addition of antineoplaston, cells at the G_1 phase were increased by 15%, compared with the control. Analysis of the expression of cell cycle-related proteins after the addition of antineoplaston showed that the p21 and p16 proteins were increased after administration, however, that the expression of the cyclin D1 and cyclin E proteins was unchanged. At 24 hours after the administration of antineoplaston, the phosphorylation of retinoblastoma (Rb) was inhibited. Furthermore, the analysis of the MAPK expression showed that the phosphorylated ERK MAPK protein began to decrease at 3 hours after antineoplaston administration. To further confirm a role of ERK MAPK in SKBR-3 cell growth, we used PD98059, an inhibitor of mitogen-activated protein kinase kinase (MEK) 1, the kinase responsible for ERK MAPK activation. PD98059 significantly reduced the levels of phosphorylated ERK MAPK, without noticeable changes in total ERK MAPK. Analysis of cell proliferation revealed that PD98059 dose-dependently inhibited cell growth compared to control (vehicle-treated cells), thereby confirming the importance of the ERK MAPK pathway in the control of cell proliferation in SKBR-3 cells. From these results, we have speculated that, in the breast cancer cell line SKBR-3, antineoplaston dephosphorylated ERK MAPK, and that the dephosphorylated ERK MAPK regulated the expression of p21, p16 proteins, inhibited the phosphorylation of Rb, and thereby causing G_1 arrest. The results suggested that antineoplaston may be an effective adjuvant therapy after breast cancer surgery.

192 POSTER

The role of SERMs on the induction of apoptosis of human breast cancer cells in vitro

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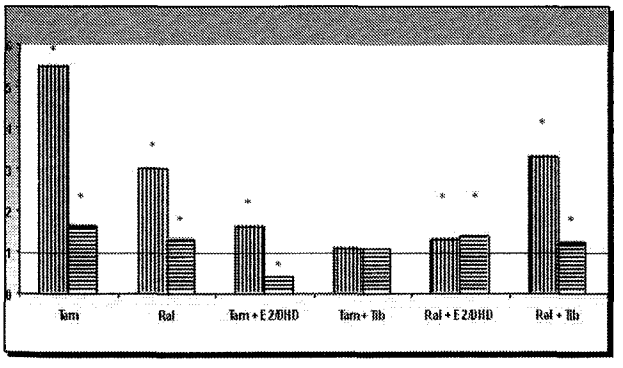
Introduction: In vitro research demonstrated that selective estrogen receptor modulators (SERMs) induce apoptosis (programmed cell death) in human mammary carcinoma cells. However the use of SERMs in vivo can cause severe climacteric complaints sometimes necessitating interrupting treatment. The combined use of tamoxifen and continuous combined HRT did not increase the risk of recurrences in breast cancer survivors (Dew et al., 2002).

Materials and methods: The SERMs tamoxifen (Tam) and raloxifene (Ral) alone or combined with estradiol (E2) plus dihydrodydrogesterone (DHD) as well as tibolone (Tib) were administered to MCF-7 cells, estrogen receptor (ER) positive and MDA-MB-231, ER negative human breast cancer cell lines, in a concentration of 10^{-6} M for 144 hours in vitro.

Proliferation was determined by measuring the expression of Cyclin D1 and apoptosis by using the DNA fragmentation assay and both performed in duplicate. The mean ratios apoptosis versus proliferation were calculated and the 95% confidence intervals assessed.

Results: Tam and Ral alone induced apoptosis in ER positive and negative breast cancer cells (figure 1). Tam and Ral combined with E2 plus DHD did induce apoptosis in ER positive breast cancer cells, however Tam plus Tib neither induced nor stimulated ER positive and negative breast cancer cells. Ral combined with Tib induced apoptosis in both cell lines.



Apoptosis/proliferation versus controls after 144 hours

Ratio>1 means induction of apoptosis. * $P < 0.05$ versus controls.

