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Potential of AG in the treatment of breast cancer

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Purpose: AG is a diterpenoid lactone, isolated from a local herb. In this study the antitumour potential of this agent was evaluated using in vitro and in vivo breast cancer models.

Methods: Human breast cancer cells, MCF-7 (hormone-dependent) and MDA-MB-231 (hormone-independent) were cultured in RPMI 1640 medium supplemented with foetal calf serum and penicillin-streptomycin. The cell killing property of AG was determined using the microculture tetrazolium (MTT) assay. Type(s) of cell death induced by AG was determined using two DNA-binding dyes, acridine-orange and propidium-iodide. Effect of AG on the cell cycle progression was determined using flow cytometry. The pharmacokinetics (plasma concentration and t1/2) of AG at various doses was carried out using NCR-Nu mice. The in vivo antitumour potential of AG was established using 6 - 8 weeks old NCR-Nu mice transplanted with MCF-7 tumour xenografts.

Results: AG displayed approximately a 4-fold selectivity in killing the hormone-dependent MCF-7 cells over the hormone-independent MDA-MB-231 cells. Generally, apoptosis seemed to be the main mode of cell death induced by AG in the sensitive MCF-7 cells. However, necrosis occurred when the cells were treated with high concentrations (30 - 100 microM) of AG. This agent induced a consistent G1-arrest in MCF-7 cells. A preliminary pharmacokinetics study showed that AG reaches maximum concentrations of 20 - 30 microM and has a half-life of 1.5 hr in the plasma of mice treated with 150 mg/kg of AG. Since the concentrations achieved in the plasma are effective in killing MCF-7 cells in vitro, a single dose of 200 mg/kg was administered (i.p) to nude mice transplanted with MCF-7 cells. AG clearly inhibited the growth MCF-7 tumours in mice for a period of 14 days.

Conclusion: The abilities of AG to selectively kill the hormone-dependent breast cancer cells in vitro and inhibit the growth of in vivo tumours, makes this compound an ideal candidate to be developed for the treatment of breast cancer, especially the hormone-dependent type.

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Survey of modalities of assessing and reporting toxicity in non-comparative prospective studies of chemotherapy in breast cancer

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Purpose: To review how toxicity, a main end-point of phase II studies, is assessed and reported in published phase II chemotherapy trials in breast cancer.

Methods: A survey was performed by hand-searching studies published in seven distinguished journals between 1995 and 1999. All selected papers were independently evaluated with an ad- hoc study report form by two investigators. Descriptive statistics, contingency tables and the chi-square test were applied.

Results: Overall, 122 papers were found; 65.6% of the papers lacked a statistical study design. Planned modalities for assessment of toxicity were inadequately reported in 21.0% of the studies. The scheduling of assessment of haematological toxicity varied greatly. Toxicity was predominantly summarized 'per patient' (69.7%). Although overall the WHO scale was more frequently adopted (45.9%), the Common Toxicity Criteria (in different versions) was more frequent in studies published in journals with a high impact factor (p=0.001), in more recently initiated studies (p=0.03), sponsored studies (p=0.0006), and studies with an identifiable statistical design (p=0.006).

Conclusion: The wide diversity in modalities of toxicity assessment and reporting observed in this study suggests that the reliability of the body of published data on the toxicity of chemotherapy in breast cancer may be questionable. Current standards should be revised and harmonized so as to improve the reliability of such data.

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The influence of genistein on the growth of experimental mouse mammary cancer 16/C and on the effectiveness of treatment with cyclophosphamide

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Genistein, an isoflavonoid present in soy products, is a phytoestrogen exhibiting various biological activities. It has been shown to be an inhibitor of tyrosine kinase and topoisomerase II activity as well as of tumor angiogenesis. In addition, genistein induces cell differentiation, apoptosis, and DNA strand breakage in the propagated in vitro cells and may reveal some estrogenic or anti-estrogenic properties.

The results of epidemiological studies suggest that the soybean consumption may contribute to the lower rate of breast cancers. Genistein was shown to inhibit the mammary tumorigenesis in rats, the proliferation and invasion of murine and human mammary cancer cells in vitro and the angiogenesis in human breast cancer cells xenografts.

The purpose of this study was to evaluate the antitumor effect of genistein alone and combined with cyclophosphamide in the mice transplanted with estrogen receptor-positive mouse mammary cancer 16/C. An influence of the route of tumor cells inoculation on the antitumor effect of these treatments and on the level of estrogen (ER) and progesterone (PgR) receptors was evaluated.

