Liarozole potentiates the all-trans-retinoic acid-induced structural remodelling in human breast carcinoma MCF-7 cells in vitro

Jimmy Van Heusdena, Marcel Borgersb, Frans Ramaekersb, Benoit Xhonneuxa, Walter Woutersc, Roland De Costerc, Gerda Smetsd, e

a Department of Molecular Cellular Biology & Genetics, University of Limburg, Maastricht/The Netherlands
b Department of Morphology, Janssen Research Foundation, Beerse/Belgium
c Department of Endocrinology & Immunopharmacology, Janssen Research Foundation, Beerse/Belgium

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Liarozole inhibits cytochrome P-450-dependent enzymes that play a key role in all-trans-retinoic acid (ATRA) catabolism. In MCF-7 cells, liarozole potentiates the antiproliferative effects of ATRA. The present study demonstrates this synergistic effect on cell differentiation of MCF-7 cell cultures as measured by immunocytochemistry for cytokeratins 8, 18, and 19, actin, E-cadherin, desmoglein and desmoplakin I & II. ATRA concentration-dependently (10^{-6} - 10^{-4} m) induced changes in actin stress fibers and cytokeratin intermediate filaments. These changes were accompanied by a more obvious interaction of these filaments with junctional complexes. Surface area and volume of the MCF-7 cells increased markedly after ATRA exposure, with extensive filopodia formation. Liarozole (10^{-6} m) alone had no effect on cell morphology, cytokeratin or actin organization, or on cellular junctions. In combination with ATRA (10^{-6} m and 10^{-4} m), liarozole potentiates the ATRA-induced effects. The MCF-7 cell cultures used showed morphological heterogeneity, consisting of at least two cellular subpopulations. This was reflected in the staining for E-cadherin, desmoglein and desmoplakins I & II. ATRA increased E-cadherin staining at cell-cell contact sites, but had no influence on the staining patterns of desmoglein and desmoplakin I & II. Similar to what has been observed for the cytokeratins, differentiation parameters, liarozole alone had no influence on E-cadherin, desmoglein or desmoplakins I & II expression, but in combination with ATRA the increase again intensified the effects on E-cadherin distribution. These effects on MCF-7 cells agree with previously obtained observations concerning the inhibition of ATRA catabolism by liarozole. Furthermore, our data support the hypothesis that the antiproliferative properties of the drug are accompanied by induction of differentiation.

Introduction

Liarozole is currently in phase III clinical trials for the treatment of relapsed prostate cancer. Liarozole exerts retinoid-mimetic effects in both animals and patients [21, 45]. Recently it has been shown that liarozole acts as a versatile inhibitor of cytochrome P-450-dependent all-trans-retinoic acid (ATRA) catabolism, inhibiting both the 4-hydroxylation of ATRA and the monohydroxylation reactions of 4-keto-ATRA [44, 46]. The drug reduces tumor growth of androgen-dependent and androgen-independent Dunning R3327 rat prostate adenocarcinoma cells [10, 11, 37, 43] and of the androgen-independent PC-3ML-B2 human prostate carcinoma xenografts [39]. Taken together, these biochemical and clinical results have given rise to the hypothesis that liarozole may exert its antitumoral effects by inhibition of the cytochrome P-450-dependent ATRA catabolism.

In MCF-7 human breast carcinoma cell cultures, liarozole has been shown to enhance the antiproliferative effects of ATRA [49]. This enhancement was dependent on the concentration of liarozole (between 10^{-12} m and 10^{-9} m) and was more than 10-fold. Under basal growth conditions, MCF-7 cells are unable to metabolize ATRA, as shown by high-performance liquid chromatography. However, pretreatment with ATRA induced a very active conversion of ATRA to more polar metabolites. This conversion was almost completely inhibited by liarozole at a concentration of 10^{-5} m [48]. Also in F9 mouse teratocarcinoma cells, liarozole enhanced the effect of ATRA on the induction of plasminogen activator more than 2-fold.