Conclusion: We demonstrated that our laboratory data mirror cell biological behaviour in vivo and we therefore suggest that ER positive breast cancer survivors using Tam or Ral may simultaneously start with E2 plus DHD or Tib to reduce side effects without compromising its efficacy.

193 POSTER

Antiproliferative activity of tamoxifen on MCF-7 breast cancer cells is modulated by weak electromagnetic field exposure

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Breast Cancer (BC) incidence has been rising ever since the second world war in industrialized countries, a trend paralleled by increasing electrification. Usage of electrical power is intricately associated with electromagnetic field (EMF) exposure. Tamoxifen, a partial estrogen receptor antagonist, is the most frequently used BC medication.

Aim of this study was to substantiate sporadic experimental communication that the anti-proliferative activity of Tamoxifen in MCF-7 BC cells can be modulated by extremely low frequency (ELF) EMF exposure.

Materials and Methods: In order to expose BC cells to reproducibly homogeneous sinusoidal 50 Hz ELF-EMF of defined electromagnetic flux density, we specifically designed tissue culture incubators delivering flux densities of either 0 μT, 1.2 μT, 10 μT or 100 μT at 37°C, 5% CO_2. MCF-7 estrogen receptor positive BC cells were incubated in MEM supplemented with 5% fetal calf serum and treated with increasing Tamoxifen (Tam) concentrations at a given magnetic flux density. After 7 days of culture resulting cell concentrations were measured using a colorimetric test, dose–response curves for Tam were recorded and EC_{50}-concentrations for Tam were calculated at each flux density.

Results: In control experiments without measurable EMF-exposure low Tam-concentrations ($<2\times10^{-7}$ M) expectedly exerted an agonistic proliferative effect on MCF-7 cells. With increasing Tam-concentration the well-known anti-proliferative effect was seen (half maximal effect at 2.2×10^{-6} M). EMF at 1.2 μT flux density shifted Tam dose–response curves to higher concentrations, resulting in a maximal proliferative effect at 1.8×10^{-6} M. In comparison to control experiments at 0 μT flux density, 2 times higher Tam-concentrations were needed to induce a half maximal anti-proliferative effect at 4.4×10^{-6} M when a 1.2μT field was applied. While 0.2μT fields exhibited a similar, if weaker curve shifting effect, higher flux densities at 10 μT or 100 μT exhibited a much less pronounced activity ("window effect").

Conclusion: Clearly, sinusoidal alternating EMF-exposure at environmental flux densities to MCF-7 BC cells modulated anti-proliferative activity of Tam. Clinically, it is interesting to note that 1.2 μT EMF induced near maximal proliferation at a Tam concentration (1 μM) that is usually achieved in serum of patients under Tam-therapy for primary or recurrent BC.

Wednesday, 17 March 2004 16:00–17:15

PROFFERED PAPERS

Psychosocial aspects

194 ORAL

Expectations for breast treatment – complex biopsychosocial determinants

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Expectations regarding the health care experience, treatment process and treatment outcome are key determinants in how the individual will view that health care experience. Within the health literature the study of expectations is often linked to measures of patient satisfaction. There is a need however, for expectations to be considered in relation to specific attributes or aspects of care, rather than being limited to generalisations about the totality of a service which tends to conflate many issues and fails to provide useful information for service improvement (Thompson & Sunol, 1995). In relation to breast care services what is often not considered is that despite an increase in the numbers of patients being diagnosed with breast cancer, many patients referred via a rapid referral system are found to have benign disease. Allied to this, the fast track referral to a breast clinic of a patient who does not have cancer can have adverse intra-personal effects due to a heightening of anxiety (Durning, Morris, Gash & Gray, 1998) and related distress (Nosarti, Roberts, Crayford, McKenzie & David, 2002). Furthermore, such expeditious referral can impact greatly on the expectations held by those individuals presenting with a breast problem.