P1. UROKINASE-TYPE PLASMINOGEN ACTIVATOR (uPA) SYSTEM IN RECURRENT BREAST CANCER: ASSOCIATION WITH TAMOXIFEN THERAPY

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The urokinase system of plasminogen activation consists of four major components, urokinase plasminogen activator (uPA), its receptor uPAR, and its inhibitors, PAI-1 and PAI-2. The prognostic impact of the tumour levels of these four factors is well established for patients with primary breast cancer, especially for uPA and PAI-1 (Jnicke F, *et al. J Natl Cancer Inst* 2001, **93**, Look MP, *et al. J Natl Cancer Inst* 2002, **94**). Furthermore, a predictive impact of UPA and PAI-1 on adjuvant chemotherapy has recently been observed (Harbeck N, *et al. Cancer Res* 2002, **62**). Extending our earlier analyses on the predictive impact of UPA and PAI-1 in recurrent breast cancer (Foekens JA, *et al. J Natl Cancer Inst* 1995, **87**), we addressed the predictive value of all four components of the urokinase system with respect to the clinical benefit patients with recurrent breast cancer derive from tamoxifen therapy. The antigen levels of the factors were determined by enzyme-linked immunosorbent analysis (ELISA) in cytosols prepared from oestrogen receptor-positive primary breast tumours from 691 hormo-na patients with recurrent breast cancer and treated with tamoxifen as first-line systemic therapy. Increasing (log-transformed) tumour levels of uPA and PAI-1 were significantly related to a lower probability of clinical benefit from tamoxifen treatment. Analysing the factors as categorical variables showed that high tumour levels of uPA ($P<0.001$), uPAR ($P<0.01$) and PAI-1 ($P<0.01$) were associated with an unfavourable clinical benefit. In the multivariable analysis for clinical benefit, uPA ($P<0.001$) provided additional information, independent of the traditional predictive factors, including menopausal status, steroid hormone receptor status, disease-free interval, and dominant site of relapse. High tumour levels of uPA, uPAR, and PAI-1 also predicted a shorter progression-free survival after start of tamoxifen therapy in a time-dependent analysis.

However, high PAI-2 levels ($P=0.015$) were related to a prolonged time of clinical benefit in the analysis without time-restriction. In conclusion, all four components of the uPA-system were predictive for the efficacy of tamoxifen treatment in patients treated for recurrent breast cancer. The role these four factors may play in tumour growth and metastasis might not only provide valuable information for the individualisation of adjuvant therapy for patients with primary breast cancer, but also for the design of novel, targeted (combination) therapies for patients with recurrent breast cancer.

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P2. EXPRESSION OF IMMUNOHISTOCHEMICAL OESTROGEN RECEPTORS α AND β IN ENDOMETRIAL CARCINOMA

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Introduction: Endometrial cancer, (EC) is the most common female genital track malignancy in European countries. EC is known to be an oestrogen-dependent disease. Oestrogens exert very powerful effects on the growth, differentiation and function of many target tissues, such as female reproductive organs, and are known to stimulate cellular proliferation in the uterus. Two oestrogen receptor types, named ERα and ERβ [1], have been identified as the chief mediators of the diverse biological functions of oestrogen and play an important role in oestrogen-dependent tissues and cancers [2,3]. The aim of the present study was to evaluate ERα and ERβ protein expression in normal and neoplastic endometrium in relation to histopathological and clinical data according to the World Health Organisation (WHO) and The International Federation of Gynaecology and Obstetrics (FIGO) 1988 criteria.

Material and Methods: Samples of normal endometrium and endometrial cancer tissue were collected from women, 14 normal endometrium (7 from proliferative and 7 from secretive phase) and 21 malignant samples (8 Grade (G)1, 7 G2 and 7 G3; stages of differentiation) according to the WHO classification. ERα and ERβ immunohistochemical analysis was performed on each paraffin-embedded block (5-m sections) and on adjacent sections stained previously with 1D5 ERα monoclonal antibodies (Dako Corp Carpintiera) using the chicken polyclonal ERβ503 IgY. Immunostaining was revealed using a streptavidin–biotin-enhanced immunoperoxidase technique. After antigen retrieval by pressure cooking in 0.1 M ethylene diamine tetraacetic acid (EDTA) buffer (pH 7.2) for 10 min and endogenous peroxidase blocking with 1% H_20_2, sections were incubated with normal donkey serum (1:40 dilution) in phosphate-buffered solution (PBS) for 30 min at 22 °C. Sections were then incubated overnight at 4°C with ERβ 503 IgY (1:1250 dilution). The complex was revealed using an anti-chicken biotinylated antibody (1:300 dilution; Vector) and streptavidin–peroxidase complex (1:1000 dilution; Dako Corp Carpintiera USA). 3,3 Diaminobenzidine tetrahydrochloride (Sigma) was used as the chromogen and the samples were counterstained with haematoxylin (Dako), dehydrated and mounted. The oestrogen receptors were counted on a semiquantitative scale from 1+ to 3+.

Results: In the samples of normal and neoplastic endometrium examined, the expression of ERα and ERβ differed between normal endometrial and cancer tissues. We observed a strong reaction for ERα in normal and neoplastic endometrium, whereas only a very slight reaction to ERβ was observed.

Conclusions: Our results demonstrate that ERα is a predominant form of oestrogen receptor in the human uterus and differential ERα and ERβ expression may play an important role in endometrial carcinogenesis and be useful information when deciding upon hormonal treatment for endometrial cancer [4].