Abbreviations. ATRA All-trans-retinoic acid. – BrdU 5-Bromo-2′-deoxyuridine. – CHAPS 3-(3-Cholamidopropyl)-dimethylammonio-1-propane sulfonate. – IEF Isoelectric focusing. – MTT 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide. – SDS Sodium dodecyl sulfate.

3) Dr. Gerda Smets, Department of Morphology-Life Sciences, Janssen Research Foundation, Turnhoutseweg 30, B-2340 Beerse/Belgium.
Drug incubations
Cells plated onto slides were incubated for 7 days with the following test conditions: ATRA at concentrations of 10^{-11} M, 10^{-10} M, 10^{-9} M, 10^{-8} M, and 10^{-7} M, and liarozole at concentrations of 10^{-11} M, 10^{-9} M, and 10^{-7} M in combination with ATRA at concentrations of 10^{-10} M and 10^{-8} M. Medium was changed on days 2 and 5. Under control conditions, cells reached confluence after 7 days.

Scanning electron microscopy (S.E.M.)
Cells grown with or without test compounds for 7 days were washed twice for 15 min in serum-free culture medium. Cells were fixed in 2% paraformaldehyde (Merck, Darmstadt/Germany) and 2.5% glutaraldehyde (Fluka, Buchs/Switzerland) buffered in 0.1 M phosphate buffer (pH 7.4) for 24 h. After being washed in 7.5% sucrose (Merck) in 0.1 M phosphate buffer (pH 7.4), cells were postfixed for 1 h at 4°C in 2% osmium tetroxide (Acros Chimica, Geel/Belgium) in 0.05 M veronal acetate (pH 7.4). The slides were washed twice for 5 min in 7% sucrose in 0.05 M veronal acetate and dehydrated through a graded series of ethanol and acetone. The samples were mounted and shadowed with 30 nm gold. They were examined and photographed in a Philips XL30 scanning electron microscope.

Transmission electron microscopy
Cells grown with or without test compounds for 7 days were fixed and postfixed as described for scanning electron microscopy. Thereafter, they were stained with 0.5% uranyl acetate (Merck) in 0.05 M veronal acetate (pH 5.2) for 40 min at room temperature. After the cells were washed twice for 5 min with 0.05 M veronal acetate (pH 5.2), they were dehydrated by ethanol and embedded in Epon. Sections 110 nm thick were cut with a diamond knife and collected on Formvar-coated grids (300-mesh). They were examined and photographed in a Philips EM410 electron microscope.

BrdU-incorporation and immunodetection
Cells grown with or without test compounds for 7 days were incubated for 2.5 h with an excess (100 μM) of 5-bromo-2'-deoxyuridine (BrdU) (Acros Chimica) in serum-free culture medium. Then cells were washed three times for 15 min with a mixture of culture medium and 0.1 M phosphate buffer (pH 7.4) (8:2 v/v). Cells were subsequently fixed in methanol (5 min, −20°C), acetone (10 s, −20°C) and air dried. Denaturation of the DNA was performed with 1% periodic acid (Merck) for 30 min at 60°C, followed by Schiff's reagent (Sigma Diagnostics, St. Louis, MO/USA) for 30 min at room temperature. Cells were washed three times with distilled water and treated with 0.01% trypsin (Life Technologies, Gent/Belgium) in 0.05 M Tris-HCl (pH 7.6). After the cells were washed in 0.05 M Tris-HCl (pH 7.6) and 0.1 M phosphate buffer, the slides were subsequently blocked in 0.5% tyrozyne in 0.1 M phosphate buffer for 1 h, incubated overnight at room temperature in a moist chamber with a primary monoclonal antibody against BrdU (1:50) (Janssen Biochimica, Beern/Belgium) and an antibody binding visualized with a secondary gold-labeled (5 nm) goat anti-mouse IgG (1:50) (Amerham International, Little Chalfont, Bucks/UK). The signal was enhanced for light microscopy with a silver enhancement kit (Amerham International). The BrdU-labeling index was defined as the proportion of BrdU-positive cells, representing cells in S-phase. It was calculated by counting 700 cells per incubation test condition (see drug conditions). Average results of six experiments are presented.

