luteinising hormone-releasing hormone (LHRH) analogues use [14]. The currently available SERMs including raloxifene, do not alleviate such symptoms and may even exacerbate some of them. In the MORE trial, hot flushes were reported more frequently in the raloxifene group than in the placebo group; however, only 0.6% of the women assigned to the raloxifene group compared with 0.1% of those assigned to the placebo group discontinued treatment because of hot flushes ($P < 0.001$) [12]. For symptomatic patients, the association of raloxifene with different drugs which have demonstrated efficacy in the control of vasomotor symptoms in breast cancer survivors, such as megoestrol acetate at low-doses (20–40 mg/day) and selective serotonin re-uptake inhibitors, is now under evaluation [15–17].

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Tibolone actions on normal and breast cancer cells

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Abstract

Tibolone and its main derivatives were studied in an original model of cultures of normal human epithelial breast (HBE) cells on proliferation, differentation and apoptosis, the three mechanisms responsible for breast homeostasis. Tibolone and its Δ4 isomer were antiproliferative, both in the absence and presence of oestradiol (E2). The oestrogenic 3α and 3β hydroxy derivatives did not display any mitogenic activities in HBE cells. Moreover, at 1 μM, they were antiproliferative. Tibolone and its Δ isomer increased the 17β hydroxysteroid dehydrogenase activity similarly to that observed with progestins [1]. Apoptosis was increased in HBE cells to a similar range as with the pure pregnane progestin, Org2058. We also studied the extent of apoptosis in hormone-dependent breast cancer cell lines. Tibolone and its Δ4 isomer also increased apoptosis, especially in ZR75-1 cells containing progesterone and

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androgen receptors [2]. We could demonstrate that these pro-apoptotic actions of tibolone and its Δ4 isomer were mediated at least partially through the bcl-2-family of proteins. Moreover, the antiproliferative and pro-apoptotic activities of tibolone, as well as Org2058, were mediated by increasing catalase activities in breast cancer cells. Thus, in breast cells, tibolone slows down the proliferation rate, increases differentiation and apoptosis. These actions seem to be optimal on breast tissue.

Keywords: Tibolone; Breast

1. Introduction

Tibolone (OrgOD14) is a progestin now proposed in hormone replacement therapy (HRT). It is metabolised in three main compounds and thus has various steroidal potencies. Its Δ4 isomer, OrgOM38 has stronger progestin and androgen potencies than OrgOD14 and there are two oestrogenic derivatives, the 3α and 3β OH metabolites [3]. Because of the concern in breast cancer with HRT, and the intrinsic properties of this treatment, we studied the effect of tibolone and of its main metabolites on proliferation, differentiation and apoptosis in normal breast cells. Apoptosis was also studied in hormone-dependent breast cancer cells. In addition, we studied some of the pathways involved in the redox state of the cells, since they are involved in proliferation and apoptosis regulation. Indeed, hydrogen peroxide (H_2O_2) was reported to have proliferative properties at low concentrations and pro-apoptotic or necrotic potencies at high concentrations.

2. Materials and methods

An original model of cultures of normal HBE cells was carried out for these studies. Some hormone-dependent breast cancer cells lines differing by their steroid receptor content, T47D, ZR75-1, were used.

3. Results

Tibolone and OrgOM38 decreased the proliferation rate of HBE cells similarly to the pure pregnane progestin Org2058. In conditions of low concentrations of serum and growth factors, when E2 exhibited a mitogenic activity [1], the 3α and 3β derivatives did not increase the growth rate. Moreover in the standard conditions of culture, they were even antiproliferative at 1 μM. The 17β hydroxysteroid dehydrogenase activity, which converts oestradiol into oestrone, a weaker oestrogen, was induced by OrgOD14 and OrgOM38. This effect was indicative of a progestin action of tibolone in these cells. We have also shown that the proportion of apoptotic cells was increased in normal HBE cells as well as in hormone-dependent breast cancer cell lines (T47D and ZR75-1). Moreover, some of the key proteins involved in the regulation of apopotosis (bcl-2 family proteins) were strongly modified by Org2058 [4] (and similarly by tibolone and its Δ4 isomer). The anti-apoptotic protein, bcl-2 was strongly inhibited by the progestins in both types of cells. We also observed drastic effects of progestins on some of the pathways involved in the redox state of the cells. Org2058 and tibolone were antiproliferative in T47D breast cancer cells. Hydrogen peroxide increased the proliferative rate of T47D at 0.1 and 1 μM, was pro-apoptotic at 10 and 100 μM and necrotic at >100 μM concentrations. In the presence of the progestin, however, the proliferative effect of H_2O_2 was blunted. We could demonstrate that this effect was linked to an increase in catalase activities.

4. Conclusions

We have shown that tibolone behaves as a progestin with mild androgenic properties in normal human breast cells and that the 'oestrogenic' derivatives do not exhibit any oestrogenic effect in this model. It is able to decrease the proliferative rate, increase apoptosis via a decrease in the bcl-2 protein and induces a marker of differentiation.

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