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1359-6349/$ - see front matter
doi:10.1016/j.ejcsup.2004.08.042

P3. EXPRESSION OF OESTROGEN RECEPTOR BETA VARIANTS AND PROGESTERONE RECEPTOR ISOFORMS IN ENDOMETRIAL CANCER

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Oestrogen is recognised as an important modifier in the development, growth and invasiveness of endometrial cancer (EC). Progesterone is antagonistic to oestrogen-mediated cell proliferation and is the principal hormone that promotes differentiation in endometrium. Among the various factors, the expression patterns of sex steroid receptors, such as oestrogen receptor (ER) and progesterone receptor (PR) are extremely important with regard to patient prognosis [1]. In our previous reports, we described the characteristic expression pattern of ER alpha (ERα) and ER beta (ERβ) in EC [2], endometriosis [3], myometrium [4] and ovarian cancer [5]. We recognise that the intact synchronised expression of ERβ interacting with ERα might be disrupted in most metastases of uterine endometrial cancers, leading to advancement and poor patient prognosis with refractoriness of hormone treatments. A novel C-terminal splice variant of ERβ, named ERβ2, was reported in 1998. ERβ2 does not bind E2 and is a dominant-negative regulator of ERα. In order to evaluate the respective contribution of ERβ and PR isoforms in EC, we quantified the absolute expression levels of ERα, ERβ1 (wild-type), ERβ2, along with total progesterone receptor (PR)-AB, and PR-B, the B isoform of PR by real-time quantitative reverse transcriptase-polymerase chain reaction (RT-PCR) assays with recombinant RNAs as standards in 60 EC and 21 normal endometria (NE). We then analysed the correlation between expression levels of each receptor and clinical characteristics including patients' prognoses. The expression of ERβ2, but not of ERβ1, was significantly, positively correlated to that of ERα and PR-B in EC. In addition, levels of ERβ2, ERα and PR-B were significantly decreased in patients who died from EC compared with those who survived. As a result of these changes, the ratio of ERβ2 to ERα and the ratio of ERβ1 to ERα significantly increased in EC patients who died over those who survived. Interestingly, the ratios of ERβ2 to ERα and ERβ1 to ERα reflected patients' prognoses better than levels of ERα or PR-B themselves. These results taken together indicate that ERβ2 may have a different function from ERβ1 and that measurement of ERβ2 has prognostic value in EC.

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P4. IUMPA-2 AS A MODULATOR OF THE EXPRESSION OF THE OESTROGEN AND PROGESTERONE RECEPTORS IN ENDOMETRIAL HYPERPLASIA SIMPLEX

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Introduction: Intrauterine medroxyprogesterone acetate (IUMPA-2) is an intrauterine distribution system of medroxyprogesterone acetate (MPA), applied in the treatment of endometrial hyperplasia simplex. The system contains 2 g of MPA, of which a fixed dose is released daily from the carrier over a period of 30 days. After completion of the process of releasing progesterone, the system undergoes an automatic biodegradation and therefore does need not be removed from the uterus. The objective of this study was to examine the influence of IUMPA-2 on cell protein synthesis in endometrial hyperplasia simplex tissue and, as a consequence, its antiproliferative effect. The cytosolic receptor level was established as a marker of the decrease in intracellular protein synthesis. We have demonstrated a relationship between the levels of both of these receptor proteins and cell proliferation.
Patients and methods: The study comprised 54 (female) patients in whom endometrial hyperplasia simplex was diagnosed. They underwent a standard, 30-day treatment with application of IUMPA-2. Histopathological material was acquired on two occasions: during the installation of the therapeutic system and 7 days after treatment completion. On both occasions, hysteroscopic-guided biopsy was performed, with the invariant size of the sample being 2 mg. The cytosolic level of both oestrogen and progesterone receptors was measured using an immunoenzymatic method in order to assess the receptor protein level in relation to the total protein level in the tissue examined (fmol/mg).
Results: Levels of cytosolic oestrogen receptor:
• before treatment: 221.52 fmol/mg (SD 96.42),
• after treatment: 49.33 fmol/mg (SD 26.87).
Levels of cytosolic progesterone receptor:
• before treatment: 614.29 fmol/mg (SD 252.31),
• after treatment: 198.49 fmol/mg (SD 62.37).
Discussion: In contrast to previous research concerning the efficiency of use of IUMPA-1 in the growth inhibition of endometrial carcinoma, a several-fold larger inhibition of marker protein production was observed in tissues exhibiting endometrial hyperplasia. It appears to be caused by a higher initial level of the progesterone receptor in the tissues affected by endometrial hyperplasia.
Conclusions:
(1) IUMPA-2 has a modulating effect on the cytosolic levels of oestrogen and progesterone receptors in endometrial hyperplasia simplex.
(2) IUMPA-2 inhibits the synthesis of cell proteins, thereby inhibiting the proliferation of the tissue affected by endometrial hyperplasia simplex.

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P5. THE VALUE OF TRANSVAGINAL ULTRASONOGRAPHY IN PREDICTING ENDOMETRIAL PATHOLOGIES IN ASYMPTOMATIC, POSTMENOPAUSAL, TAMOXIFEN-TREATED BREAST CANCER PATIENTS

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Purpose: There is no established ultrasonographic endometrial cut-off value for the diagnosis of endometrial pathologies in asymptomatic, postmenopausal, tamoxifen-treated patients. We attempted to look for the most accurate cut-off value.
Materials and methods: Multiple ultrasonographic cut-off points were evaluated by logistic regression analysis, based on 279 patients who had transvaginal ultrasonographic examinations and were followed by endometrial histopathological analysis. Performance characteristics were calculated and correlations to endometrial histological findings assessed. Using the different ultrasonographic endometrial cutoff values, a search was also made for potential cases of various endometrial pathologies that could have been missed.
Results: There was a gradual increase in specificity and a gradual decrease in sensitivity of the ultrasonographic studies with an increase in endometrial thickness. More endometrial pathologies were identified with an increase in the cut-off values. By using a cut-off value of 7.5 mm, we could have missed one case (20.0%) of endometrial cancer, and the same principle was applied when a cut-off value of 12.5 mm was used. However, with this latter cut-off value, we could have avoided performing endometrial sampling in 59.1% of cases. With higher cut-off values, more endometrial pathologies, including three more cases of endometrial cancer, could have been missed, while more cases of endometrial sampling could have been avoided.
Conclusion: In asymptomatic, postmenopausal, tamoxifen-treated breast cancer patients, the use of higher endometrial cut-off values are associated with an increased possibility of missing endometrial pathologies, including endometrial cancers, while the amount cases of endometrial sampling may be reduced.