Immunocytochemistry
Cells grown with or without test compounds for 7 days were washed twice with serum-free culture medium. Cells were fixed in methanol (5 min, −20°C) and acetone (10 s, −20°C) and air dried. Cells were subsequently blocked in 5% normal goat serum in 10 M phosphate buffered saline (PBS) (pH 7.4) for 1 h, incubated for 1 h at room temperature in a dark moist chamber with the primary monoclonal antibody and visualized with the immunofluorescence technique using a secondary fluorescein isothiocyanate (FITC)-labeled goat anti-mouse
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Fig. 1. Effect of 10^{-6} M ATRA on the heterogeneous MCF-7 cell population, analyzed by S.E.M. A majority of small cells (a) and a minority of large cells (b) is observed under control conditions. Occasionally, glandular structures (c) could be detected. 10^{-6} M ATRA increased the size of both cell populations (d). It induced membrane ruffling in the small cells and the formation of filopodia in the large cells. – Bars 0.5 mm (a, b, d), 0.1 mm (c).

Tab. 1. Primary monoclonal antibodies used for immunocytochemistry.

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IgG (Southern Biotechnology Associates, Birmingham, AL/USA). Slides were air dried and mounted in Mowiol (Hoechst, Frankfurt a.M./Germany). Nuclei were stained with 4′,6-diamidino 2-phenylindole (DAPI; diluted 1:40000) (Sigma Chemicals). The characteristics and references of the primary antibodies used in this study are summarized in Table 1.

One- and two-dimensional gel electrophoresis
Equal numbers of control and ATRA-treated cells (4.5 × 10^6) were washed with 10 mM PBS (pH 7.4) and scraped into sodium dodecyl sulfate (SDS)-electrophoresis sample buffer [18] and boiled for 10 min. For one-dimensional gel electrophoresis, samples were diluted 1:4, and proteins were electrophoretically separated on 12% SDS-containing polyacrylamide gels (Mini Protean II Cell; Bio-Rad Laboratories, Nazereth/Belgium). Gels were stained with Coomassie Brilliant Blue R250.
For two-dimensional gel electrophoresis the samples were prepared as described above. Before loading onto isoelectric focusing (IEF) gels, an equal volume of buffer containing 2% Nonidet P-40 (Fluka), 5% β-mercaptoethanol (Merck), 5% Bio-Lyte carrier ampholytes (pH 3–10) (Bio-Rad Laboratories) and 9 M urea (Merck) was added, followed by addition of 1 M 3-[C-cholamidopropyl]-dimethylammonio]-1-propane sulfonate (CHAPS) (Sigma) (1:4 v/v) and urea until saturation [9]. Two-dimensional gel electrophoresis (Mini-Protean II 2-D system; Bio-Rad Laboratories) was performed as described by O’Farrell [27], with the following modifications.

In the first dimension, IEF was performed in 4% polyacrylamide rod gels containing 8 M urea, 1.3% Bio-Lyte carrier ampholytes (pH 3–10) and 12.5 mM CHAPS. For IEF no preelectrophoresis was performed. Gels were run at increasing voltages starting at 500 V for 10 min, followed by 1000 V for 20 min and 1500 V for 100 min. For the second dimension, the rod gels were applied directly onto the stacking gel of a 10% SDS-containing polyacrylamide gel. The gels were stained with Comassie Brilliant Blue R250. The stained protein bands were quantitated by digitizing with a RXS videocamera attached to a Macintosh IIx computer. Integration of the optical density was performed with the Image 1.44 software (NIH, Bethesda, MD/USA). Signals were corrected for background.

Statistical analysis
Where appropriate, data were analyzed using the two-tailed Mann-Whitney U-test using the Stat View II software (Abacus Concepts, Inc., Berkeley, CA/USA). Significance was defined at the level of P < 0.05.