When genistein was administered from day 4th (during 10 days) after orthotopic tumor cells transplantation, the statistically significant stimulation of tumor growth was observed in the treated mice in comparison with untreated control group (3780 and 2834 mm3, respectively at day 24). Also in mice inoculated subcutaneously, stimulation of tumor growth by genistein was observed as compared to the control mice (2893 and 2540 mm3, respectively at day 27). After the treatment with CY alone or with genistein combined with CY, the statistically significant inhibition of primary tumor growth was observed in both s.c. and orthotopically inoculated mice.

On the other hand, in mice bearing s.c. tumors, genistein down-regulated the ER and PgR levels. No differences in these receptors level in mice transplanted orthotopically with 16/C tumor and treated or not with genistein were observed. In tumors from control mice transplanted s.c. the ER and PgR level was significantly higher than in tumors transplanted orthotopically.

In conclusion, genistein, known from its antitumor activity in some tumor models, stimulated 16/C mouse mammary cancer growth. On the other hand, in mice bearing 16/C tumor, genistein reduced ER and PgR level when the cells were injected s.c., but not after orthotopic transplantation of tumor cells.

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Primary chemotherapy, using outpatient (OP) continuous infusional (CI) 5-Fluoruracil, epirubicin and cyclophosphamide (IFEC), produces surgically significant down-staging in locally advanced breast cancer (LABC)

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Introduction: In the neoadjuvant setting response rates (RR) of 75 to 80% have been reported. ECF regime (epirubicin, cisplatin and CI 5-FU) produces high RR, but is a toxic regime requiring hospitalisation. CI 5-FU alone in ABC produces (RR) of 20-30%, following failure of bolus 5-FU regimes. We describe our experience in treating women with LABC with OP primary IFEC (CI 5FU 200mg/m2/day, E 60mg/m2, C 600mg/m2, Q= 21 days).

Methods and Patients: From 1998-2000 chemo-naive women with histologically or cytologically confirmed breast cancer, inoperable (IO) or unwilling for mastectomy (M), were treated with IFEC (total 6 cycles if responding). Staging was according to clinical assessment, triple assessment (histology, mammogram and ultrasound), and metastatic screening (CXR or CTScan, and bone scan). Responses and toxicity were graded according to WHO criteria.

Results: 33 women,(with 34 tumours), age range 29-71 (2 <30yrs, 1= 30-40yrs, 14 =40-50yrs, 10=50-60yrs, 6 >60yrs), PS 0 (18) or 1 (15), received IFEC.14/34 tumours (41%) were T4, 12 (35%) were T3, 7 (21%)

were T2 and 1 (3%) was T1. 21 tumours (62%) were N0, 8 (23%) were N1 and 5 (15%) were N2, and 4 (12%) also had M1 disease. Overall RR (CR +PR) was 94%. After 6 cycles 6 tumours (17.6%) had a CR, 26 (76.4%) had a PR, 2 (6%) had SD. None had progressed. At presentation, 17/34 tumours (50%) were inoperable (IO), 16(47%) would have been suitable for M and 1(3%) suitable for WLE. Post IFEC, of the 17 IO tumours, 1 (6%) achieved CR, did not require surgery, 4 (23.5%) had WLE, 8 (47%) had M and 4 (23.5%) were not operated upon due to M1 disease. Post IFEC, of 16 tumours previously suitable for M, 5 (31%) did not require SR, 8 (50%) had WLE and 3 (19%) had M. Over 95% of DI was delivered for E in 80% patients, for C in 79% and for 5FU in only 29% patients. Less than 75% DI for 5FU was delivered in 24% of patients. IFEC had the following toxicity: 100% grade 2 alopecia, 30% grade 2 and above PPE, 12% grade 4 neutropenia, with 3% neutropenic sepsis. Indwelling catheter complications included: 4/33 (12%) line site infection, 2 (6%) required re-siting of catheter, and 2 (6%) line-associated thrombus.

Conclusion: Primary IFEC is an OP treatment, producing high RR and surgically significant downstaging of LABC. IO tumours are rendered operable, and M can be avoided. Toxicity is acceptable.

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Mammary ductoscope in diagnosis of breast nipple discharge

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Breast nipple discharge is one of the symptoms of breast cancer. However, patients with breast nipple discharge dose not necessarily have breast cancer. Therefore, it often cause panic among the patients and difficulties in diagnosis for the doctors. About 3-6% of patients who came to our breast surgical outpatient service had the nipple discharge as the chief complaint. According to various reports, 5-21% of patients with nipple discharge had breast cancer, 35-48% had intraductal papilloma, 17-36% had ductal ectasia, and other diagnoses including fibrocystic disease, mastitis, etc. were also observed. Other studies indicated that the different types of discharge revealed differential risks of breast cancer. For example, the rates of confirmed breast cancer among patients of bloody and serous discharge were 27% and 34%, respectively. The common diagnostic methods used for years include palpation, mammography, breast ultrasonography, cytological test, and galactography. Recently, the Medical Science Company in Japan developed a new tool, mammary ductoscope (0.8mm in diameter), for the diagnosis of breast of breast nipple discharge. We applied this new device to 15 patients whose cause of nipple discharge was unable to be identified through the traditional diagnostic methods during the period from April

1999 to July 2000. Except one Patient with narrow mammary ducts, the rest (25) patients were all-capable of taking the examination and underwent the procedure successfully. Among them, 3 were diagnosed with early stage breast cancer, 9 with intraductal papilloma, 3 with ductal ectasia, and 8 with fibrocystic disease.