P6. DNA BINDING OF TAMOXIFEN IN HUMAN UTERINE AND COLON TISSUE AFTER ADMINISTRATION OF A SINGLE 14C-LABELLED THERAPEUTIC DOSE

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Tamoxifen, recently approved for the prevention of breast cancer in healthy women, is known to increase the incidence of endometrial cancer and there is some concern that tamoxifen therapy may be associated with an elevated risk of colorectal malignancy. There is much controversy as to whether DNA adduct formation is a contributing factor in tamoxifen-induced carcinogenesis in women, since a genotoxic mechanism is known to occur in rat liver. The present evidence is conflicting regarding whether tamoxifen adducts are formed in human tissues. We have therefore employed the sensitive technique of accelerator mass spectrometry to unequivocally determine if ^{14}C-tamoxifen DNA adducts are detectable in uterine and colon tissue of patients administered a single therapeutic dose of ^{14}C-radiolabelled drug (20 mg, 1.85 MBq) 18 h prior to hysterectomy or colorectal surgery, respectively.
Quantitation of ^{14}C-radiolabel in tissues confirmed the presence of tamoxifen and its metabolites at a concentration of 204 ± 23 fmoles (mean ± standard error of the mean (SE), n = 10) and 653 ± 135 fmoles (n = 10) tamoxifen equivalents/mg in uterine and colon tissue, respectively. Following this single dose, plasma levels of tamoxifen equivalents at the time of surgery were comparable in the two patient populations (19 ± 2 ng/ml, n = 20) and similar to published values. We demonstrate that tamoxifen does bind irreversibly to DNA from both the uterus and colon of treated patients, with the extent of damage detected being 237±77 DNA adducts/10^{12} nucleotides in uterine endometrium and 4356 ± 1050 adducts/10^{12} nucleotides in colon tissue. Similar levels were present in the myometrial layer, a tissue in which tumours do not arise as a consequence of tamoxifen treatment. In colon, the levels of CYP3A4 protein, the enzyme responsible for conversion of tamoxifen to a reactive electrophile were 10-fold higher than those detected in uterine tissue of untreated patients (1.01 ± 0.14 pmols/mg protein (n = 10) compared with 0.10 ± 0.03 (n = 4)), but did not correlate with levels of DNA binding. These results show conclusively that tamoxifen is able to bind to human uterine and colon DNA, but the extent of DNA damage is 4–5 orders of magnitude below that detected in rats that develop liver tumours after chronic tamoxifen treatment. The role of this low level of damage and the importance of alternative epigenetic mechanisms in the development of tamoxifen-induced cancer remains to be determined.

P7. EFFECTS OF TIBOLONE ON OESTROGEN AND PROGESTERONE RECEPTOR EXPRESSING HUMAN ENDOMETRIAL CANCER CELL LINES

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Tibolone is a tissue-specific compound used for treatment of climacteric complaints and prevention of osteoporosis. It is converted by steroid metabolising enzymes in the liver and intestine into three active metabolites: δ-4-isomer (exerting progestagenic and androgenic effects) and two hydroxy-metabolites: 3-OH-tibolone and 3-OH-tibolone (exerting oestrogenic effects). The aim of the current study was to gain more insight into the progestagenic, oestrogenic and tibolone-specific effects on the endometrium, using human endometrial cancer cell lines expressing different amounts of steroid receptors.
The following cell lines were used:
- Parental Ishikawa cells: progesterone receptor (PR)- and oestrogen receptor (ER)-negative.
- PRAB-36 cells (Ishikawa sub-cell line): PRA- and PRB-positive, ER-negative.
- ECC1: PR-negative, ER-positive.
- ECC1-AB21 (ECC1 sub-cell line): PRA-, PRB- and ER-positive.

The cells were cultured for 10 days in the presence or absence of tibolone (100 nM), metabolites of tibolone (100 nM), medroxyprogesterone acetate (MPA) (1 nM), E_2 (1 nM) or MPA+E_2, before growth was measured, or morphology assessed. The molecular mechanism underlying the counterbalancing effect between the progestagenic and oestrogenic activities of tibolone will, in the near future, be studied using microarrays.
Growth experiments showed tibolone-induced growth inhibition in progesterone-responsive endometrial carcinoma cells; tibolone-induced growth-stimulation in oestrogen-responsive endometrium carcinoma cells; and, interestingly, in endometrial cell lines expressing both PR and ER, tibolone and the combination of E_2+MPA, inhibited growth. Upon reviewing the morphology of the cells, it was observed that progestagens induced flattening of the cells in a single layer, while oestrogens induced growth in multiple layers. Culture in the presence of tibolone, or E_2+MPA, verified in PR- and ER-expressing ECC1–AB21 cells, was observed to be undistinguishable from culturing in the presence of MPA alone. These results indicate that, in endometrial cancer cell lines expressing both ER and PR, the oestrogenic, growth-stimulating, properties of tibolone are effectively counterbalanced by its progestagenic, growth inhibiting properties.