Results
Liarozole enhances ATRA effects on MCF-7 cell morphology
MCF-7 cell monolayers showed morphological heterogeneity. As illustrated in the scanning electron micrograph, about 80 to 90% of the cells in MCF-7 cultures exhibited a small, round and tightly packed morphology (Fig. 1a), occasionally forming glandular structures (Fig. 1c). About 10 to 20% of the cell population consisted of large cells (Fig. 1b), mostly single growing. They were obviously more voluminous, exhibited a lower ratio of nuclear to cytoplasmic area and formed sparse loose intercellular junctions.

Increasing ATRA concentrations were tested for their influence on cell morphology. Both cell populations had an increased surface area and became clearly more voluminous at ATRA concentrations of 10^{-8} M to 10^{-6} M (Fig. 1d), with the induction of membrane ruffling, especially in the population of small cells, and the formation of extensive filopodia, especially in the large cells. Furthermore, all cells appeared to have lower ratios of nuclear to cytoplasmic area. At 10^{-6} M ATRA, virtually no small cells were noted. The morphological distinction between small and large cells diminished to a large extent, but was still evaluable by scoring membrane ruffling and filopodia, respectively. Below 10^{-7} M ATRA, again small cells were observed as under control conditions. Liarozole alone at 10^{-6} M did not induce morphological changes. However, simultaneous addition of 10^{-4} M liarozole and 10^{-10} M to 10^{-8} M ATRA resulted in a clear potentiation of the ATRA-induced effect (data not shown).

Synergistic effects of liarozole and ATRA on inhibition of MCF-7 cell proliferation
The concentration-dependent effects of ATRA and liarozole on proliferative activity of MCF-7 cells are shown in Figure 2. As presented in Figure 2a, the BrdU-labeling index for the subpopulation of small cells under control conditions was 36.8 ± 1.3% (n = 6). The BrdU-labeling index decreased only slightly when going from 10^{-11} M to 10^{-7} M ATRA. Liarozole alone up to a concentration of 10^{-5} M had no effect on the BrdU-labeling index. At 10^{-5} M, liarozole lowered the BrdU-labeling index to 27.8 ± 0.5% (n = 6). Simultaneous application of 10^{-4} M liarozole and ATRA, however, resulted in a significant 10-fold potentiation of the antiproliferative effect of ATRA. Although ATRA (10^{-8} M) alone had only a minor effect on the BrdU-labeling index, ATRA in combination with liarozole (10^{-6} M) resulted in a 2-fold decrease of the number of cells in S-phase. At concentrations of 10^{-8} M ATRA alone and at 10^{-7} M and 10^{-6} M ATRA in combination with 10^{-4} M liarozole, the BrdU-labeling index could not be evaluated because there were virtually no small cells noted.
The antiproliferative effects of ATRA could be established in both subpopulations of MCF-7 cells. As presented in Figure 2b, the BrdU-labeling index for the large MCF-7 cells under control conditions was 42.2 ± 0.8% (n = 6). Cells responded in a concentration-dependent manner to increasing ATRA concentrations. The BrdU-labeling index of the large MCF-7 cells decreased 2-fold when going from $10^{-11}$ M to $10^{-7}$ M ATRA. Under these conditions, the drug concentration necessary to attain 50% growth inhibition was $5.6 \times 10^{-8}$ M. Again, liarozole alone up to $10^{-6}$ M had no effect. At $10^{-5}$ M, liarozole lowered the BrdU-labeling index to 29.5 ± 1.1% (n = 6). In large cells too, liarozole at $10^{-6}$ M significantly potentiated the antiproliferative effect of ATRA by 10-fold. Note that liarozole is unable to potentiate the effect of $10^{-6}$ M ATRA.

Liarozole potentiates the ATRA modulation of cell adhesion molecules in MCF-7 cells

The presence of desmosomes and adherens junctions in MCF-7 cells under control conditions is illustrated in the transmission electron micrographs in Figure 3. Intermediate filaments were associated with desmosomes (Fig. 3b). Typical adherens junctions were only present at the apical zone of neighboring cells (Fig. 3c), consistent with the polarization of the MCF-7 cells (Fig. 3a).