In summary, the mammary ductoscope can assist in making differential diagnoses as well as locating the focus, which makes it a good option for diagnosing breast nipple discharge with unknown clinical origin.

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T1 breast cancer and axillary lymph node metastases

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Purpose: Determination of factors associated with the incidence of axillary lymph node metastasis (ALNM) in T1 tumors and cases in which axillary dissection could be omitted.

Methods: Data from 245 patients with T1 primary invasive breast cancer (size $\leq$ 20 mm) who underwent either mastectomy or wide local excision of the tumor and axillary dissection were reviewed.

Results: ALNM was found in 78 of 245 patients with T1 tumors (31.8%). Tumor size was found to be the only independent predictor of ALNM, having a directly analogous relationship with the probability of invaded nodes: T1a ($\leq$5 mm) tumors had 0% ALNM, whereas T1b (5 mm < T1b $\leq$ 10 mm) and T1c (10 mm < T1c $\leq$ 20 mm) tumors had 27.5% and 35.3% ALNM respectively. Among the other factors studied (patient's age, tumor size, hormone receptor status, histologic type and grade of the tumor) only the histologic grade of the tumor cells appeared to correlate with the incidence of lymph node involvement, but this was not statistically significant.

Conclusion: Only tumor size has statistically significant correlation with the incidence of ALNM. Routine axillary dissection could be omitted only in patients at minimal risk of ALNM (ductal carcinoma in situ and T1a) and when treatment decisions were not influenced by lymph node status (e.g. elderly patients with clinically negative axilla). Axillary dissection (at least levels I and II) should be performed in all cases with primary invasive breast cancer with tumor size > 5 mm.

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Long-term outcome of male breast cancer. A single institution experience

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Background: Male breast cancer is almost an orphan disease. It comprises only 1% of all cases of breast cancer, and treatment strategies are basically extrapolated from those effective in women.

Aims: To analyze the clinical features, treatments and long-term outcome of breast cancer in males.

Patients and Methods: Records of 5914 patients with cancer (1317 with breast cancer) treated at our institution from January 1994 to March 2001 were searched looking for males with breast cancer.

Results: Twenty-one men with breast cancer were treated. Median age was 65 years (range 39-87). Histology was infiltrating ductal carcinoma in 19 patients, intraductal carcinoma in 1 and papillary carcinoma in 1. Stage at diagnosis was 0 in 1 patient (5%), I in 3 (14%), II in 4 (19%), III in 6 (28%) and IV in 7 (33%). Treatment for stages I-III included mastectomy with axillary dissection followed by anthracycline-based chemotherapy plus postoperative irradiation and tamoxifen. Therapy for stage IV patients included palliative hormonotherapy and chemotherapy. Disease-free survival at 5 years was 27%. Overall survival was 36% at 5 years.

Conclusions: Male breast cancer, presenting mostly in aged men, is usually diagnosed in advanced stages with fair prognosis, that might be improved with diagnostic and treatment programs specifically tailored to this population.

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Down-regulation of the zeta-chain in sentinel node biopsies from breast cancer patients

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Background: In several neoplastic diseases, immunosuppression has been shown to correlate with disease stage, progression and outcome. As the prognosis for metastatic breast cancer still is pessima, additional strategies are being sought to improve survival. Local immunosuppression in sentinel node biopsies from 24 breast cancer patients was evaluated as a possible way to select patients for immunotherapy.

Method: Sentinel node biopsy was performed in 24 women operated for primary breast cancer. Specimens were snap-frozen and double-stained for the zeta-chain of the T-cell receptor. The degree of down-regulation of the zeta-chain was evaluated in three different areas of the lymph nodes: primary follicles, secondary follicles and paracortex.

Results: We observed immunosuppression of varying degree in all 24 sentinel node biopsies. A high degree of down-regulation (more than 50% of T-cells not expressing the zeta-chain) was seen in the primary follicles of 6 patients (25%), the secondary follicles of 13 patients (72%) and the paracortex of 19 patients (79%).

Conclusion: Local immunosuppression was seen in sentinel node biopsies of breast cancer patients. In addition to possible prognostic implications, the sentinel node might be the appropriate location for detection of early-stage immunological down-regulation, which might open the possibility to select those patients who could benefit from immunotherapy.