P8. EXPRESSION OF IMMUNOHISTOCHEMICAL OESTROGEN RECEPTORS α AND β IN OVARIAN CANCER TISSUE

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Introduction: In European countries, epithelial ovarian cancers (EOC) are a common cause of death from gynaecological malignancies, but little is known about the oestrogen dependency of EOC. Oestrogens have effects on the growth, differentiation and function of many target tissues, such as female reproductive organs, and especially in the ovaries and ovarian cancer tissues. ERβ is the predominant form in human ovaries [1,2]. The aim of this study was to evaluate ER-α and ER-β expression in ovarian cancer.
Material and methods: Samples of ovarian cancer tissue were collected from women; 70 samples of ovarian epithelial cancers were examined for the presence of ERα and 17 ovarian cancers for the presence of ER-β. ERα and ERβ immunohistochemical analysis was performed on each paraffin-embedded block (5-m sections) and on adjacent sections stained previously with 1D5 ERá monoclonal antibodies (Dako Corp Carpintiera) using the polyclonal ER (Upstate Corp.). Immunostaining was revealed by a streptavidin–biotin-enhanced immunoperoxidase technique. After antigen retrieval by pressure cooking in 0.1 M ethylene diamine tetraacetic acid (EDTA) buffer (pH 7.2) for 10 min and endogenous peroxidase blocking with 1% $H0_2$, sections were incubated with normal donkey serum (1:40 dilution) in phosphate-buffered solution (PBS) for 30 min at 22 °C. Sections were then incubated overnight at 4 °C with ER (1:1250 dilution). The complex was revealed using anti-chicken biotinylated antibody (1:300 dilution; Vector) and streptavidin–peroxidase complex (1:1000 dilution; Dako Corp Carpintiera, USA). 3,3 Diaminobenzidine tetrahydrochloride (Sigma) was used as the chromogen and the samples were counterstained with haematoxylin (Dako), dehydrated and mounted. The oestrogen receptors were counted on a semiquantitative scale from 1+ to 3+.
Results: In the ovarian cancer samples examined, more than 60% showed expression of ER-α. ERβ was observed in more than 90% of EOC.
Conclusion: Our preliminary results reveal that ER-α and ERβ expression in ovarian cancer tissues. ER and ER expression may play an important role in ovarian carcinogenesis and be useful in the early diagnosis and treatment of ovarian cancers [3,4,5].

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P9. IMMUNOHISTOCHEMICAL PROFILE OF ENDOMETRIAL BIOPSIES FROM PATIENTS TREATED WITH LHRH AGONISTS AND TAMOXIFEN REFLECTS THE ANTIPROLIFERATIVE PROPERTIES OF LHRH AGONISTS

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We previously presented the results of a prospective study [1] conducted with postmenopausal breast cancer patients receiving a combination of luteinising hormone-releasing hormone (LHRH) agonists (goserelin (Zoladex) 3.6 mg, monthly) and tamoxifen (Nolvadex 20 mg, daily) as adjuvant therapy. The aim of the present study was to investigate the safety profile of this combined treatment on the endometrium. Among the 85 patients screened, no benign or atypical endometrial lesions were observed. The mean duration of the follow-up was 3.7 years. The combination of LHRH agonists and tamoxifen prevents endometrial growth induced by tamoxifen. With the agreement of the patients, hysteroscopies and endometrial biopsies were performed after at least 3 years of therapy. To try to explain the remarkable protection of the endometrium by LHRH, we compared the immunohistochemical profile of Ki67 (clone MIB1, Dako) and p53 (clone DO-7, Dako) in endometrial biopsies collected from women on the combined therapy and on tamoxifen alone. All endometrial biopsies from patients on the combined treatment presented an atrophic endometrium at histological examination and showed no immunostaining for Ki67 and p53. In contrast, a nuclear immunostaining of Ki67 and of p53 was noticed in the endometrium of patients treated with tamoxifen alone, irrespective of the histological appearance of the endometrium, as well as in the proliferative endometrium from premenopausal women who were used as controls. These results show that LHRH agonists have an antiproliferative effect that explains its growth inhibitory action on the endometrium [2,3].

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P10. COMBINED ADMINISTRATION OF ANASTROZOLE, 5-DEOXY-AZACYTIDINE AND VINORELBINE-TARTRATE INDUCE THE D2 STAGE OF APOPTOSIS IN AN ADVANCED BREAST INFILTRATING DUCTAL CARCINOMA CHARACTERISED BY OVEREXPRESSION OF BCL-2, 5'CPG ISLAND HYPERMETHYLATION OF THE AROMATASE (*CYP19*) GENE AND ER

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Postmenopausal women produce oestrogens through the aromatase (*CYP19*) gene which is present in high concentrations in breast cancer tissue and its stroma. Anastrazole is a reversible aromatase inhibitor which interrupts the metabolic transformation of androgenic precursors into oestrogens inhibiting its production within the tumour deposit. This mechanism makes aromatic inhibitors, such as anastrozole, superior to other endocrine therapies. Although breast cancer is sensitive to endocrine intervention in sequence, eventually resistance will occur. We obtained cancer cells from a treatment-resistant post-menopausal patient with advanced infiltrating or invasive ductal carcinoma (IDC). Methylation-specific polymerase chain reaction (MSP) detected coincident aberrant 5'CpG island hypermethylation of aromatase (*CYP19*) and oestrogen receptor (ER) which caused their transcriptional silencing. Loss of ER and *CYP19* expression was associated with poor histological differentiation, high growth fraction and poor clinical outcome. Polymerase chain reaction (PCR) exhibited overexpression of anti-apoptotic oncogene, bcl-2, which causes chemoresistance. After combined administration with the aromatase inhibitor anastrozole, DNA methyltransferase inhibitor 5-deoxyazacytidine and microtubule depolymerising agent vinorelbine-tartrate, we observed phosphorylation and downregulation of bcl-2, CpG island demethylation and re-expression of *CYP19* and ER protein and inhibition of the metabolic transformation of androgenic precursors into oestrogens. All of these events induced apoptosis in the tumour cells as measured by TdT-mediated-DUTP-biotin nick-end labelling (TUNEL). Furthermore, transmission electron microscopy showed irreversible D2 apoptotic signs, such as the disintegration of tumour cells to membrane-bound small bodies (apoptotic bodies) which were phagocytosed by adjacent tumour cells leading to a bystander killing effect. In conclusion, this therapeutic approach may revolutionise treatment against advanced breast infiltrating ductal carcinomas that are resistant to treatment due to the potential advantages offered in comparison to conventional therapy. These include a well defined mode of action, selectivity and the circumvention of resistance to treatment.