The effect of ATRA on the expression of cell adhesion molecules was investigated. MCF-7 cells were negative for P-cadherin under all conditions tested (data not shown). The staining results of E-cadherin showed a clear relationship with the morphological heterogeneity of the cell population. The subpopulation of small cells (Fig. 4a) showed a clear E-cadherin staining along the cell membranes, accompanied by some punctate cytoplasmic staining. In large cells (Fig. 4f), E-cadherin staining was predominantly faint, solely localized in the cytoplasm, while at the cell-cell contacts E-cadherin staining was completely negative. With $10^{-8}$ M (Fig. 4c) to $10^{-6}$ M ATRA (Fig. 4b), the ruffled membrane of the small cells showed an intense E-cadherin signal, indicative of an up-regulation of this constituent. Upon treatment with $10^{-8}$ M (Fig. 4b) and $10^{-6}$ M ATRA (Fig. 4g), the cell junctions at the filopodia of the large cells now showed E-cadherin staining. $10^{-6}$ M liarozole alone (Figs. 4d, 4j) had no effect on E-cadherin staining, but in combination with $10^{-8}$ M and $10^{-6}$ M (Figs. 4e, 4j) ATRA, the drug intensified the ATRA-induced changes at the level of the cell-cell contacts, as scored by an increased ruffling of the membrane, indicative for an up-regulation of E-cadherin.

Similar to E-cadherin staining, staining for desmoplakin I & II and desmoglein showed a clear relationship with the different cell types of the cultures (data not shown). Under control conditions, the subpopulation of small cells presented a clear punctate staining along the cell membranes. The subpopulation of large cells was either completely negative or showed some dot-like staining at cell-cell contacts. The small cells retained a dot-like staining at $10^{-8}$ M to $10^{-6}$ M ATRA (data not shown). Similarly, after treatment with $10^{-8}$ M to $10^{-6}$ M ATRA, the large cells showed no difference in the adhesion molecule staining patterns as compared to control cells (data not shown). They remained either completely negative or showed a dot-like reactivity at cell-cell contacts. Liarozole alone or in combination with $10^{-6}$ M and $10^{-8}$ M ATRA had no effect on the staining patterns for desmoglein and desmoplakins I & II (data not shown).

Enhanced effects of ATRA in combination with liarozole on the cytoskeletal organization of MCF-7 cells

In parallel with the changes in cell shape, a reorganization of cytoskeletal filaments was observed. The effect of ATRA (ranging from $10^{-11}$ M to $10^{-6}$ M) alone or in combination with liarozole ($10^{-6}$ M) on the organization of the cytoskeleton was tested by immunocytochemical staining for cytokeratins 8, 18, 19, actin, and vimentin.

The MCF-7 cell clone used in this study was negative for vimentin under all conditions tested (data not shown). The results for the cytokeratin intermediate filaments are essentially the same for the different cytokeratin subtypes. Under control conditions, the staining pattern for cytokeratins in both MCF-7 cell subpopulations was characterized by a compact network of filaments (Fig. 5a). $10^{-7}$ M and $10^{-8}$ M (Fig. 5b) ATRA changed the organization to such an extent that a clear cytokeratin filamentous network running across the whole cell became visible. Also at a concentration of $10^{-8}$ M ATRA (Fig. 5c), this effect was still visible, although less pronounced as...
compared to $10^{-6}$ M ATRA. With $10^{-6}$ M lirazolone alone (Fig. 5d), the immunocytochemical staining results resembled those for control conditions. However, $10^{-6}$ M lirazolone in combination with $10^{-8}$ M and $10^{-6}$ M (Fig. 5e) ATRA potentiated the ATRA-induced effect, as illustrated by the presence of clear cytokeratin filaments and numerous cell-cell contact sites. At numerous foci of cell-cell adhesion points, cytokeratin filaments could be seen to interact with desmosomal junctions (Figs. 5b, c).

