1. Objective

To study the result of endometrial assessment in patients who were treated with tamoxifen.

2. Methods

Clinical and ultrasonographical data from 207 consecutive patients, who had been referred for endometrial assessment, were prospectively collected. The proportion of patients with endometrial pathology was studied, based on ultrasonographical or histopathological findings.

3. Results

An endometrial malignancy was histologically confirmed in 7 patients (3.4%): 4 well-differentiated and 1 moderately differentiated endometrioid adenocarcinomas, 1 serous papillary carcinoma and 1 metastatic breast cancer. The mean uterine volume and double endometrial thickness were 74 ml and 9 mm, respectively in all patients without endometrial malignancy; 88 ml and 17 mm in 41 patients with benign polyps; and 171 ml and 17 mm in patients with endometrial malignancy. Benign endometrial polyps were removed in 41 patients (19.8%). During a follow-up period ranging from 3 to 56 months none of the patients has clinically presented with endometrial cancer. No mortality from endometrial cancer occurred after a median follow-up of 24 months.

4. Conclusion

A high proportion of patients who are treated with tamoxifen have benign or malignant endometrial pathology. Ultrasonography is a reliable tool to detect endometrial pathology. However, a large randomised trial assessing the impact of endometrial monitoring on the mortality from endometrial cancer is needed to prove that endometrial monitoring improves survival in patients on tamoxifen.

Abstract: P22

Apoptosis and anti-apoptosis in oestrogen-receptor negative endometrial cancer cells in response to anastrozole, 4-hydroxytamoxifen and medroxyprogesterone acetate

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1. Purpose

Several studies have addressed the potential of anti-oestrogens and aromatase inhibitors to induce apoptosis in breast cancer cells *in vitro*. To our knowledge, the effect on endometrial cancer cells has so far not been evaluated. Our aim was to determine if anastrozole (ANAST) and 4-hydroxytamoxifen (4-OHT) had any effects on proliferating oestrogen receptor (ER)-negative endometrial cancer cell lines, in terms of apoptosis and the cell cycle distribution.

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2. Methods

Using multiparameter-flow cytometric (MP-FCM) analysis with a DAKO/PAS flow cytometer, cultures of 2 ER-negative endometrial cancer cell lines (HEC 1A and MFE 319), not treated with any hormones, as well as cultures treated with 5 μM or 10 μM of 4-OHT, ANAST or medroxyprogesterone acetate (MPA) for 2, 4 or 6 days, were evaluated for their reactivity with M30-cytodeath and Bcl-2, as well as for cell cycle parameters such as the S phase and the G0/G1 fraction.

3. Results

MP-FCM analysis revealed that the effects of the three drugs tested were similar in terms of increasing M30 and decreasing Bcl-2 expression in both cell lines. In the MFE 319 cell line, at 2 days, the effect was dose-dependent only with 4-OHT in terms of M30 expression, and with ANAST and MPA in terms of Bcl-2 expression. At the 4-day interval, the effects on M30 and Bcl-2 expression were dose-dependent in all three drugs, i.e. an increase in the dose produced an increase in M30 or a decrease in Bcl-2 expression. In the same cell line, the effects on M30 and Bcl-2 expression were also time dependent, at 4 days the levels of M30 and Bcl-2 expression were higher and lower, respectively, in relation to the 2-day interval. At 6 days, the effects of all drugs were very much attenuated.

In the HEC 1A cell line, however, this effect on M30 and Bcl-2 expression was neither dose-dependent nor time-dependent in all three drugs, i.e. an increase in the dose did not produce either an increase in M30 or a decrease in Bcl-2 expression. 4-OHT, ANAST and MPA induced apoptosis in the HEC 1A line, with mean values of 11, 12 and 15%, respectively, compared with the control with a mean value of 2.4%. At 4 and 6 days, the effects of all three drugs were very much attenuated in relation to the 2-day interval, i.e. M30 levels decreased and Bcl-2 levels increased despite treatments. In the HEC 1A cell line, 4-OHT showed the strongest Bcl-2 down-regulatory effect to 11%, in comparison with the control of 43%, and compared with ANAST and MPA both showing a mean decrease to 16%.

In the MFE 319 cell line, ANAST induced apoptosis in 12%, 4-OHT in 11% whereas MPA showed a value of 13% in relation to the control population with only 1% M30 reactivity. The downregulatory effect for Bcl-2 in MFE 319, was stronger for ANAST, with 27%, than for 4-OHT and MPA showing values of 36 and 31% respectively, compared with the control with a value of 55%.

In the HEC 1A cell line, at 2 days, 5 and 10 μM 4-OHT caused a dose-dependent increase in the G1 fraction of 16 and 57% and a decrease in the S phase fraction (SPF) of 16 and 55% in relation to the control. This compared with an increase of 10 and 16% and a decrease of 12 and 16% for the same doses of ANAST at 2 days. MPA showed similar effects, with an increase of 10% in the G1 fraction and a decrease of 10% in the SPF with 5 μM at 2 days. However, 10 μM of MPA caused a decrease in the G1 fraction of 43% and a decrease in the SPF of 12% in relation to the control at 2 days. The effects on the cell cycle of all three drugs, were less evident at 4- and 6-day intervals.

In the MFE 319 cell line, all three drugs showed a dose-dependent increase in the G1 fraction as well as a dose-dependent decrease in the S phase fraction at 2 days. 5 and 10 μM 4-OHT caused a dose-dependent increase in the G1 fraction of 8 and 9% and a decrease in the S phase fraction of 8 and 58%, at 2 days, in relation to the control. This compared with an increase of 8 and 21% and a decrease of 13 and 67% for the same doses of ANAST, and an increase of 5 and 19% and a decrease of 13 and 34% for MPA at 2 days. These effects were less evident at the 4- and 6-day intervals.

4. Conclusion

The results show that it is possible to use MP-FCM to quantify the effect of ANAST, 4-OHT and MPA on apoptosis induction in ER-negative endometrial cancer cell lines by M30-cytodeath expression. This appears to be directly related to Bcl-2 down-regulation. Effects on the cell cycle showed decreased SPF. Therefore, also other aromatase inhibitors and more pure anti-oestrogens such as 4-OHT, deserve a closer look at their ability to modify apoptosis and Bcl-2 expression in ER-negative endometrial tumours.