P11. POTENT PHYTO-OESTROGENICITY OF 8-PRENYLNARINGENIN AND CANCER-CHEMOPREVENTIVE ACTIVITY OF XANTHOHUMOL, PRENYLATED FLAVONOIDS DERIVED FROM HOPS (*HUMULUS LUPULUS* L.)

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8-Prenylnaringenin, a prenylated flavanone, has been identified as the oestrogenic principle of hops (*Humulus lupulus* L.) [1]. Based on specific and sensitive *in vitro* bioassays (Ishikawa Var-I, yeast oestrogen screen, receptor binding), 8-prenylnaringenin proved to be a highly potent phyto-oestrogen with oestrogenic acitivities considerably greater than that of other well-established phyto-oestrogens such as coumestrol, genistein, and daidzein [1]. The oestrogenic activity was confirmed by *in vivo* assays with ovariectomised mice and rats (acute uterine vascular response, vaginal mitosis, uterine response, bone protective effect) after subcutaneous or oral administration [2,3]. Xanthohumol, a prenylated chalcone, is oestrogenically inactive, but was shown to have high cancer-chemopreventive properties *in vitro* at all stages of carcinogenesis including a strong anti-proliferative activity [4,5].

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P12. BODY FAT DISTRIBUTION AFFECTS STEROID RECEPTOR EXPRESSION IN BREAST CANCERS FROM POSTMENOPAUSAL WOMEN AND MAY PREDICT PROGESTERONE RECEPTOR (PR) EXPRESSION IN THOSE WITH AN OESTROGEN RECEPTOR (ER)-POSITIVE LESION

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Background: Approximately 1 in three ER-positive breast cancers diagnosed after the menopause is PR-negative. PR is an end-product of the nuclear oestrogen–oestrogen receptor binding. Furthermore, postmenopausal oestrogens are from the aromatisation of adrenal androgens in peripheral fat tissue. We evaluated the effect of parameters reflecting body fat and body fat composition on ER and PR-expression in postmenopausal women with breast cancer.
Patients and methods: Between January 1st, 2002 and September 2003, 390 postmenopausal women with a primary operable breast cancer were evaluated upon admission for body weight, height, abdominal and hip circumference. Waist-to-hip ratio (WHR) and BMI [Weight/(Length in metres)^2] were calculated. We compared mean values for BMI and WHR between ER and PR and for their combined expression. Tumours with an ER-PR+ phenotype ($n = 3$) were excluded. For all breast cancers and for those with an ER-positive lesion, we compared the frequency of PR positivity for those in the lowest (Q1) and those in the highest quartile (Q4) of BMI and WHR, respectively. We also aimed to estimate the number of patients to include in order to show a statistical difference ($P < 0.05$) if the percentage of PR positivity in those that are ER-positive does not change. For steroid measurements, we used the H-score and defined steroid receptor-negativity for ER and PR as a value of <100/300.
Results: When the total number of patients reaches 468 and the current percentage for PR-positivity in those who are ER-positive does not change, WHR may significantly predict PR positivity in women with an ER-positive breast cancer comparing Q1 with Q4 (see Table 1–3).
Conclusion: Although mean values for BMI are not different between postmenopausal breast cancers with a different steroid receptor expression pattern, mean WHR predicts ER, PR and the combination. In women with an ER-positive breast cancer, we see a trend for WHR to predict PR-expression, but larger numbers are required before this is likely to reach significance.

Table 1
BMI and WHR according to ER and PR level (mean ±SD)

	ER 0-100	ER 100–300	*P*-value	PR 0–100	PR 100–300	*P*-value
N	79	311		195	195	
BMI	26.4 ± 5.5	26.6 ± 4.5	0.703	26.9 ± 4.8	26.3 ± 4.9	0.225
WHR	0.88 ± 0.74	0.91 ± 0.09	0.026	0.91 ± 0.09	0.89 ± 0.04	0.065

N, number; SD, standard deviation. See text for other definitions.