Gel electrophoretic analysis of the ATRA-treated cells showed a decrease in total cytokeratin content, as compared to the histone content (Fig. 6). Two-dimensional gel electrophoresis (Fig. 7) showed a clear decrease of all three cytokeratins 8, 18, and 19 at $10^{-6}$ M ATRA, in relation to several other proteins which remained present at a constant level. Densitometrical analysis showed a decrease of cytokeratins 8, 18, and 19 of approximately 50% after treatment with $10^{-8}$ M ATRA.

Similar to the changes described for cytokeratins, ATRA treatment resulted in profound effects on the actin-containing stress fibers. Under control conditions, actin was seen as stress fibers along the cell membrane and as a more diffusely staining meshwork in the cytoplasm (Figs. 5f, k). Treatment with $10^{-5}$ M and $10^{-6}$ M (Figs. 5g, I) ATRA induced numerous actin-containing stress fibers throughout the cell. In addition, numerous focal adhesions became apparent. After treatment with $10^{-8}$ M ATRA (Figs. 5h, m), actin staining predominantly resembled what was observed under control conditions. $10^{-6}$ M lirazolone alone induced no actin reorganization (Figs. 5i, n), but when the drug was given simultaneously with $10^{-8}$ M and $10^{-6}$ M (Figs. 5j, o) ATRA, it clearly enhanced the formation of stress fibers and focal adhesions.

Two-dimensional gel electrophoresis (Fig. 7) showed an increase of approximately 40% in actin content after treatment with $10^{-8}$ M ATRA.

**Discussion**

The present study confirms and extends previous results [49] showing that lirazolone potentiates the effects of ATRA. To study this synergism we used MCF-7 cells. MCF-7 cell cultures have been shown to exhibit a profound heterogeneity [4, 50]. For example, Resnitzoff et al. [30] showed that MCF-7 cells consist of different subpopulations, which originate from a single clone. Moreover, there exist several clones of this cell line which exhibit biological and phenotypical differences. The MCF-7 cell line used in this study consists of a subpopulation of small cells and a subpopulation of large cells. The phenotype of these MCF-7 cells was evaluated by immunocytochemistry and revealed a lack of vimentin filaments and estrogen receptor expression, albeit heterogeneously.

Since its establishment the MCF-7 cell line [38] consists of several size variants. The relative numbers of small and large cells depends on the passages used [50]. The majority of early passages consist mainly of large cells, as described by Bani et al. [2]. In later passages (over 150), as used in this study, small cells have outnumbered the large cells. The heterogeneity within the cell line implies different responsiveness to nutrients, mitogenic and differentiation-inducing signals. At high concentration, ATRA changed the balance between large and small cells. At $10^{-8}$ M ATRA, large cells by far outnumbered the small cells, but the effect was concentration-related. At

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**Fig. 4.** Effects of ATRA and lirazolone on the distribution patterns of E-cadherin in small (a-e) and large (f-j) MCF-7 cells. Conditions tested: control (a, f), $10^{-7}$ M ATRA (b, g), $10^{-8}$ M ATRA (c, h), $10^{-6}$ M lirazolone (d, i), $10^{-4}$ M lirazolone in combination with $10^{-6}$ M ATRA (e, j). Bar 0.1 mm.
concentrations of $10^{-6}$ M and $10^{-5}$ M ATRA, small cells outnumbered the large cells, comparable to control cultures.

As shown by BrdU-incorporation studies, the antiproliferative effects of ATRA on MCF-7 cells were potentiated by lirozole, as indicated previously by means of an MTT assay [49]. The immunocytochemical detection of BrdU enabled the quantification of the differential ATRA-sensitivity in the sub-populations of small and large cells. Liarozole ($10^{-6}$ M) potentiated the antiproliferative effects of ATRA by 10-fold. The combination of liarozole with $10^{-7}$ M and $10^{-8}$ M ATRA

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**Fig. 5.** Effects of ATRA and liarozole on the organization of the cytoskeleton, comprising cytokeratins (a-e) and actin (f-o) in small (a-d) and large (k-o) MCF-7 cells. Conditions tested: control (a, f, k), $10^{-4}$ M ATRA (b, g, l), $10^{-5}$ M ATRA (c, h, m), $10^{-6}$ M liarozole (d, i, n), $10^{-8}$ M liarozole in combination with $10^{-5}$ M ATRA (e, j, o). – Bars 0.5 mm (a-e), 0.5 mm (f-o).
showed similar BrdU-uptake rates for both cell populations. Thus, liarozole is able to potentiate the effects of ATRA to the same extent in both cell populations. Liarozole was unable to potentiate the effect of 10⁻⁴ M ATRA in large cells. This favors our hypothesis that liarozole potentiates the antiproliferative effects of ATRA via inhibition of its catabolism.

