1994, Le tamoxifène dans le traitement du cancer du sein, J. Gynécol. Obstét. Biol. Reprod. 23, 751.

Charlier, G, A. Chariot, N. Antoine, M.P. Merville, J. Gielen and V. Castronovo, 1995, Tamoxifen and its active metabolite inhibit growth of estrogen receptor-negative MDA-MB435 cells, Biochem. Pharmacol. 49, 351.

Coezy, E., J.L. Borgna and H. Rochefort, 1982. Tamoxifen and metabolites in MCF7 cells: correlation between binding to estrogen receptor and inhibition of cell growth, Cancer Res. 42, 317.

Fisher, B., *J.* Costanrino, C. Redmond, R. Poisson, D. Bowman, J. Couture, V.D. Nikolay, N. Wolmark, D.L. Wickerman, E.R. Fisher, R. Margolese, A. Robidoux, H. Shibota, J. Terz, A.H.G. Poterson, M.I. Feldman, W. Farrar, J. Evans, H.L. Lickley and M. Ketner, 1989, A randomized clinical trial evaluating tamoxifen in the treatment of patients with node-negative breast cancer who have estrogen-receptor positive tumors, New Engl. J. Med. 320, 479.

Fuqua, S.A.W., G.C. Chamness and W.L. McGuire, 1993, Estrogen receptor mutations in breast cancer, J. Cell. Biochem. 51, 135.

Gupta, V., N. Kamath, G.T. Tkalcevic and S.V. Singh, 1994, Potentiation of tamoxifen activity by verapamil in α human breast cancer cell line, Biochem. Pharmacol. 47, 1701.

Jeng, M.H., P. Ten Dijke, K.K. Iwata and V.C. Jordan, 1993, Regulation of the levels of .three transforming growth factor β mRNAs by estrogen and their effects on the proliferation of human breast cancer cells, Mol. Cell. Endocrinol. 97, 115.

Osborne, C.K., D.H. Boldt, G.M. Clark and J.M. Trent, 1983, Effects of tamoxifen on human breast cancer cell cycle kinetics: accumulation of cells in early G1 phase. Cancer Res. 43, 3583.

Shapiro, L., A.M. Fannon, PJD. Kwong, M.S. Lehmann, G. Griibel, J.-F. Legrand, J. Als-Nielsen, D.R. Colman and W.A. Hendrickson, 1995, Structural basis of cell-cell adhesion by cadherins. Nature 374, 327.

Soule, H.D., J. Vazquez, A. Long, S. Albert and M. Brennan, 1973, A human cell line of a pleural effusion derived from a breast carcinoma, J. Natl. Cancer Inst. 51, 1409.

Strobl, J.S., V.A. Peterson and K.A. Woodfork, 1994. A survey of human breast cancer sensitivity to growth inhibition by calmodulin antagonists in tissue culture, Mol. Endocrinol. 2, 167.

Wiseman, H., M. Cannon, H.R. Amstein and B. Halliwell, 1993, Tamoxifen inhibits lipid peroxidation in cardiac microsomes, Biochem. Pharmacol. 45. 1851.