Table 2
BMI and WHR according to ER/PR (mean ± SD)

	ER – PR–	ER+PR–	ER+PR+	*P*-value
N	73	122	192	
BMI	26.6±5.5	26.2±4.4	26.9±4.8	0.395
WHR	0.89±0.07	0.90±0.09	0.91±0.08	0.069

Table 3
PR positivity (%) in all patients and in those with an ER-positive tumour per quartile (Q)1 and Q4 for BMI and WHR

All	Q1	Q4	*P*-value	ER+	Q1	Q4	*P*-value
N	97	97		*N*	79	79	
BMI	45.4%	51.5%	0.473	BMI	55.8%	61.5%	0.516
WHR	45.4%	58.8%	0.084	WHR	57.1%	67.9%	0.186

P13. IL-1 SYSTEM AND SEX STEROID RECEPTOR EXPRESSION IN HUMAN BREAST CANCER: IL-1α PROTEIN SECRETION IS CORRELATED WITH MALIGNANT PHENOTYPE

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Purpose: The interleukin-1 (IL-1) system plays an important role in human pathology and is involved in the local control of malignant disease. However, little is known about its expression in breast cancer and its correlation with prognostic parameters, such as receptor status and grading.
Experimental design: The expression of IL-1α and other IL-1 family members was analysed by reverse transcriptase-polymerase chain reaction (RT-PCR), enzyme-linked immuno-sorbent assay (ELISA) and immunohistochemistry in breast cancer cell lines, tumour-derived fibroblasts, and breast cancer tissue biopsies, and compared with sex steroid receptor status and grading.
Results: In breast cancer cell lines, IL-1α and β gene expression was present in the phenotypically most malignant cell lines, whereas ERα and PR mRNA expression was confined to lines that exhibit a rather benign phenotype. Only the highly malignant receptor-negative tumour cell line, MDA-MB 231, expressed IL-1α protein, and none of the cell lines secreted IL-1β. Breast cancer tissues expressed various amounts of IL-1α, IL-1β and IL-1 Ra mRNA, but consistently high levels of IL-1tIR. IL-1α protein expression was detected in tumour cells and/or adjacent stroma in 88%, and epithelial protein expression was correlated with both, increasing de-differentiation ($P = 0.002$; $r = 0.469$), and decreasing epithelial ERα expression in the neighbouring tumour epithelium ($P = 0.001$; $r = 0.457$).
Conclusion: We have demonstrated the presence of a functional IL-1 system in breast cancer and found that IL-1α is inversely correlated with local sex steroid receptor expression. We hypothesise that the unphysiological expression of IL-1α in less differentiated and ERα-tumours might contribute to their local invasiveness and malignant behaviour.

P15. SOME PECULIARITIES OF THE INFLUENCE OF TAMOXIFEN ON THE ENDOMETRIUM OF POST-MENOPAUSAL PATIENTS

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The aim of our research was to study the influence of tamoxifen on the endometrium, in post-menopausal patients, and to perform an hyperplastic processes during therapy. Two groups of patients were formed: 155 received tamoxifen for more than 1 year (the study group) and 121 did not receive anti-oestrogen therapy (the controls). The groups were standard with regard to their age, duration of postmenopause, time to beginning of the postmenopause and co-morbid diseases. Investigations were supported by anamnesis, transvaginal sonography, hysteroscopy with aspirate biopsy in cases of increasing M-echo of more than 5 mm and morphological analysis of bioptate. Results in the study group showed an increasing M-echo in 95 (61%) patients, hyperplasic processes were found using hysteroscopy among 43 (28%) of patients and 31 (20%) of them had fibrotic polyps and 12 (8%) – adeno-cystosis hyperplasia. In 49 (32%) of cases atrophy of the endometrium was found, but morphological investigations showed hyperplasia in the basal part of endometrium, with large amounts of sclerosing spiral arteries. Three (2%) patients had endometrial adenocarcinoma with a high level of differentiation status. In the control group, 22 (18%) had an increased M-echo and 20 (17%) of them had hyperplastic processes that consisted of 7 (6%) polyps, 11 (9%) adenohyperplasia and 2 (2%) atypical hyperplasia. Two (2%) patients had highly differentiated adenocarcinoma of the endometrium. In conclusion, tamoxifen causes proliferation of the stromal component and hyperplasia of the basal part of the endometrium, as can be seen by an increasing M-echo. The most frequent consequences of basal hyperplasia are fibrotic polyps of the endometrium.

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P14. TIBOLONE AND ITS METABOLITES INHIBIT THE INVASION OF HUMAN MAMMARY CANCER CELLS

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Invasion of mammary cancer cells leads to metastasis and is the major determinant of the fatal outcome of the disease. Since tumours are considered as micro-ecosystems consisting not only of cancer cells, but also of living and non-living host elements, invasion models offering living tissue as a confronting partner for the test cancer cells are more relevant to tumour behaviour *in vivo* than models offering non-living host material only. So, in addition to the collagen type I invasion assay, we also include the chick heart assay as a model to evaluate the effect of possible anti-invasive agents. For tibolone and its metabolites, collagen type I gels were incubated with human T47-D mammary carcinoma cells, while embryonic chick heart fragments were confronted with human MCF-7/6 mammary cancer cells. The interaction of the cancer cells with their substrates was evaluated in living cultures after 1 day and on histological sections after 8 days. At 100 μM, tibolone and its 3-β-OH metabolite inhibited invasion of T47-D cells into collagen type I, while in this assay 3-α-OH, sulphated 3-α-OH and δ 4 failed to do so. In the chick heart assay, tibolone and 3 of its metabolites (3-α-OH, 3-β-OH and δ 4) inhibited invasion of MCF-7/6 cells. In the latter assay, the treated cancer cells were found to grow around the chick heart fragment without any signs of occupation or destruction of the host tissue. Among the many hundreds of compounds tested during recent years in the chick heart assay, tibolone and its principal metabolites share their anti-invasive effect with tamoxifen, retinoic acid, the citrus flavone tangeretine and the hops-derived prenylated chalcone xanthohumol. The mechanism of action of tibolone and its metabolites is now under study, and we can already exclude effects on directional migration and on cell-cell adhesion. In conclusion, tibolone and 3-β-OH tibolone possess anti-invasive activities in at least two different invasion assays using human mammary carcinoma cells. This may add to the beneficial effects of tibolone in cancer patients.
Vanhoecke BW is a recipient from the Gent University (BOF nr. B/00222/01).