The synergism between liarozole and ATRA was observed on their differentiation-inducing effect on the heterogeneous MCF-7 cell population. As recently described by Bani Sacchi et al. [2], E-cadherin staining is closely related to this morphological heterogeneity. The subpopulation of small cells showed a clear E-cadherin staining along the cell membranes. In contrast, in the subpopulation of large cells, E-cadherin staining was faint and solely localized in the cytoplasm. However, upon treatment with ATRA, E-cadherin staining was increased at the cellular membrane of both cell populations. These results are similar to those recently described for the SK-BR-3 human breast cancer cell line [1] and suggest induction of differentiation. The increased E-cadherin staining at the junctional complex was concomitant with the observed cytoskeletal changes. This is not surprising in light of the association of the cytoskeleton with cell-cell junctions [15, 40]. Liarozole potentiated these ATRA-induced effects. The increased E-cadherin staining at cell-cell contacts might also have functional effects on the cells, as for example increased cell aggregation as seen for the invasive MCF-7/6 cell line variant after ATRA treatment [5]. Given that E-cadherin expression is correlated with a differentiated non-invasive phenotype [40] and that ATRA enhances the functionality of E-cadherin [3], liarozole might exert anti-metastatic effects in vivo [39], where the drug has been shown to have retinoid activity [21, 45]. This would have important clinical implications for the use of liarozole in the treatment of prostate cancer, in which decreased expression of E-cadherin has been correlated with tumor progression [42].

The expression of desmosomal proteins (desmoglein and desmoplakins I & II) was related to both MCF-7 cellular subtypes, but ATRA treatment had no influence on their staining. Also in FR1C B fetal rat intestine cells the expression of desmoplakins I & II did not change upon retinoid treatment [28].

The changes in cell morphology and the level of cell-cell junctions were accompanied by a profound reorganization of the cytoskeleton. ATRA treatment changed the organization of cytokeratin filaments so much that a clear network of cytokeratin filaments became visible, accompanied by a 50% down-regulation of cytokeratin expression. Liarozole potentiated this ATRA-induced reorganization of cytokeratin filaments. These effects were also seen when MCF-7 cells were treated with the neurohypophyseal peptide oxytocin [7]. Cytokeratin modulation by liarozole was also observed concomitant with a shift in the differentiation status in prostate tumors [37]. Down-regulation of cytokeratins in cancer cells is considered as an induction of differentiation by some authors [17, 41].

Analogous to the changes described for cytokeratins, ATRA had a profound effect on actin organization. ATRA induced numerous actin-containing stress fibers and focal adhesions. This effect which also suggests induction of differentiation [19, 25], was enhanced by liarozole. Furthermore, the occurrence of actin-containing stress fibers has been correlated with anti-metastatic properties [47]. Similar changes in the organization of actin fibers have been described in connection with ATRA-treated F9 testicular teratocarcinoma cells.
[19], FRIC B fetal rat intestine cells [28] and UMR 106-66 osteogenic sarcoma cells [25]. ATRA has been found to stimulate actin polymerization [24]. In contrast to down-regulation of cytokeratins, actin content was up-regulated upon ATRA treatment.

In conclusion, liarozole not only potentiated the antiproliferative effects of ATRA on MCP-7 cells, but also ATRA-induced differentiation. This potentiation is most probably due to inhibition of ATRA catabolism and agrees with previously obtained biochemical findings on the synergistic interaction of ATRA and liarozole in MCP-7 cells.

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References