P16. DISSEMINATION OF PROBAND-MEDIATED INFORMATION DOES NOT MEET THE EXPRESSED WISHES OF FAMILIES WITH A *BRCA1/2* GENE MUTATION

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Background: Genetic counselling for hereditary breast and/or ovarian cancer (HBOC) is usually based on a protocol of non-directive counselling from the international guidelines used for Huntington families. When a *BRCA1/2* gene mutation is found in a family, the possibility of predictive counselling and testing is also offered to the other family members, but only through informing the proband. We have examined the efficiency of information transfer from the proband to the other relatives, and we compared the level of transferred information to the needs in these families.
Patients and methods: Fourteen families (with a *BRCA1/2* mutation) with 107 subjects participated in the study. Subjects were eligible for participation if they were first-degree relatives of an affected person with breast cancer, ovarian cancer, or another primary cancer, or if they were first-degree relatives of a known or probable mutation carrier. Data were collected using semi-structured interviews.
Results: This study clearly reveals that the transfer of information from probands to their relatives is highly defective. In contrast, and surprisingly, almost all participating relatives (97.8%) wanted to be informed about the various aspects concerning HBOC, and even wanted to have a predictive genetic test (96.6 %).
Conclusions: The results of this study lead to the conclusion that the current practice of dissemination of proband-mediated information is inefficient and does not meet the needs expressed by members of high-risk cancer families for whom preventive measures (such as clinical surveillance, prophylactic oophorectomy and prophylactic mastectomy) are available. We therefore propose to inform relevant relatives with an informative letter, without revealing any personal genetic test results.

P17. NEOADJUVANT HORMONAL THERAPY IN BREAST CANCER: RESPONSE IS NOT ALWAYS REFLECTED IN A SIZE REDUCTION BY CLINICAL EXAMINATION OR IMAGING

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Introduction: Locally advanced breast cancer treated by mastectomy is associated with a high risk of loco-regional recurrence, metastatic disease and poor outcome. Neoadjuvant hormonal therapy is used to reduce the size of the tumour and the number of micrometastasis, in order to improve outcome. A reduction of tumour volume on clinical examination and imaging is generally regarded as response.

Case report: A 69 year old woman came to the gynaecology clinic with a palpable mass in her left breast. She was referred by the haematology department, where she was treated for low-grade Non-Hodgkin's lymphoma stage IV in 1993. In 1996, she experienced a mild cerebrovascular accident (CVA) and in 1999 she was put on anti-coagulantia because of an inferior infarction of the right lung. In 2000, she developed a myelodysplastic syndrome.

Clinical examination of the left breast showed a palpable tumour with a diameter of 6×3 cm in the superomedial quadrant. The left axilla contained a small mobile lymph node.

Mammography showed a distortion of the breast tissue behind the left areola, while ultrasound demonstrated a poorly defined hypo-echogenic lesion in the left breast together with pathologically enlarged lymph nodes in the left axilla between 1 and 2.3 cm in diameter.

Magnetic resonance imaging (MRI) showed a zone extended over 6 cm in the superolateral quadrant of the left breast with a pathological time–intensity curve. A true-cut biopsy was performed and showed an infiltrating lobular carcinoma with 100% oestrogen receptor-positivity, no receptors for progestogen and overexpression of the HER2/Neu oncoprotein.

Liver ultrasound, chest X-ray and bone scintigraphy showed no indication for metastasis. CA 15.3 levels were 25.5 /ml. Therefore, it concerned an extensive local process (T3N1M0).

As there was an important co-morbidity in this patient, a neo-adjuvant hormonal therapy (letrozole) was the first choice of treatment. In case of sufficient tumour regression, surgical treatment would be considered.

The patient was re-evaluated after 6 months. Breast palpation still demonstrated the same pronounced mass superomedial in the left breast. Ultrasound still showed an inhomogenic mass superior in the left breast and hypo-echogenic lymph nodes with diameters between 10 and 19 mm. Mammography now revealed a zone with relatively higher density behind the left areola and above that a few small nodules.

MRI showed two proximate circular lesions with identical position compared with the previous MRI, but with less captation of contrast. CA 15.3 levels were 29.1 μ/ml.

Clinically speaking, there was no sense of regression of the disease and one could say there was a certain stability. Preceded by haematological work-up, a mastectomy of the left breast was performed followed by axillary lymph node resection.

Pathology results showed dense fibrous breast tissue with multiple foci of infiltrating, well-differentiated lobular adenocarcinoma. The infiltrating component was located in the 9-cm fibrous zone. It was impossible to determine the maximal tumour diameter. The M.A.I was 0. The receptor status was identical to that in the true-cut biopsy, except for the overexpression of the HER2/Neu oncoprotein which was no longer detectable. All six prelevated lymph nodes were invaded by lobular adenocarcinoma without capsular penetration. There was a remarkable amount of dense fibrosis within the lymph nodes.

Although clinical examination and imaging techniques did not show any tumour regression after neo-adjuvant treatment with letrozole, tumour load reduction was proven by pathology reports. Because of partial response in neo-adjuvant setting, further adjuvant treatment with letrozole was given.

Conclusions: Tumour response is not always reflected by a reduction in tumour size on clinical examination or imaging (mammography or magnetic nuclear imaging). The true response can only be assessed by pathology.

P18. IUMPA-2: PROGESTERONE RECEPTOR LEVEL IN ENDOMETRIAL HYPERPLASIA SIMPLEX AS A PROGNOSIS FOR THE LOCAL EFFECT OF INTRAUTERINE DRUG DELIVERY SYSTEM

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Grant of The State Committee of Scientific Research nr 4P05E 058 19

Introduction: IUMPA-2 (Intrauterine Medroxy progesterone Acetate) is a drug delivery system aimed at the local intrauterine distribution of progestagen. Its therapeutic effect is due to a well-known action of the mechanism of action of the steroid hormone on target tissues through passive diffusion. The aim of this study was to examine cytosolic progesterone receptor levels to assess the potential effect of blocking the process of oestrogen binding to the cytosolic oestrogen receptor. The inhibition of oestrogen binding to the cytosolic oestrogen receptor inhibits the expression of cellular DNA and subsequent protein synthesis and, as a consequence, reduces further tissue hyperplasia.

Materials and methods: The study comprised 165 patients, divided into the following groups:

1. Forty nine patients with uterine leiomyoma, whose endometrium was histopathologically normal,
2. Fifty four patients, in whom simple endometrial hyperplasia was detected,
3. Sixty two patients with endometrial carcinoma.

Histological material was collected by way of a hysteroscopic-guided biopsy, with the size of the sample set at 2 mg. In our study, we used an immunoenzymatic method to measure the cytosolic progesterone levels, we assessed the receptor protein level in relation to the total protein level in the tissue examined (fmol/mg).

Results:

- Cytosolic progesterone receptor level in normal endometrium: 567.19 fmol/mg (standard deviation (SD) 289.25).
- Cytosolic progesterone receptor level in simple endometrial hyperplasia: 614.29 fmol/mg (SD 252.31).
- Cytosolic progesterone receptor level in endometrial hyperplasia: 147.67 fmol/mg (SD 256.19).

Discussion: The results show that progesterone receptor levels in normal endometrium and in simple endometrial hyperplasia, are very similar. The examined receptor level is sufficient grounds for the local application of progesterone, in keeping with the rule that the higher the receptor level the better the results of the hormonal therapy. Our results, are consistent with our previously obtained data, as well as with data in the literature.

Conclusions: It is justified to use apply IUMPA-2 to treat endometrial hyperplasia simplex on account of high cytosolic progesterone receptor level.

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P19. PHYTO-OESTROGENS

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Phyto-oestrogens could be a valid substitute in the treatment of postmenopausal symptoms. This suggestion is often made based on epidemiological studies and, to a much lesser extent, based on data from mature and well-designed clinical trials. Phyto-oestrogens have a modest activity on hot flashes. The scientific support, from mostly underpowered clinical trials, shows an improvement in almost 40% of the patients compared with 20% placebo and 80% of oestrogen-treated patients. The biologically effective dose is in the order of 50–100 mg per day. The effect of isoflavones, particularly genistein, on bone mineral metabolism is well documented, both in preclinical and clinical work. A daily dose of 50–100 mg genistein compares well with 1 mg oestradiol. The treatment with phyto-oestrogens of both hot flashes and bone demineralisation takes time: 4 months to 1 year. Isoflavones are well known to protect individuals from cardiovascular diseases and the health claim of soy proteins is well documented, with sound scientific support. The daily dose again is minimally 50 mg isoflavones or 25 g of soy protein. Phyto-oestrogens are well tolerated and doses up to 300 mg daily are non-toxic. Nutrition-derived phyto-oestrogens even protect against endometrial, breast and colon cancers. By virtue of this wealth of qualities, phyto-oestrogens indeed appear excellent alternatives to classic hormonal replacement therapy when prevention of osteoporosis and cardiovascular disease is the goal. Even in the treatment of hot-flashes they can be useful. At least, that is what we can predict from nutrition studies. Now that more purified compounds, mixtures and extracts become available in definable quantities, it is time for clinical studies. Then we can learn how much, when and for whom we can advise the use of phyto-oestrogens in the future.

P20. CONCOMITANT ENDOMETRIAL AND OVARIAN HORMONE-SENSITIVE MALIGNANCIES: A REPORT OF TWO CASES

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Case 1: A 71-year old patient presented with postmenopausal bleeding for more than one year. The patient had diabetes mellitus type 2 and arterial hypertension, was obese (body mass index (BMI) 41.74 kg/m^2) and had a supernormal bone density (Z-scores of 3.8 SD for L1-L4 and 3.6 SD for right and left hip). On vaginal ultrasound and computerised tomographic (CT)-scan, an endometrial thickness of 50.1 mm and a 200 x 110 x 150 mm ovarian mass above the uterus were found. Pipelle endometrial biopsy led to the diagnosis of a well-differentiated endometrioid adenocarcinoma. A total abdominal hysterectomy with bilateral salpingo-ovariectomy, peritoneal washing and omentectomy were performed. On histology, the diagnosis of an extensive grade I endometrioid adenocarcinoma of the endometrium with superficial myometrial invasion, as well as a large unilateral stage Ia endometrioid adenocarcinoma of the left ovary was made. There was endometriosis of the contralateral ovary.
Case 2: A 43-year old nulligravida presented with a one year history of menometrorrhagia and dysmenorrhoea. The patient had type 2 diabetes mellitus, was obese, and had a history of primary subfertility. Vaginal ultrasound showed a myomatous uterus with irregular endometrial lining. A total abdominal hysterectomy with bilateral salpingo-ovariectomy was performed. The histological examination confirmed the uterine myomata and demonstrated a well differentiated stage Ia endometrioid adenocarcinoma of the endometrium, as well as a unilateral Müllerian stage Ia adenosarcoma of one ovary: the epithelial component was borderline malignant and the stromal component was a low grade sarcoma. Endometriosis in the contralateral ovary was found.
Conclusion: In both cases independent synchronous primary Müllerian tumours of the endometrium and one of the ovaries is suggested based on the clinical presentation and the histological features. Chronic endogenous oestrogenic impregnation may have promoted the development of both the well differentiated endometrial carcinoma, and the malignant degeneration of ovarian endometriosis.